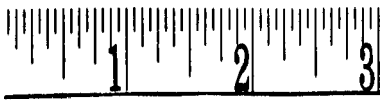
DOCTORAL DISSERTATION SERIES

TITLE CULTURE STUDIES IN THE GENERA PLEOSPORA, CLATHROSPORA, AND LEPTOSPHAERIA

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CULTURE STUDIES IN THE GENERA *PLEOSPORA*, *CLATHROSPORA*, AND *LEPTOSPHAERIA*

by

Emory Guy Simmons

A dissertation submitted in partial fulfillment
of the requirements for the degree of
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University of Michigan

1950

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CHAPTER I

STATEMENT OF PROBLEM

The classification of Pyrenomycetes is at present in a chaotic condition. Any natural and usable classification depends upon a more detailed knowledge of the life cycles, development, and variability of a much larger number of species than has been studied. Such information is not always easy to obtain because of technical difficulties involved in making proper observations and in obtaining significant data.

The systematic arrangement of families and, to some extent, of genera in the Pyrenomycetes is based upon the type of perithecial development. Delimitation of species is established primarily on the morphology of the perithecial stage, often on data derived from a very limited number of collections. It is desirable, therefore, to extend the procedure of species circumscription to reflect (a) a knowledge of the pattern of variation of the natural population and (b) a knowledge of the range and the mechanism of variation in both the ascus and the conidial stages and of the correlation of these two stages.

The determination of these factors is hampered by

certain unsolved problems:

(1) work on the cytology and the developmental morphology of the perfect fruiting body has been retarded by the difficulty of obtaining perithecia in culture and by the technical difficulties of staining and of interpreting the complex hyphal structures within the perithecia;

(2) the determination of genetic behavior and of the cause and mechanism of variation in these organisms also depends upon the routine formation of perithecia in culture under controlled conditions;

(3) the knowledge of variability of imperfect stages is not on a firm basis because of the lack of known genetic connections with ascus stages and the lack of comparative studies of variation in pure strains.

The range of these problems is so great that it soon became obvious that no complete and final study could be made of any one of them. There was made, rather, a preliminary survey of this field by a cultural study of several species in three genera of the Pleosporaceae with more detailed excursions into several problems which presented possibilities of positive results.

The first line of investigation was concerned with the behavior of various strains and species in the formation of conidia and the variation of conidia within and between isolates. These problems were studied in some detail.

Secondly, because of the importance of production of perithecia in culture for studies in cytology, genetics, or ascospore variation, special attention was given to this problem. In addition, a preliminary study was made on the variation of ascospore length in single pure cultures of three different perithecial strains.

CHAPTER II

INTRODUCTION AND HISTORICAL ACCOUNT

Comparatively little experimental work has been done on perithecial development of the fungi under discussion, or on determination of the validity of species circumscription in these genera, or on clarification of the systematic value of conidial stages connected with different species. The following review of the literature summarizes the present state of our knowledge concerning these problems.

Pyrenomycetes are those fungi which form an enclosed fruiting body (a perithecium) containing a hymenial layer of asci.

Lindau (1897) divided this subclass into four orders: Perisporiales, Hypocreales, Dothideales, and Sphaeriales. Later workers erected other orders: Myriangiales (Starbäck, 1899), Hemisphaeriales (Theissen, 1913), and Pseudosphaeriales (Theissen and Sydow, 1918). These separations were based upon structure and ontogeny of the fruiting body; however, our knowledge of the groups is limited to details of comparatively few forms.

The Pleosporaceae have a pseudosphaeriaceous type of development and produce solitary fruiting bodies beneath the

surface of stems and leaves of herbaceous and woody plants. The genera considered here differ in their types of ascospores as follows:

Pleospora -- ascospores with transverse and longitudinal septa; spores not flattened.

Clathrospora -- ascospores with transverse and longitudinal septa; spores flattened.

Leptosphaeria -- ascospores with transverse septa only; spores not flattened.

In addition to the ascospores borne in sac-like asci in the fruiting body, species of these genera also may have asexual conidial stages of different sorts borne free upon the mycelium or within fruiting structures.

I. PERITHECIAL DEVELOPMENT IN PLEOSPORA, CLATHROSPORA, AND LEPTOSPHAERIA

Perithecial development in members of the genera Pleospora and Leptosphaeria has been described only by a few investigators and has not been described for any member of the genus Clathrospora. Previous ontogenetic studies have been limited to Pleospora herbarum (Pers.) Rab., P. Tropaeoli Halsted, Leptosphaeria Lemaneae (Cohn & Wor.) Sacc., and L. Opuntiae Dodge.

Gibelli and Griffini (1874) described and figured the origin and early development of the perithecia of

Pleospora Sarcinulae Gib. & Griff. [= P. herbarum (Pers.) Rab.]. They indicated that the fruiting structure arises from one or two cells of an otherwise undifferentiated hypha. Swelling and proliferation of these cells leads to the formation of a spherical body with an outer region of dark-colored cells and an inner region of light-colored pseudoparenchyma having a central core of vertical, parallel, filamentous hyphae (the paraphyses) and asci. Because of the opacity of the peripheral cells, Gibelli and Griffini were unable to follow the actual development of structures within the fruiting bodies. They gave no opinion concerning the sexual potential of any of the hyphae involved in the formation of perithecia.

Bauke's description (1877) of the early perithecial development of P. herbarum in his cultures was in agreement with Gibelli and Griffini. He extended his description of the changes which take place within the outer layers of dark-colored cells by means of observations made on vertical sections taken from developing fruiting bodies. Statements were made that a number of slender, parallel hyphae or paraphyses arise from a layer of parenchymatous cells located near the base of the perithecium initial and grow upward through the central parenchyma causing the compression and gelation of this latter tissue. Asci are produced later as outgrowths of the basal cells of the paraphyses and extend

upward between the paraphyses which, at length, become pressed together and lose their protoplasmic contents. On the basis of these observations, Bauke declared that hyphae which develop into perithecia are purely vegetative in character and that at no time in the perithecial development does any morphologic evidence of sexual fusion of hyphae occur.

Miyabe (1889) gave a more detailed account of the developmental morphology of P. herbarum. His observations were, in essence, a refinement of the findings of Bauke. Miyabe described the formation of the perithecial initial with a center composed of pseudoparenchyma. He indicated the presence of certain internal cells of variable number and position and with highly refractive contents which develop eventually into short, simple or branching chains of cells giving rise to a closely packed bundle of paraphyses and, later, to the asci themselves. He described the dissolution of the central pseudoparenchyma during the development of the paraphyses and the compression of the peripheral pseudoparenchyma, of the paraphyses, and of the basal cells during the formation of asci. In agreement with Bauke, Miyabe stated that he was unable to observe any sexual process connected with the formation of perithecia and, in addition, that he could find no trace of a sexual process inside young perithecia before the formation of

paraphyses and asci. He considered the initial cells of the perithecium to be of vegetative origin and the formation of the perithecium to be entirely a vegetative process.

Cavara and Mollica (1907), unlike their predecessors, ascribed a definite sexual nature to certain growth elements in the form of P. herbarum they studied. A summary of their observations made on stained sections of material obtained from cultures follows:

The perithecial initial originates in an act of conjugation between two morphologically identical hyphae. There is a reciprocal orientation of nuclei in paired cells of adjacent hyphae, and, occasionally, the absence of a nucleus in one member of the pair of cells can be correlated with the presence of two nuclei in the other member of the pair. (These authors were not able to offer cytologic proof that the pair of nuclei are from different cells instead of being sister nuclei, but they carried out the remainder of their discussion on the assumption that a diploidization does occur.)

Hyphal neighbors of the joined cells form a knot of mycelium around the diploidized (?) cell and gradually, through successive divisions, form a pseudoparenchymatous tissue around this cell and its progeny. The diploidized cell divides to form chains or other groupings of similar cells containing relatively large nuclei and dense cytoplasm.

Later the diploid cells increase in number and, following the dissolution of the vegetative or haploid cells, finally occupy all the space within an outer few layers of thick-walled and heavily pigmented cells.

Still later, in an internal region near the base of the perithecial initial, there appears a group of cells differentiated from other cells by reason of the presence of two or of four nuclei in each. Cavara and Mollica inferred from their observations that the tetranucleate cells are derived from the binucleate ones, either by division of the two nuclei of the latter or through fusion of the binucleate cells in pairs. (The explanation of this nuclear behavior and that which follows was very involved, and the authors often clouded their observations with more or less obscure assumptions.)

From these tetranucleate cells there then arise series of uninucleate cells forming vertical, linear elements which these authors designated as paraphyses. Shortly afterward a pair of nuclei is found in one intercalary cell of each of several of the vertical series. This pair of nuclei could originate either in a division of the pre-existing nucleus of the cell or in consequence of the fusion of two cells of contiguous vertical elements. (The authors favored the latter explanation, although they admitted the lack of cytologic proof.) This cell, uninucleate after the fusion

of the pair of nuclei previously present, was called the mother-cell of an ascus and was considered to develop into a mature ascus containing eight spores.

No cytologic evidence more detailed than the size, position and color density of the nuclei in the different stages of perithecial development was offered by Cavara and Mollica. Their conclusions, based on their observations and inferences, were: (1) perithecial development is initiated after an act of conjugation between physiologically differentiated cells; (2) the entire centrum of the mature fruiting structure, including paraphyses and asci, develops from the conjugated cells; and (3) nuclear phenomena of division, pairing, and fusion subsequent to the initial act of conjugation have the single significance of separating and of recombining chromosomes to the end of transmitting and of distributing hereditary characters to the ascospores. These authors admitted the possibility of only one nuclear fusion which is sexual in nature, namely, the initial fusion in the hyphal elements preceding the formation of the perithecial initial.

Klebahn (1918) added nothing to the more complete understanding of the ontogeny of this species when he described material derived from cultures of Pleospora Sarcinulae Gib. & Griff. [= P. herbarum (Pers.) Rab.]. He remarked upon the formation of perithecial initials with

central cavaties filled with vertical, parallel, thread-like hyphae. He supposed these threads to be paraphyses between which asci later would appear but was unable to find asci in these particular cultures over an extended period of time. In some of his cultures which were left undisturbed for 11 months he reported the eventual formation of asci with mature spores.

Nuclear phenomena in the development of P. herbarum have been described by Miyabe (1889), who recorded his observations of nuclei in the ascus, and by Cavara and Mollica (1907). No intranuclear phenomena in the species have been described. Students who have worked with strains of this fungus agree, in most points, as to the internal morphology of different stages of development of its perithecia. However, the questions of the exact site of nuclear pairing, of the origin of the ascus, and of the possibility of any sexual phenomena in this species remain controversial.

The only other species of Pleospora about which information derived from cultured material is available is P. Tropaeoli Halsted. Halsted (1893) described the early stages of development of a perithecium as being characterized by a slender filament becoming septate, turning olive-brown, and becoming thick-walled. He stated that frequently two or more such threads take part in the formation of a perithecium but that he was unable to discover morphologic

evidence of sexual phenomena, although there were "many hints of the presence of fertilization." No description of the further development of perithecia was given. Halsted remarked, however, that he was unable to find paraphyses in the fruiting bodies observed.

Knowledge of developmental morphology in Leptosphaeria is limited to two species, L. Lemaneae (Cohn & Wor.) Sacc. and L. Opuntiae Dodge. Observations for these species were made on material from nature rather than on material from pure cultures.

Woronin (1870) described the earliest evidence of perithecial formation in L. Lemaneae as the swelling of the tip of a hypha and the subsequent delimitation of the enlarged portion by the formation of a cross-wall. A second dilated hypha from the same mycelium then becomes applied to the first swollen cell. Woronin failed to discover the subsequent stages in development, but, from analogy with other fungi familiar to him, he inferred that the pair of inflated cells becomes surrounded by neighboring hyphae until a mass of tissue is formed. This outer tissue develops into a cortical layer of brown, polyhedral cells, while the internal cells proliferate into a tissue of indistinct, small cells forming the perithecial centrum and, eventually, mature asci. Woronin stated that paraphyses are not found in this species.

Brierley (1913) made observations on the development of Leptosphaeria Lemaneae from microtome sections and from macerations of material from nature. He made elaborate attempts at obtaining reproduction of this species in pure culture but was unsuccessful. His description of the developmental phenomena observed may be summarized as follows:

It is found, though rarely, that two hyphae become applied to each other and that adjoining, slightly dilated cells (terminal pairs, intercalary pairs, or terminal with intercalary) show the appearance of fusion. In such cases one cell apparently is devoid of a nucleus while its partner contains two nuclei or a single nucleus of about twice the size of a paired nucleus.

Fine hyphal branches arise from cells immediately adjacent to this diploid cell and become applied closely to it.. The enveloping hyphae increase in size and number until a small spherical mass is formed around the large, more deeply staining uninucleate cell.

The fusion nucleus divides, and the cell containing it becomes septate into a number of multinucleate cells, from which ascogenous hyphae arise. These hyphae branch freely and irregularly in the perithecial tissue and the nuclei pass into the branches in pairs. Walls are laid down and, apparently, asci may arise from any of the binucleate cells thus formed. This binucleate stage of the ascus lasts

only for a short while, the nuclei soon fusing. Various stages in ascus development from the binucleate, dilated hyphal tips to mature asci are to be found within the same perithecium.

During the development of the asci, the enveloping hyphae develop into a structure having a wall layer of somewhat elongate and flattened polyhedral cells, an internal few layers of thin-walled cells, and a central mass of very delicate cells among which the asci are formed.

Brierley stated that his specimens reveal no paraphyses. Instead, he observed that the region above the tips of the asci is filled with a pad of sterile, thin-walled cells which degenerate, thus forming the ostiolar canal. He remarked, also, that the apparent fusion of hyphal elements which marks the initiation of perithecial development is exactly paralleled by similar mycelial dilations of purely somatic occurrence. From his studies he found it difficult to believe that the former fusions are reproductive in nature.

Dodge (1937), in his study of L. Opuntiae, dealt only with the organization of the perithecial cavity. Earlier stages of development were not described. His observations may be indicated by quoting part of his summary: "The perithecial cavity develops largely through the differential growth of the peripheral tissues as a wall while the cells

at the center elongate vertically by adding intercalary cells so that one sees streamers of hyphae extending across the cavity and attached to the wall cells above and below. Ascogenous elements arise from the fertile tissue at the base and grow up between the disorganizing vertically growing space-making hyphae." His main points were based on the facts that the interthecal elements are attached to the wall cells both above and below, that the asci arise from fertile tissue at the base of the perithecium, and that paraphysoid elements found in mature perithecia are the remains of the original sterile, vertical threads.

The developmental morphology of species of Leptosphaeria, thus, is very imperfectly known, and cytologic details are practically non-existent. Only through an extension of experimental work covering a large number of species can there be derived sufficient data for the full determination of the type or types of perithecial development within the genus.

II. SPECIES CIRCUMSCRIPTION IN PLEOSPORA, CLATHROSPORA, AND LEPTOSPHAERIA

The basis of species segregation by workers in the genera Pleospora, Clathrospora, and Leptosphaeria rests, at present, upon the relative magnitude and quality of different characters of the mature perfect stage. Chief among these

characters in common use (Wehmeyer, 1948) are those of the perithecia (size, wall thickness, formation of setae); of the asci (size, shape); and of the ascospores (shape, size, septation, color). Substratum preferences and conidial connections have been used in a few instances in the circumscription of species by Diedicke (1903), by Noack (1905), by Drechsler (1923), and by others.

The use of data derived on the basis of these criteria has resulted in the describing and in the naming of large numbers of species in these genera. The tendency in such nomenclatorial procedure has been to describe as a new species any collection or morphologically similar group of collections which does not satisfy a previous description completely, although a great latitude in the overlapping of character similarities of the "species" being differentiated has been allowed. The primary objections to such circumscription of species in these genera are that the investigator cannot know the genetic origin and constitution of a particular specimen, that he does not know the environmental history of the collection, and that he cannot be certain always of the maturity of the critical structures. In short, it is impossible for him to be certain of the specific identity of the organism without being informed concerning the pattern of development and the innate variability of the species.

The worker in the systematics of these genera is plagued by the lack of these data. In order to approximate a natural classification of known species, he must be able to understand the condition of existence of the organisms in nature. In this respect he must find answers to such questions concerning the natural state of affairs as the following:

(1) Are all of the similar organisms found in a collection actually derived from sources (ascospores, conidia, hyphae) which are genetically identical?

(2) Are the perithecia of a given collection the product of one, of two, or of several spores or hyphae?

(3) Are single spores of a specimen capable of reproducing the species, or does the completion of the life cycle depend upon some system of heterocaryosis or of hybridization?

(4) What are the effects of such factors as temperature, moisture, light, altitude, nutrient supply, physical nature of substratum, time of year of initiation of growth, or presence of other organisms upon the development and ultimate morphology of the critical elements? Do these factors have individual specific effects upon the growth of the organism or do they act in conjunction with or in opposition to each other?

The answers to these questions and to related questions

are not known in any specific case.

In order to reconcile the system of species segregation in these genera as it exists in nature and as it is described in the literature on the basis of more or less artificial criteria, it is necessary that controlled experimental work be done involving the reconstruction or approximation of the conditions prevailing in nature. For this purpose it is essential that the experiments make use of individual organisms (i. e., the progeny of single ascospores or conidia) and that as many isolates as possible of different collections of morphologically similar organisms be examined and compared. Only in this manner can a knowledge of the actual variation-potential of a species and its strains be derived and a concrete basis for species delineation in these genera be founded.

III. CONIDIAL STAGES OF PLEOSPORA, CLATHROSPORA, AND LEPTOSPHAERIA

Mycological literature contains numerous statements concerning ontogenetic connections between species of Pleospora and of Leptosphaeria and members of the Fungi Imperfecti. Usually these assertions are based on observations that a perithecial stage and an imperfect stage are closely associated in nature; either both organisms usually are found on the same host, or both are found intermixed in the same

collection, or one develops on a site previously occupied by the other, or one develops upon the surface of the other. Infrequently, conclusions concerning such connections are based on experimental studies in which one stage is seen to develop from pure cultures of spores of the other stage.

The recorded observations of some of the earlier mycologists are open to doubt, primarily because of the inadequacy of the culture techniques employed (when culture techniques actually were used), but also because of the probability of the incorrect determination of source materials or of the inclusion of more than one genetically different but morphologically similar species in a single experiment.

A classic example of the confusion raised over the ontogenetic connection of imperfect stages with a perithecial stage is the history of studies made on Pleospora herbarum (Pers.) Rab. Tulasne (1863) listed no less than sixteen binomials in his discussion of the pleomorphism of the species, including members of the form-families Sphaerioidaceae, Moniliaceae, Dematiaceae, and Tuberculariaceae. This list has been augmented and depleted in turn through the years following studies by several investigators (Fuckel, 1869; Hallier, 1869; Gibelli and Griffini, 1874; Bauke, 1877; Kohl, 1883; and Mattirolo, 1888). Using artificial culture techniques, Miyabe (1889) conclusively established the

insertion of a single imperfect stage, Macrosporium parasiticum Thm. [= Stemphylium botryosum Wallr.], in the life cycle of P. herbarum. Since the time of Miyabe, His conclusions have been affirmed by a number of other investigators (Brefeld, 1891; Prillieux and Delacroix, 1893; Cavara and Mollica, 1907; Teodoro, 1923; Bolle, 1924; Kidd and Beaumont, 1924; Briant and Martyn, 1929; Brinkman, 1931; Cocchi, 1931; Ellis, 1931; Smith, 1940; Groves and Skolko, 1944a; and Hughes, 1945).

No other single species of either Pleospora or Leptosphaeria has received such detailed attention, but different authors have indicated conidial connections of at least 45 named species of Pleospora and 50 named species of Leptosphaeria. Most of these connections, as has been stated, have been based on field observations, but a few have been worked out in controlled culture and have become established in literature on pleomorphism. Among the latter may be mentioned:

(?Pleospora) Pyrenophora avenae Ito & Kuribayashi

Imperfect -- Helminthosporium avenae Eidam

(Ito, 1930; Dennis, 1935)

Pleospora infectoria Fckl.

Imp. -- Alternaria tenuis auct.

(Mattirolo, 1888; Cavara and Mollica, 1907)

P. Alternariae Gib. & Griff. (Syn. of P. infectoria Fckl.)

Imp. -- Alternaria tenuis auct.
(Gibelli and Griffini, 1874; Peglion, 1903; Ghimpu, 1931)

P. pellita (Fr.) Rab.

Imp. -- Dendryphium penicillatum Fr. (Syn. of Helminthosporium papaveri Sawada)
(Tulasne, 1863; Fuckel, 1869; Bauke, 1876)

P. calvescens (Fr.) Tul. (Syn. of P. pellita (Fr.) Rab.[1])

Imp. -- Helminthosporium papaveri Sawada
(Christoff, 1930; Barbacka, 1936)

P. Rehmiana Staritz

Imp. -- Phoma medicaginis Malbr. & Roum.
(Hungerford, 1936; Remsberg and Hungerford, 1936)

P. trichostoma (Fr.) Ces. & de Not. (This species is interpreted at present by Wehmeyer (1949) as including several binomials erected by Diedicke (1903), namely, Pleospora Bromi, P. teres, P. Tritici-repentis, P. Avenae, and P. graminea.)

Imp. -- Helminthosporium form-species (The Pleospora segregates named by Diedicke were indicated (1903) to be connected respectively with

---

[1] the modern interpretation of the species as indicated by Wehmeyer (1949).

Helminthosporium Bromi Died., H. teres Died., H. Tritici-repentis Died., H. Avenae Br. & Cav., and H. gramineum Rab. Drechsler (1923) observed such connections in P. teres and P. Tritici-repentis.)

Leptosphaeria avenaria Weber

Imp. -- Septoria avenae Frank

(Weber, 1922; Jørstad, 1930; Grove, 1934)

L. Coniothyrium (Fckl.) Sacc.

Imp. -- Coniothyrium Fuckelii Sacc.

(Stewart, 1910; Martin, 1929; Wakefield and Moore, 1936)

L. Salvinii Catt.

Imp. -- Helminthosporium sigmoideum Cav. and Sclerotium Oryzae Catt.

(Tullis, 1933a; Tullis, 1933b; Tullis and Cralley, 1933; Tullis, Jones, and Davis, 1934; Cralley and Tullis, 1935)

Aside from phytopathological applications, the knowledge concerning these conidial connections has not been employed to any great extent in mycological work. The hope that a correlation of imperfect with perfect stages would aid in the construction of a natural system of taxonomy has been hindered by the scarcity of reliable data. Moreover, the present limited understanding of what constitutes a

genetic entity in the Fungi Imperfecti prevents the resolution of this problem from a systematic standpoint.

CHAPTER III

MATERIALS AND METHODS

I. LIST OF COLLECTIONS USED[2]

Pleospora ambigua (Berl. & Bres.) Wehm.

1. On *Lupinus albicaulis* var. *shastensis*, Mt. Shasta, Calif, 1941. Leg. *W. B. Cooke* 15739.
2. On *Aster shastensis*, Mt. Shasta, Calif., 1946. Leg. *W. B. Cooke* 18234.
3. On *Lupinus subalpinus*, Mt. Rainier National Park, Wash., 1948. Leg. *E. G. Simmons* 1424.
4. On *Chrysanthemum leucanthemum*, Mt. Rainier National Park, Wash., 1948. Leg. *E. G. Simmons* 1812.
5. On *Artemisia tacomensis*, Mt. Rainier National Park, Wash., 1948. Leg. *E. G. Simmons* 1966.
6. On *Valeriana sitchensis*, Mt. Rainier National Park, Wash., 1948. Leg. *E. G. Simmons* 2338b.

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[2] All specimens were identified by Doctor L. E. Wehmeyer. The collection of *Pyrenophora alternarina* Whitehead & Dickson was obtained through the courtesy of Marvin D. Whitehead, who states that it is the type specimen. The name given by Whitehead and Dickson is used here in reference, although it is listed later as *Pleospora infectoria* Fuckel, from which species Wehmeyer considers the specimen to be morphologically indistinguishable.

Pleospora asymmetrica Wehm. inedit.

7. On Lupinus volcanicus, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1937b.
8. On Agrostis sp., Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 2187.
9. On Festuca viridula, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 2188.
10. On Muhlenbergia filiformis, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 2200a.

Pleospora comata Niessl

11. On Silene Macounii, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1969.

Pleospora helvetica Niessl

12. On Lupinus subalpinus, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1613.
13. On Aster foliaceus, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1670.
14. On Pedicularis contorta, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1673.
15. On Arnica Parryi, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1935.
16. On Lupinus volcanicus, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1937a.
17. On Ligusticum purpureum, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1952b.

18. On Achillea millefolium var. alpicola, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1958.

Pleospora herbarum (Pers.) Rab.

19. On Chrysanthemum leucanthemum, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1812a.

Pleospora herbarum (Pers.) Rab. var. occidentalis Wehm.

20. On Artemisia tacomensis, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1929.

Pleospora infectoria Fckl. (obtained as Pyrenophora alternarina Whitehead & Dickson)

21. On oat straw (Avena sativa), Madison, Wisc., 1948. Leg. M. D. Whitehead.

Pleospora laxa Ell. & Holw.

22. On Potentilla flabellifolia, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1951.

Pleospora niegusensis Bub.

23. On Helianthella sp., Pullman (Whitman Co.), Wash., 1948. Leg. W. B. & V. G. Cooke 23487.

24. On Balsamorrhiza sagittata, near Moran, Wyo., 1948. Leg. E. G. Simmons 1370.

Pleospora oligasca Bub.

25. On dead stems of a rosaceous plant, between Red Lodge, Mont., and N. E. entrance of Yellowstone

National Park, Wyo., 1948. Leg. E. G. Simmons 1366.

Pleospora richtophensis E. & E. var. pallida Wehm. inedit.

26. On Ranunculus sp., Blue Mt. Summit Pass, Ore., 1948. Leg. E. G. Simmons 1374.

27. On Aster foliaceus, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1926.

Pleospora rubicunda Niessl var. americana Wehm. inedit.

28. On Potentilla flabellifolia, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1951a.

Pleospora trichostoma (Fr.) Ces. & de Not.

29. On old wheat straw, Riley Co., Kansas, 1949. Leg. T. E. Brooks 1945.

Clathrospora Cookei Wehm. inedit.

30. On Stipa californica, Mt. Shasta, Calif., 1946. Leg. W. B. Cooke 18095.

Clathrospora diplospora (E. & E.) Wehm. in press

31. On Monardella odoratissima, Mt. Shasta, Calif., 1946. Leg. W. B. Cooke 18094.

32. On Castilleja miniata, Mt. Shasta, Calif., 1947. Leg. W. B. Cooke 20284.

33. On Anemone occidentalis, Mt. Shasta, Calif., 1947. Leg. W. B. Cooke 20285.

Clathrospora Elynae Rab.

34. On Juncus mertensianus, Mt. Shasta, Calif.,

1946. Leg. W. B. Cooke 18427.

Clathrospora permunda (Cke.) Sacc.

35. On Ligusticum purpureum, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1952a.

Clathrospora rainierensis Wehm. inedit.

36. On stems of a composite, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1746d.

37. On Muhlenbergia filiformis, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 2209.

Clathrospora Simmonsii Wehm. inedit.

38. On stems of Carex ablata, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1930.

39. On leaves of Carex ablata, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1931.

Leptosphaeria Erigerontis Berl.

40. On Ligusticum purpureum, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1952.

Leptosphaeria filiformis Wehm. inedit.

41. On stems of a composite, Mt. Rainier National Park, Wash., 1948. Leg. E. G. Simmons 1746a.

Leptosphaeria heterospora (de Not.) Niessl

42. On Trisetum spicatum, Mt. Shasta, Calif., 1947. Leg. W. B. Cooke 20223.

43. On Poa epilis, Mt. Shasta, Calif., 1947. Leg. W. B. Cooke 20477.

II. LIST OF MEDIA AND PLANT SUBSTRATA USED

The principal medium employed in the isolation, maintenance, and comparison of all isolates was Leonian's agar (1924). This substratum is considered to be particularly suitable for the culture of certain imperfect fungi. Leonian's agar also was used to a great extent in attempts at reproducing the life cycles of the different isolates in artificial culture, since data in the literature do not indicate that the type of organisms being studied here must grow on any specific medium in order to produce perithecial stages.

Isolates also were grown on several other types of media so that the influence of different substrata upon vegetative growth, conidial production, and perithecial formation could be studied.

The artificial media and other substrata employed at different times are defined below. The uses of each and specific variations from the formulae given are indicated later in their proper connections. All media and plant substrata were sterilized by means of autoclaving with steam at 121° C. for 20 minutes.

Leonian's medium: KH_2PO_4, 1.25 g.; $MgSO_4 \cdot 7H_2O$, 0.625 g.; peptone, 0.625 g.; maltose, 6.25 g.; malt extract, 6.25 g.; distilled water, 1000 ml.

Leonian's agar: Leonian's medium solidified by the addition of 20.0 g. agar to 1000 ml. of the liquid medium.

Oatmeal agar: 40 g. rolled oats, ground, then cooked while being stirred in small amount (200 ml.) of water; added, while hot, 800 ml. of distilled water in which had been dissolved 20.0 g. agar.

Mineral-peptone agar: a variation of Leonian's agar containing KH_2PO_4, 1.25 g.; $MgSO_4 \cdot 7H_2O$, 0.625 g.; peptone, 0.625 g.; agar, 20.0 g.; distilled water, 1000 ml.

Mineral agar: KH_2PO_4, 1.25 g.; $MgSO_4 \cdot 7H_2O$, 0.625 g.; agar, 20.0 g.; distilled water, 1000 ml.

Malt agar: malt extract, 20.0 g.; agar, 20.0 g.; distilled water, 1000 ml.

Ellis' synthetic medium: NH_4NO_3, 0.04 g.; $MgSO_4$, 0.24 g.; KH_2PO_4, 0.27 g.; maltose, 0.72 g.; agar, 20.0 g.; distilled water, 1000 ml. (Ellis, 1931)

Plain agar: agar, 20.0 g.; distilled water, 1000 ml.

Aster agar: decoction of Aster stems heated slightly in 300 ml. water, 250 ml.; agar, 20.0 g.; distilled water, 750 ml.

Plant stem substrata: 12 cm. lengths of dead stems of the following listed plants: Dactylis glomerata, Poa compressa, Paeonia sp., Anemone sp., Aster sp. Stems were autoclaved in test tubes 175 mm. long to which had been added 5 ml. of distilled water.

Filter paper cones: a filter paper disc was converted into a cone; the cone was placed on its base and its vertex was then depressed so that the truncated base of the original cone supported a small paper-walled well. This cone was placed in a small glass capsule-dish to which any desired liquid medium was added before autoclaving.

III. ISOLATION METHODS

In attempting an expansion of the criteria for the identification and the circumscription of species of Pleospora, Clathrospora, and Leptosphaeria it is considered essential that cultures of undoubted purity be compared.

Using aseptic techniques, perithecia of identified specimens were removed from the host tissues and were crushed in distilled water. Ascospores then were transferred to hanging drops of sterile tap water in concavity slides. These preparations were allowed to remain at room temperature (22°--26° C.) until germination occurred or, in the instance of spores which revealed no evidence of germination, for at least two weeks. Since most of the spores of the majority of preparations showed evidence of germination within 24 hours, the time period of two weeks was considered ample for the expression of spore viability under the conditions imposed. In the case of some perithecia which did not yield germinating spores in water, spore transfers were made to

Leonian's medium or to stem decoction of Aster in concavity slides. In some cases germination was induced by this treatment. Drawings were made of spores in different stages of germination in the drop cultures. Subsequently, individual germinated ascospores were isolated manually with the aid of fine needles and were transferred to Petri plates containing Leonian's agar. In some instances when single-spore isolates failed to become established on this medium, single asci with germinated spores or groups of germinated spores of undoubted origin were used in obtaining cultures. Careful microscopic checks were made in every case in order that the purity of the different isolates might be insured. After a length of time sufficient for the formation of well-established colonies by the different isolates which survived on Leonian's agar, subcultures were initiated on several artificial media and on sterilized stems of a few herbaceous plants.

CHAPTER IV

CULTURE STUDIES: GENERAL DATA

It is a common observation of many collectors that the types of fungi being considered here often produce mature perithecia in nature after overwintering on dead stems or leaves. Because of this character, preliminary test-cultures usually were allowed to remain at room temperature (22°--26° C.) for periods of one to fifteen days, after which they were removed to a cold chamber at 5° C. It was found that under these conditions Clathrospora diplospora (Collection No. 33) would develop perithecial primordia and, eventually, mature perithecia. Using this evidence of the effect of prolonged cold treatment upon the formation of perithecia, subsequent cultures of all isolates were treated in a similar manner. In most instances an initial period of growth at room temperature was permitted until the individual inoculates had formed well-established colonies. Only then were they transferred to the lower temperature.

In Table I are summarized the general results of this treatment of all species used in this study. Notations are included on establishment of growth and on production of conidia and of perithecia in culture, although the conidial

TABLE I

SUMMARY OF CULTURAL RESULTS FOR ALL ISOLATES OF SPECIES OF PLEOSPORA, CLATHROSPORA, AND LEPTOSPHAERIA

Key to Abbreviations:

No.	= collection number designated in List of Collections Used
Age	= age in months of collection at the time of attempts of isolation of ascospores
No germ.	= no germination of ascospores
Germ. only	= germination of ascospores but no further growth on transfer
Est.	= germination of ascospores and growth established in culture
Imp.	= conidial stage produced in culture
Per.	= perithecial stage produced in culture

TABLE I (continued)

SUMMARY OF CULTURAL RESULTS FOR ALL ISOLATES OF SPECIES OF PLEOSPORA, CLATHROSPORA, AND LEPTOSPHAERIA

No.	Name	Age	No germ.	Germ. only	Est.	Imp.	Per.
1	P. ambigua	75	X				
2	..	16			X		
3	..	5			X		X
4	..	5	X				
5	..	3			X		
6	..	5	X				
7	P. asymmetrica	2		X			
8	..	2		X			
9	..	2		X			
10	..	3	X				
11	P. comata	2½	X				
12	P. helvetica	3		X			
13	..	4		X			
14	..	4		X			
15	..	2½		X			
16	..	3		X			
17	..	3	X				
18	..	3		X			
19	P. herbarum	4	X				
20	P. herbarum var. occidentalis	3	X				
21	P. infectoria	(18)	(X)		X	X	
22	P. laxa	2½		X			
23	P. niegusensis	10½			X		X
24	..	5			X		
25	P. oligasca	10	X				
26	P. richtophensis var. pallida	5		X			
27	..	2		X			
28	P. rubicunda var. americana	3		X			
29	P. trichostoma	½			X		X
30	C. Cookei	17	X				
31	C. diplospora	34	X				
32	..	21			X		X
33	.. - I	4			X		X
	.. - II	4			X	X	
34	C. Elynae	14			X	X	

TABLE I (continued)

SUMMARY OF CULTURAL RESULTS FOR ALL ISOLATES OF SPECIES OF PLEOSPORA, CLATHROSPORA, AND LEPTOSPHAERIA

No.	Name	Age	No germ.	Germ. only	Est.	Imp.	Per.
35	C. permunda	2½			X		
36	C. rainierensis	12	X				
37	..	14			X		X
38	C. Simmonsii	2½		X			
39	..	2½		X			
40	L. Erigerontis	2½		X			
41	L. filiformis	3		X			
42	L. heterospora	4	X				
43	..	1			X	X	

and perithecial stages are considered separately and in greater detail in subsequent chapters.

The results indicated in Table I reveal the following information:

1. Ascospores from perithecia of 14 of the 43 collections gave no evidence of germination. It is possible that the age of a specific collection of fungus may determine whether or not the perithecia present will yield viable ascospores. In P. asymmetrica, C. diplospora, and L. heterospora the oldest specimens did not yield viable spores, whereas the younger ones did. Such age-viability correlation, however, was not found in all species. In P. ambigua, P. helvetica, and C. rainierensis viable ascospores were obtained from collections equally as old or older than similar specimens in which spores were not viable.

2. In 17 of the remaining 29 collections ascospores from the perithecia germinated in drops of liquid media but did not become established when transferred to agar media. It is possible that this failure was due to mechanical injury to the young mycelium or to drying. However, it should be noted that where spores of two or more collections of a single species were germinated, the young mycelia, when transferred, either all grew or all died. This would indicate that, in the species which failed to grow, the nutritional or other environmental requirements of the organisms

were not met by the conditions imposed upon them. (Early attempts at germination of spores were carried out on solid media, but this technique was discontinued when it was found that the incidence of contamination was high and that making critical observations for drawings was difficult.)

3. Three isolates from different species in the remaining 12 collections (in addition to the cultures of P. infectoria received from Whitehead) produced conidia in culture; six other isolates of ascospores from these 12 collections produced perithecial primordia and mature perithecia. In no case was it observed that a conidial strain also produced perithecia or that a perithecial strain also produced a conidial stage.

The 31 collections from which ascospores either did not germinate or did not become established on solid media will not be considered beyond their listing in Table I.

The descriptions and behavior of isolates of ascospores from the 12 specimens which became established in culture (plus the P. infectoria cultures) are outlined in succeeding pages. In the descriptions of the different isolates the following listed growth characters are considered: (1) gross morphology of isolates under different conditions of culture, (2) formation of distinctive hyphae or of other remarkable morphologic elements, (3) the production of conidial stages, (4) the production of perithecial stages.

I. DESCRIPTIONS OF CULTURES

Pleospora ambigua (Berl. & Bres.) Wehm.

(Collections Nos. 2, 3, 5; Figure 1)

Ascospores from perithecia in these three collections were germinated successfully and were continued in pure culture. Cultures of this species from Collection No. 2 were derived from a single ascospore; other cultures (Collections No. 3 and No. 5) were derived from the mycelia of at least two sister ascospores. Ascospores from these collections produced one to six fragile, septate germ hyphae following the swelling of cells of the spores. Usually all cells of a germinating spore became decidedly swollen and hyaline immediately preceding or during the time of germination. Cultures of these isolates were kept at room temperature for 14--20 days before removal to the cold chamber. Differences in the gross morphology of these three isolates were not apparent. On plain agar, Aster agar, peptone agar, and mineral agar as well as on stems of Poa, Paeonia, Aster, Dactylis, and Anemone a very scant superficial mycelium was produced. No other distinctive morphologic elements were produced. An abundant to heavy mycelial growth was produced on Leonian's agar, oatmeal agar, and malt agar. On Leonian's agar these isolates produced a moderately heavy to dense

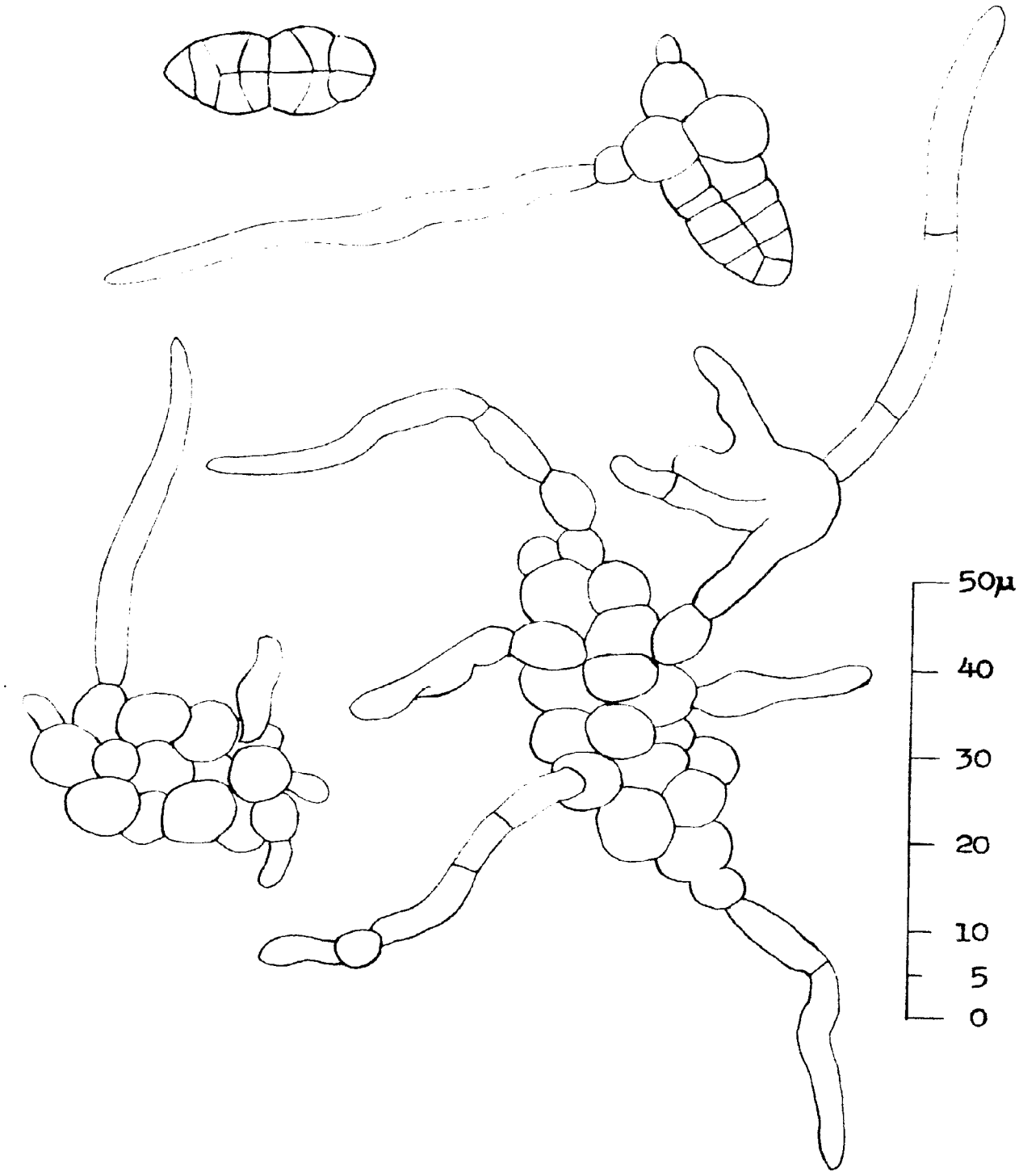


FIGURE 1

ASCOSPORES OF PLEOSPORA AMBIGUA

cottony, gray[3] to olive-gray aerial mycelium and, in the substratum, an olive-gray to dark brown or black, opaque growth. Anastomoses were common between neighboring hyphae. Conidia were not found in cultures of any of these isolates. Fruiting bodies containing mature ascospores were produced on Leonian's agar only in cultures derived from spores of Collection No. 3 of this species.

Pleospora infectoria Fckl.
(Pyrenophora alternarina Whitehead & Dickson)
(Collection No. 21)

The initial cultures of this species were received from M. D. Whitehead, Agricultural and Mechanical College of Texas. In correspondence Whitehead indicates that the cultures used here were derived in each case from single ascospores. In order to complete the data for this species, ascospores were obtained from the specimen which accompanied the cultures and were placed in a hanging-drop preparation of Leonian's medium. Germination was not obtained in spores derived from the specimen 18 months after the date of collection. (This specimen had been sent to Wehmeyer for his opinion on its identity and presumably had not been

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[3] Color values cited are used in a general sense in comparison of colonies of different species. They are not derived from a standard system of color names.

fumigated.)

The following account of cultural characters of this species is based on observations made on subcultures of Whitehead's isolates. These subcultures were allowed to remain at room temperature for 20 days, after which they were removed to the cold chamber. On Leonian's agar a very minute amount of fine white mycelium was produced and became extended over the entire surface of the medium. Neither aerial hyphae nor conidia were observed on this substratum. In cultures on unsolidified Leonian's medium, however, a distinct submerged mat of olive-brown mycelium with an aerial covering of pale gray hyphae was produced. Conidia were very abundant in these liquid cultures. On oatmeal agar light to dark gray, rapidly spreading, dense cottony colonies were produced. On this medium a heavy subsurface growth was common, the mycelium becoming black, opaque and causing the formation of a tough pseudostromatic layer in the substratum. Conidia were produced abundantly on oatmeal agar. No evidence of the formation of perithecial primordia was seen in any culture of isolates of this species.

Pleospora niegusensis Bub.

(Collections Nos. 23, 24; Figure 2)

Collection No. 23: Germination of ascospores occurred by decided swelling of the ascospore cells and by the

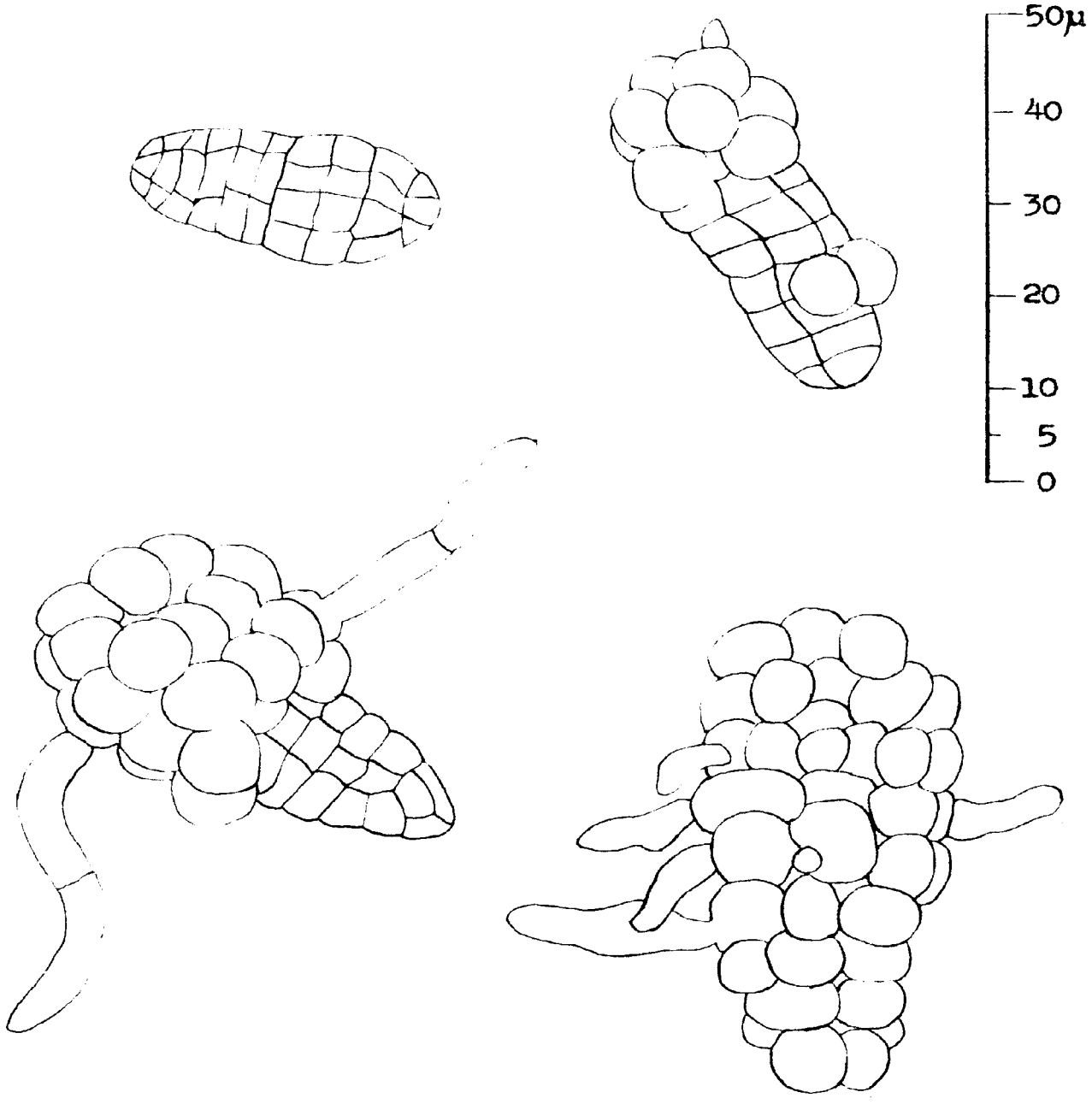


FIGURE 2

ASCOSPORES OF PLEOSPORA NJEGUSENSIS

production of as many as six germ hyphae by most of the spores observed. Single-spore isolations and mass transfers of germinated spores were made on Leonian's agar. These cultures were maintained at room temperature for 12--15 days and then were removed to the cold room. Differences between the many single-spore and mass-transfer cultures with respect to their development and gross morphology could not be discerned. On Leonian's agar the rate of growth of the colonies was decidedly slow. Within the 12--15 days of growth at room temperature a small (5 mm. in diameter), crateriform mass of blackened mycelium was produced over the site of inoculation in each of the cultures. After removal to the cold room, growth of the colonies proceeded until the medium was covered and penetrated by distinctly radiate, dark olive-brown hyphae. A very loose cottony, gray aerial mycelium was produced over the entire colony. At no time were conidia of any type observed in these cultures. Mature perithecia were produced in abundance in all cultures maintained on Leonian's agar.

Collection No. 24: Ascospores germinated readily on Leonian's medium. Single-spore transfers to Leonian's agar did not survive, but mass-transfers of germinated spores did become established. Cultures on Leonian's agar were left at room temperature for 21 days and then were transferred to the cold room. Within eight weeks a heavy growth consisting

of a medium gray to dark olive-brown matted mycelium on and in the substratum and a loose cottony, light olive-gray to dark gray aerial mycelium with superficial, dense cottony patches of white hyphae was formed. Later the aerial mycelium became less prominent or disappeared from large areas of the colony, and the colony became heavy, black-brown, opaque in the center (a circular area about 4 cm. in diameter) with a circumference of distinctly radiate, olive-brown subsurface mycelium. Conidia were not observed in any of the cultures. No evidence of the formation of perithecial primordia was found at any time.

Pleospora trichostoma (Fr.) Ces. & de Not.

(Collection No. 29; Figures 3 and 4)

Ascospores obtained from this specimen germinated readily in hanging-drop preparations of Leonian's medium. Swelling of cells of the spores accompanied the production of germ hyphae. A large number of single-spore and single-ascus isolates were made on Leonian's agar, which medium proved to be decidedly favorable to the growth of this organism, and on filter paper cones moistened with unsolidified Leonian's medium. Among the cultures of single-spore isolates on Leonian's agar, two types of gross morphology of colonies became apparent by the third day.

Type I: A single spore in culture yielded compara-

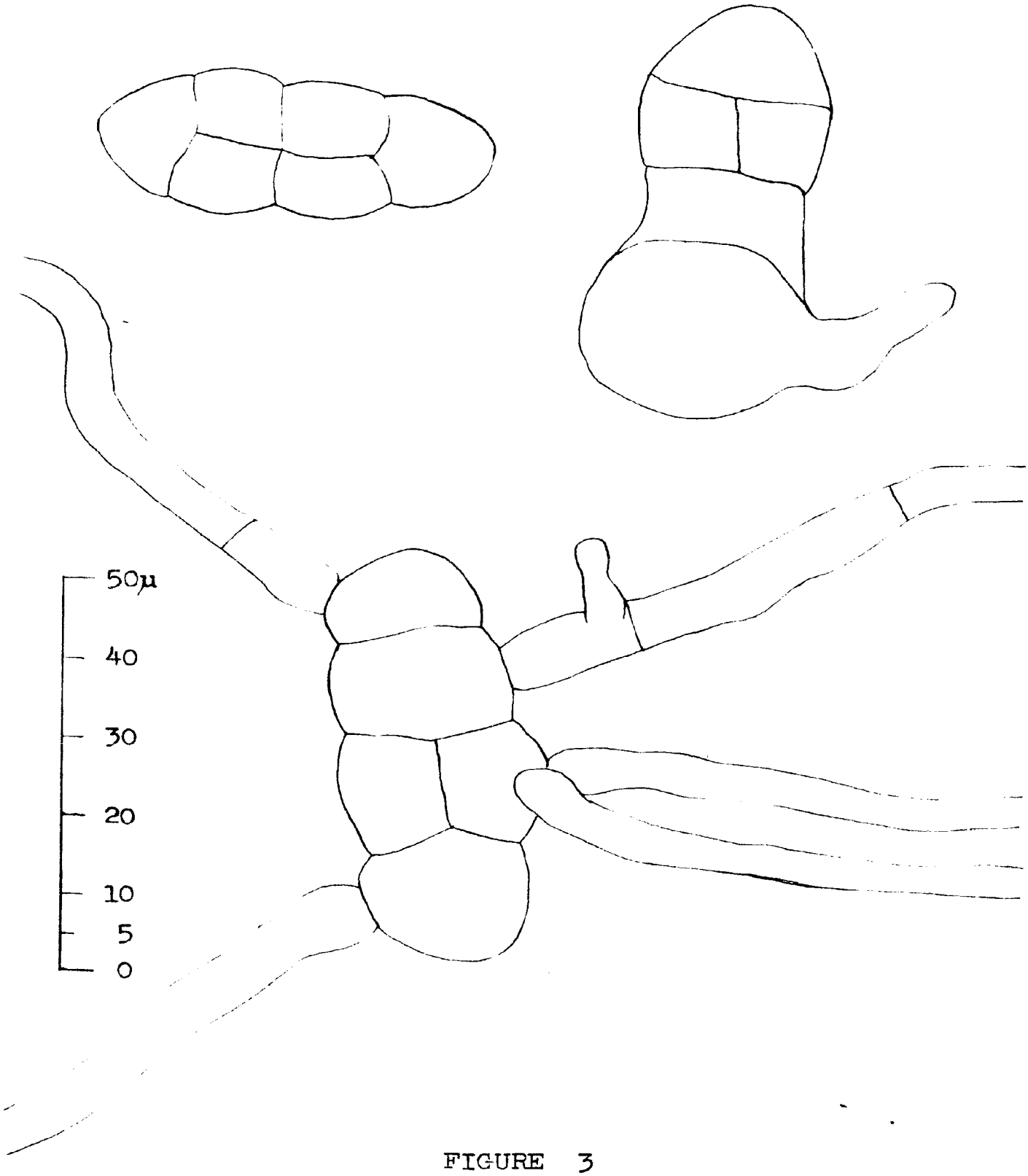


FIGURE 3

ASCOSPORES OF PLEOSPORA TRICHOSTOMA

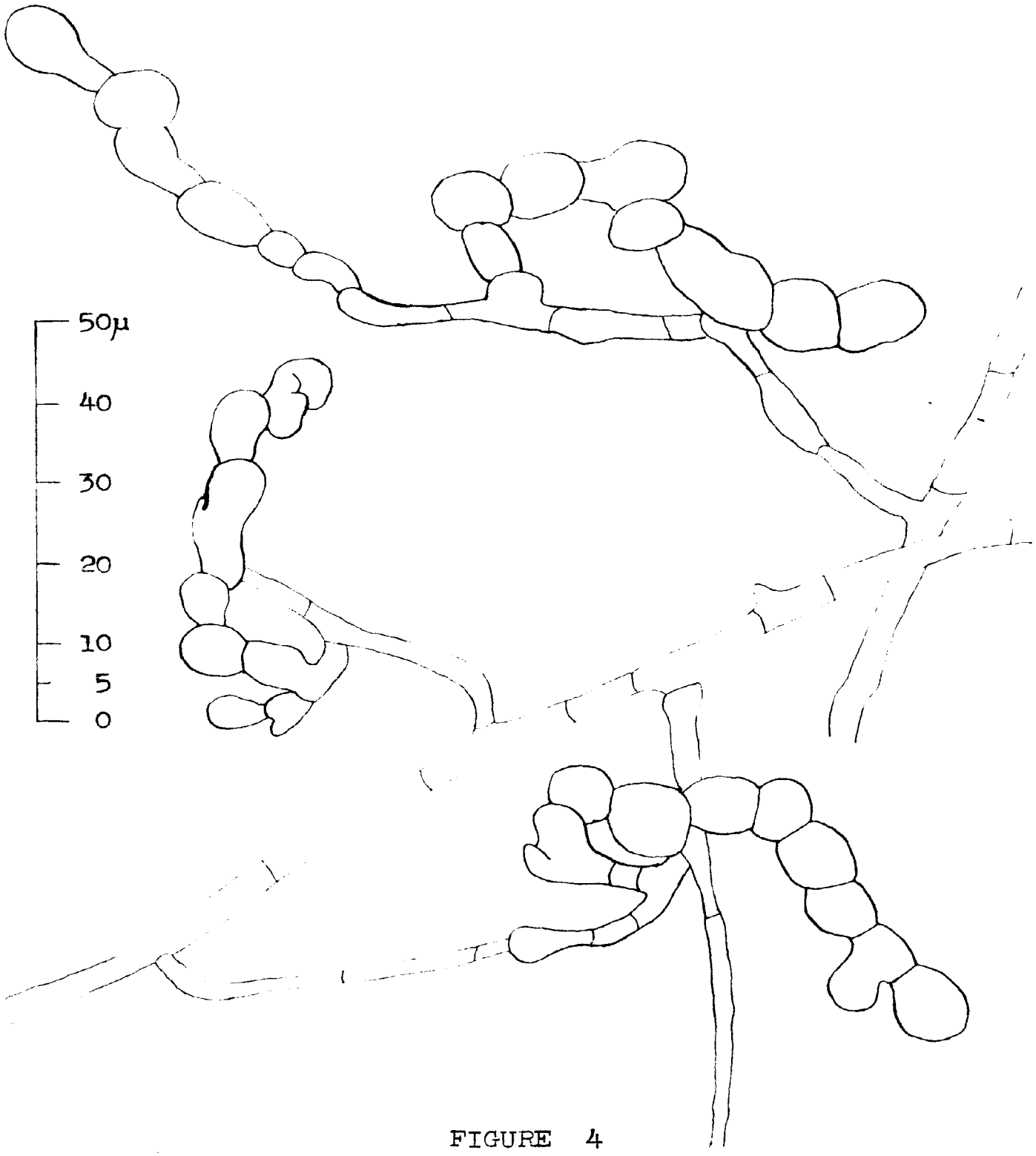


FIGURE 4

INFLATED HYPHAL CELLS PRODUCED ON LEONIAN'S AGAR BY ISOLATES OF PLEOSPORA TRICHOSTOMA

tively scant, light gray mycelium and produced dark inflated cells (Fig. 4) in the substratum which, in the aggregate, became visible to the unaided eye by the third day; by the fourth day these structures appeared orange-brown to olive-green in color and occupied an area about 3 cm. in diameter in colonies which had reached a diameter of 4--5 cm.

Type II: A single spore in culture yielded relatively heavy, light gray mycelium but did not produce aggregates of darkened cells visible to the unaided eye; by the fourth day the central area of the colony became olive-green, but no discrete darkened structures were visible.

Cultures derived from single asci exhibited the growth characteristics of Type I. Differences between the types of gross morphology of the various isolates became less pronounced within a week because of the production in all cultures of a heavy gray to olive-gray aerial mycelium.

Cultures on Leonian's agar were allowed to remain at room temperature for 18 days, after which they were removed to the cold room. Cultures on filter paper cones moistened with liquid Leonian's medium were kept at room temperature for 10 days, after which they were remoistened with sterile distilled water and were placed in the cold room. Isolates on filter paper produced a loose cobwebby, light to medium gray aerial mycelium. Conidia were not observed in any culture of this species. Perithecial primordia were produced

in all cultures of both types. Mature ascospores were found in all cultures derived from single asci and in both types of cultures derived from single spores whether grown on Leonian's agar or on moistened filter paper.

Clathrospora diplospora (E. & E.) Wehm. in press

(Collections Nos. 32, 33; Figures 5 and 6)

Ascospores from perithecia in Collection No. 32 germinated readily in Leonian's medium and in Aster decoction 21 months after the collection was made. Ascospores from Collection No. 33 germinated in distilled water and in Leonian's medium four months after the date of collection but failed to germinate on the same media after 21 months. The cultural characters of these isolates were so different that their descriptions will be noted separately.

Collection No. 32 (Fig. 5): Ascospores became swollen preceding and during the period of production of germ hyphae. Transfers of single germinated spores to Leonian's agar from the hanging-drop preparations were not successful; mass transfers of germinated spores to Leonian's agar were successful and subcultures were made from these isolates. Cultures on Leonian's agar were kept at room temperature for 12 days, by which time small (about 5 mm. in diameter), pulvinate masses of white mycelium, which apparently were not spreading, were produced. These cultures then were removed

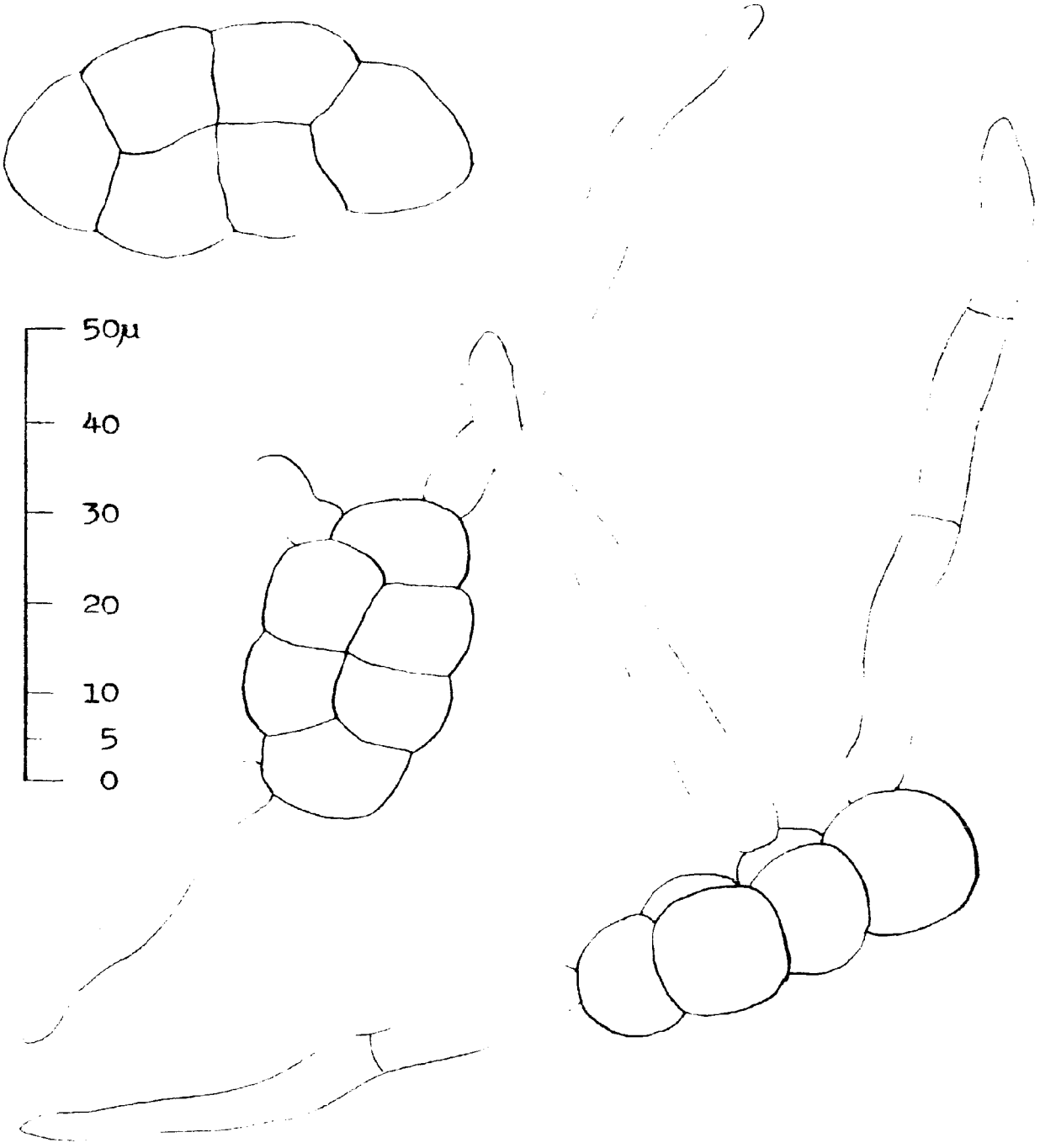


FIGURE 5

ASCOSPORES OF CLATHROSPORA DIPLOSPORA
(COLLECTION NO. 32)

to the cold room. After a period of six months at the low temperature, these colonies had increased to a diameter of 10 cm. (the limits of the culture dishes), but the amount of mycelium outside a central area about 6 cm. in diameter was scarcely noticeable. The mycelial growth was primarily on the surface of the substratum or within it. This mycelium was olive-brown to black and opaque and was produced in abundance in the center of the colony. A light gray, loose cottony aerial mycelium was produced overall, extending to the limits of the medium but becoming relatively compact only over the site of inoculation. Small, thin-walled perithecial primordia were produced in abundance in these colonies, but only a few comparatively large (up to 450 μ diameter) individuals contained mature ascospores within eight months. Conidia were not observed at any time in these cultures.

Collection No. 33 (Fig. 6): Ascospores from perithecia of this specimen (identified by Wehmeyer as a large-spored form of Clathrospora diplospora) germinated on Leonian's medium, swelling of cells of the spores occurring during the formation of germ hyphae. Six single-spore isolates readily became established on Leonian's agar. Colonies on Leonian's agar, on oatmeal agar, and on malt agar seldom were left at room temperature for more than seven days before being removed to the cold room. Two distinct types of growth

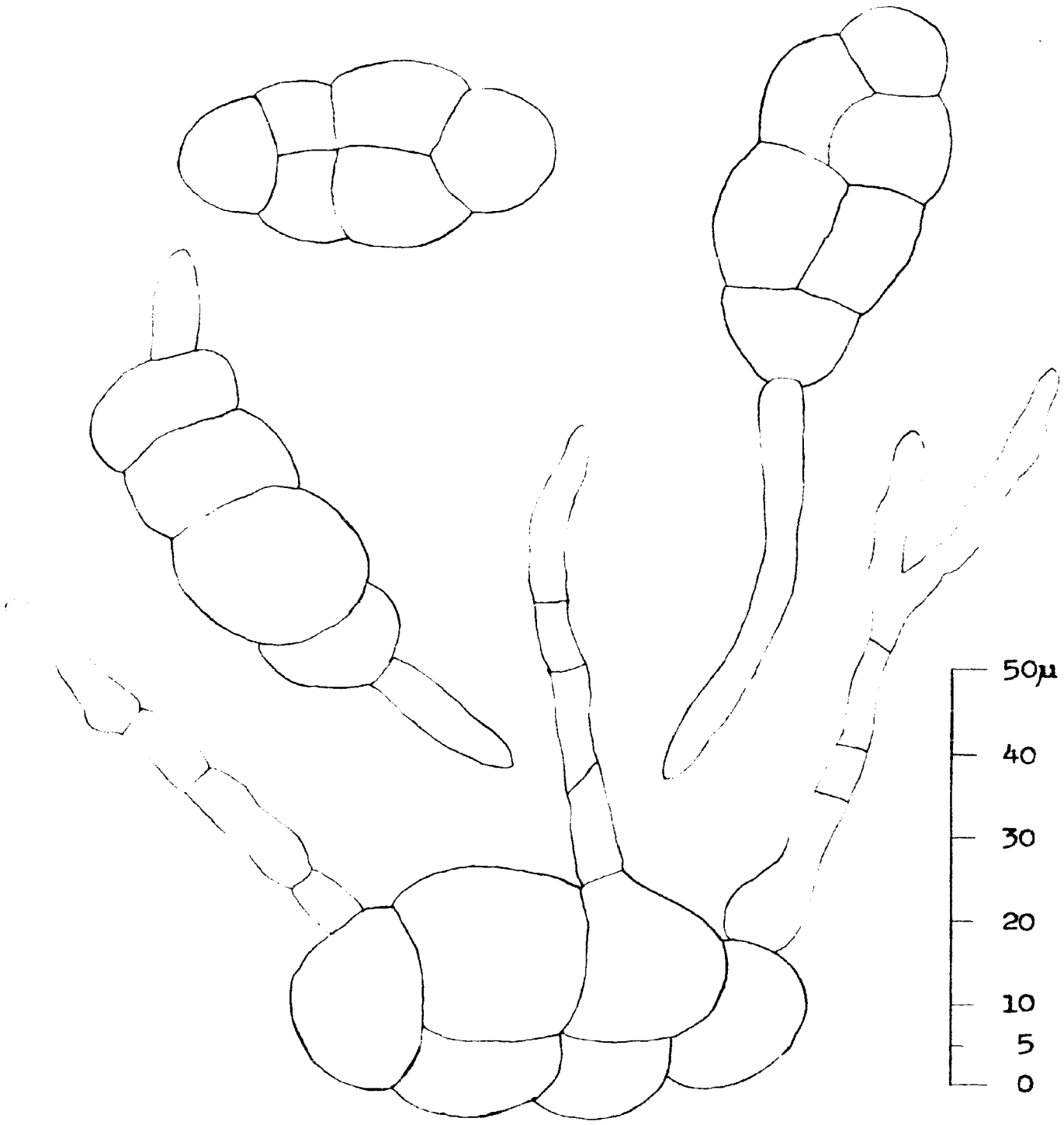


FIGURE 6

ASCOSPORES OF CLATHROSPORA DIPLOSPORA
(COLLECTION NO. 33)

became evident in the different single-spore cultures of this organism.

Type I: On plain agar, Aster agar, Leonian's agar, mineral-peptone agar, and mineral agar as well as on stems of Poa and Dactylis a very scant surface and aerial mycelium was produced within 10 days. A mycelium visible to the unaided eye was not produced on stems of Paeonia, Aster, and Anemone. A moderately heavy to heavy mycelial growth was produced on oatmeal agar and on 2% malt agar. Within 30 days after being placed at the low temperature, colonies on Leonian's agar and on malt agar produced a dark olive-black mycelium which spread slowly over the surface and beneath the surface of the substratum. A very small amount of whitish aerial mycelium was evident, chiefly over the centers of the colonies. Numerous black-brown, spherical perithecial primordia were present on the surface of or partially submerged in the substrata. Mature ascospores were not found within these structures until after a period of about four months at low temperature, at which time mature spores were present in abundance. Conidia were not observed in these cultures at any time.

Type II: On plain agar, Aster agar, mineral-peptone agar, and mineral agar as well as on stems of Poa and Dactylis a very scant surface and aerial mycelium was produced. A moderate amount of mycelial growth was formed on stems of

Aster and Anemone, and an abundant to very heavy growth was produced on Leonian's agar, oatmeal agar, malt agar, and on stems of Paeonia. On Leonian's agar within 10 days at room temperature Type II cultures produced heavy, greenish-gray colonies about 6 cm. in diameter with a dense, white, cottony aerial growth. Concentric zonation was moderately marked in these cultures. Conidia were produced in great abundance. On the same medium at a low temperature colonies of white mycelium about 9 mm. in diameter were produced within 10 days. A moderate amount of aerial mycelium was present in the latter cultures, but zonation was not evident. On oatmeal agar within 10 days at room temperature Type II cultures produced heavy, dark olive-brown colonies about 7 cm. in diameter with an abundant, matted aerial mycelium which was black near the center of the colonies and dark yellow-green at the periphery. Concentric zonation was marked in these cultures and conidia were produced in great abundance. On oatmeal agar at the low temperature colonies of white mycelium about 8 mm. in diameter were produced within 10 days. A moderate amount of aerial mycelium was present in these cultures, but zonation was not evident. On Mineral-peptone agar and on mineral agar colonies of scant whitish-tan mycelium about 3 cm. in diameter and with a very scant aerial mycelium were produced at room temperature within 10 days. Conidia were produced sparingly under these conditions;

zonation was faint. On the same media at the low temperature for 10 days colonies of barely discernible white mycelium about 10 mm. in diameter and with a very scant aerial mycelium were produced. Conidia were not found on colonies formed under these conditions and zonation was not evident. Perithecial primordia were not found in any of the cultures of Type II listed above within a period of 12 months.

Clathrospora Elynae Rab.

(Collection No. 34; Figure 7)

Germination of ascospores from perithecia of this species was obtained on Leonian's medium in hanging-drop preparations. Production of germ hyphae was accompanied by slight swelling of cells of the spores and by fragmentation of the spores into their cellular components. Two single-spore transfers from hanging-drop preparations made on two different days became established on Leonian's agar. Subcultures on Leonian's agar were left at room temperature for seven days, as a rule, before being transferred to the cold room. On plain agar, Aster agar, mineral-peptone agar, and mineral agar as well as on stems of Poa and Paeonia a scant to moderate amount of surface and aerial mycelium was formed. An abundant to very heavy mycelial growth was produced on Leonian's agar, oatmeal agar, malt agar, and on stems of Aster, Dactylis, and Anemone. Conidia were formed abundantly

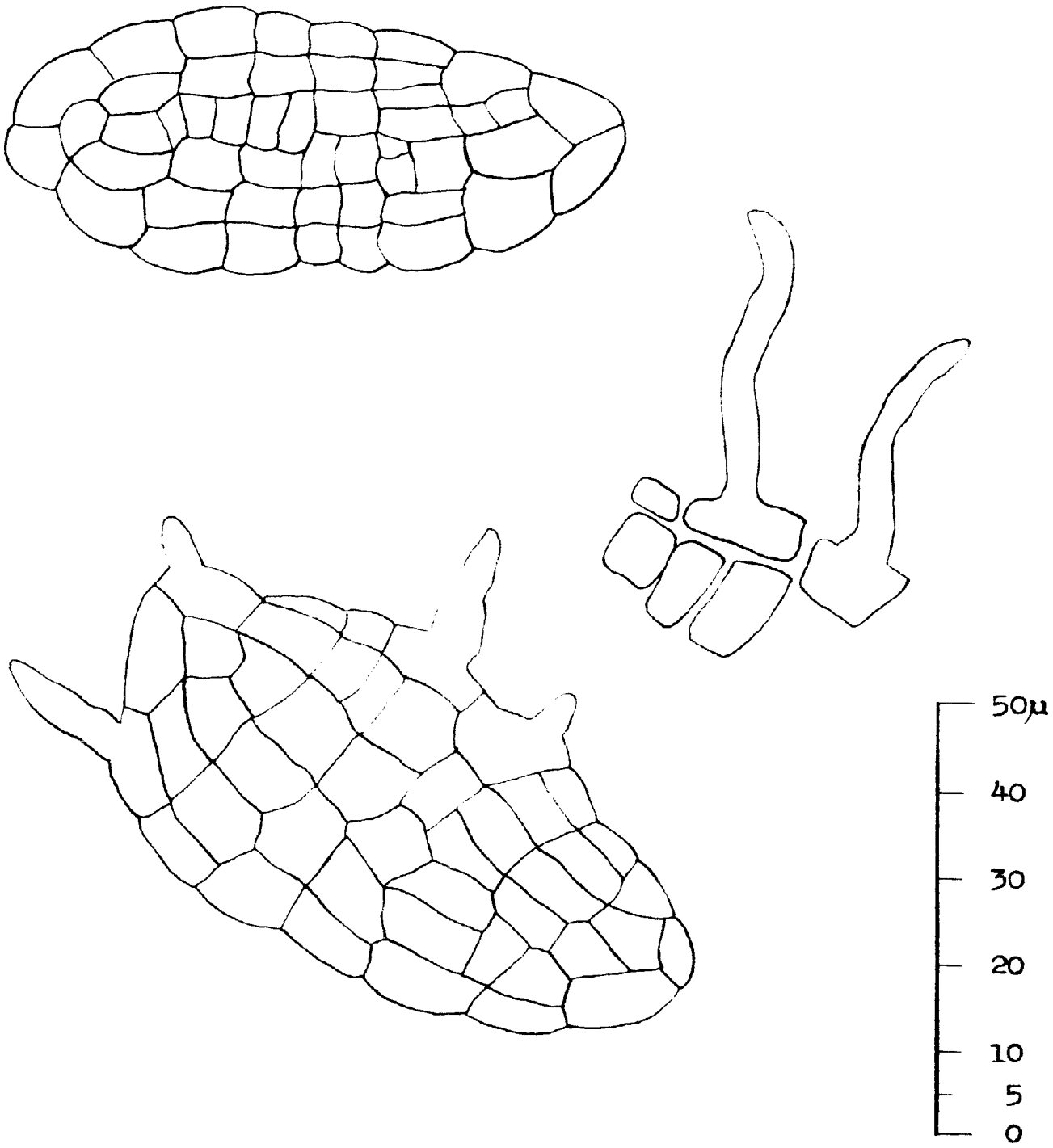


FIGURE 7

ASCOSPORES OF CLATHROSPORA ELYNAE

in colonies on all substrata except oatmeal agar, on which medium production of conidia was relatively fare. On Leonian's agar within 10 days at room temperature cultures produced heavy, greenish-black colonies about 6.5 cm. in diameter with a scant, whitish, cottony aerial mycelium. Concentric zonation was marked in these colonies. Conidia were produced in abundance. On the same medium at the low temperature colonies of scant, white mycelium about 9 mm. in diameter were produced within 10 days. A scant amount of aerial mycelium was present in the latter cultures; zonation was not evident. On oatmeal agar within 10 days at room temperature cultures produced heavy, greenish-black colonies about 7.5 cm. in diameter with a dense, whitish, cottony aerial mycelium. Conidia were produced sparingly under these conditions and zonation was only moderately marked. On oatmeal agar at the low temperature colonies of scant, white mycelium about 10 mm. in diameter were produced within 10 days. A moderate amount of aerial mycelium was present in these cultures, but zonation was not evident. On mineral-peptone agar and on mineral agar colonies of scant, white mycelium 3--5 cm. in diameter and with or without a very fine, white aerial mycelium were produced at room temperature within 10 days. Conidia were not found in cultures grown under these conditions; zonation was faint. On the same media at the low temperature for 10 days colonies

of scant, white mycelium 10--12 mm. in diameter were produced. A fine, white aerial mycelium was present in these cultures, but zonation was not apparent. Perithecial primordia were not found in any of the cultures of this specimen within a period of 12 months.

Clathrospora permunda (Cke.) Sacc.

(Collection No. 35; Figure 8)

Ascospores germinated in Leonian's medium with decided swelling of cells of the spores and with the production of one or, rarely, of two branching, septate germ hyphae. Establishment of single-spore isolates on Leonian's agar was not successful, but successful mass-transfers of germinated spores were accomplished. These cultures were maintained at room temperature for 14--20 days and then were removed to the cold room. On Leonian's agar the rate of growth of the colonies was very slow. Colonies about 10 mm. in diameter and consisting of a medium brown, radiate mycelium with a white, cobwebby aerial covering were produced at room temperature within two to three weeks. After removal to the lower temperature the darkened surface and subsurface mycelium did not advance more than a few millimeters in three months. Within three months at the low temperature the light surface and aerial mycelium spread widely over the substratum but became obscure by the end of this time.

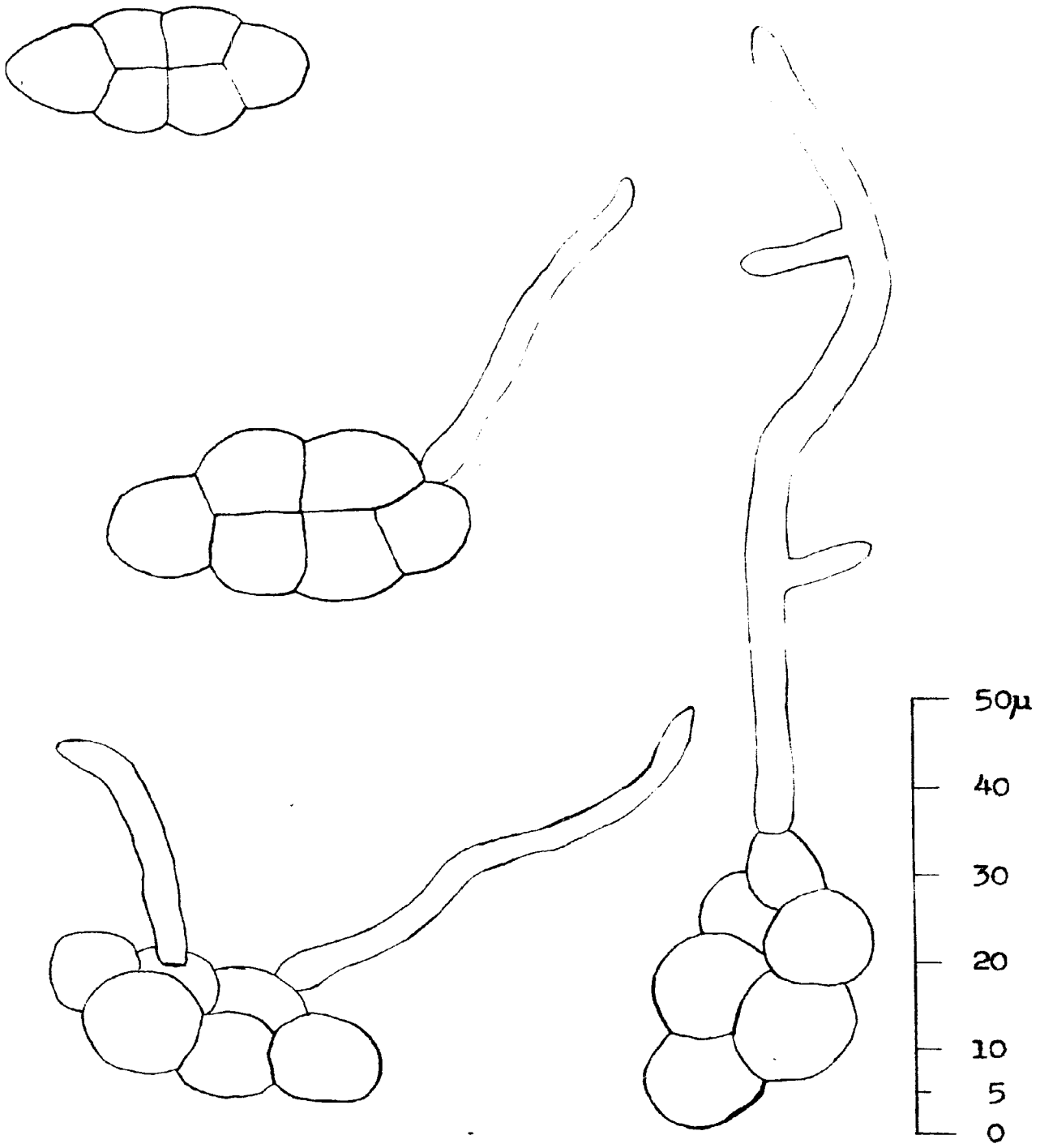


FIGURE 8

ASCOSPORES OF CLATHROSPORA PERMUNDA

Neither perithecial primordia nor conidia were found in these cultures at any time within a period of 10 months.

Clathrospora rainierensis Wehm. inedit.

(Collection No. 37; Figure 9)

Ascospores germinated in hanging-drop preparations of Leonian's medium 14 weeks after the collection was made. Single-spore and single-ascus isolates of germinated material were obtained on Leonian's agar. These cultures were allowed to remain at room temperature for 21 days and then were placed at the low temperature. The rate of growth of these cultures at either temperature was extremely slow. Within 21 days at room temperature colonies of heavy, matted, pinkish-white mycelium about 2 cm. in diameter and with aerial portions of white, cottony mycelium were produced. Within 60 days these colonies had extended to cover an area about 6 cm. in diameter. This additional growth at low temperature also was heavy, matted, and pinkish-white in color. Conidia were not observed in any of the cultures within a period of 14 months. Two to four mature perithecia were produced in each of three cultures on Leonian's agar within a period of nine months. Perithecial primordia were not produced in abundance.

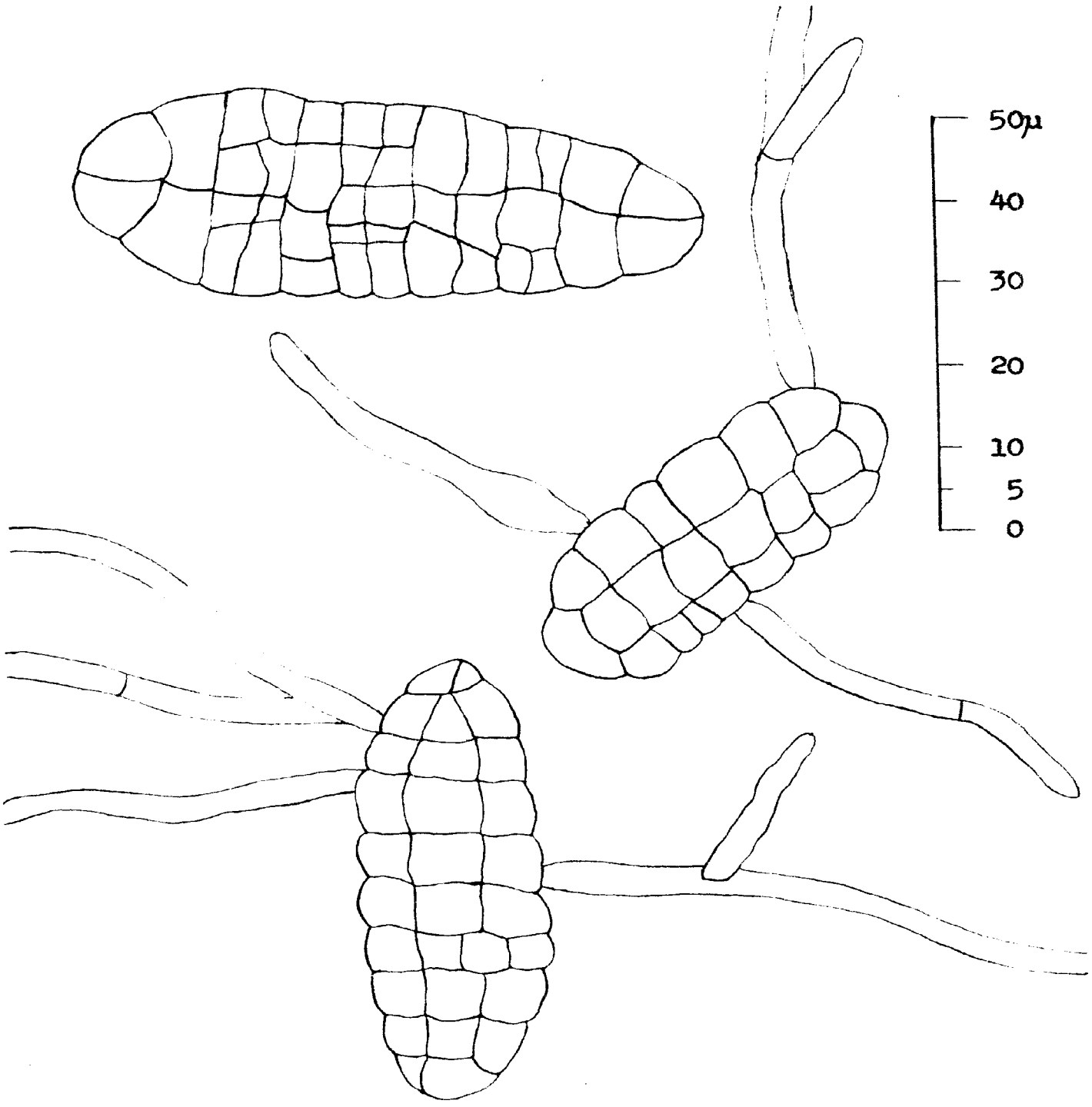


FIGURE 9

ASCOSPORES OF CLATHROSPORA RAINIERENSIS

Leptosphaeria heterospora (de Not.) Niessl

(Collection No. 43; Figure 10)

Germination of ascospores was obtained in water in hanging-drop preparations made five weeks after collection of the material. Swelling of cells of the spores accompanied production of branching, septate germ hyphae from the terminal cells of the spores. Often two or three hyphae were produced by a single cell. In no instance were central cells of the spores seen to germinate. Five single-spore isolates became established on Leonian's agar. Subcultures on Leonian's agar were left at room temperature for 7--14 days before being transferred to the cold room. On plain agar, *Aster* agar, mineral-peptone agar, and mineral agar as well as on stems of *Poa*, *Paeonia*, *Aster*, *Dactylis*, and *Anemone* a scant to moderate amount of surface and aerial mycelium was produced. Heavy mycelial growth was formed on Leonian's agar, oatmeal agar, and malt agar. These latter three media became black, opaque beneath the colonies. Conidia were formed abundantly in colonies on all substrata except stems of *Paeonia*, on which material production of conidia was quite limited. On Leonian's agar within 10 days at room temperature cultures produced heavy, gray-green to greenish-black colonies about 5.5 cm. in diameter with a copious, gray-green to dark olive, cottony aerial mycelium. Concentric zonation

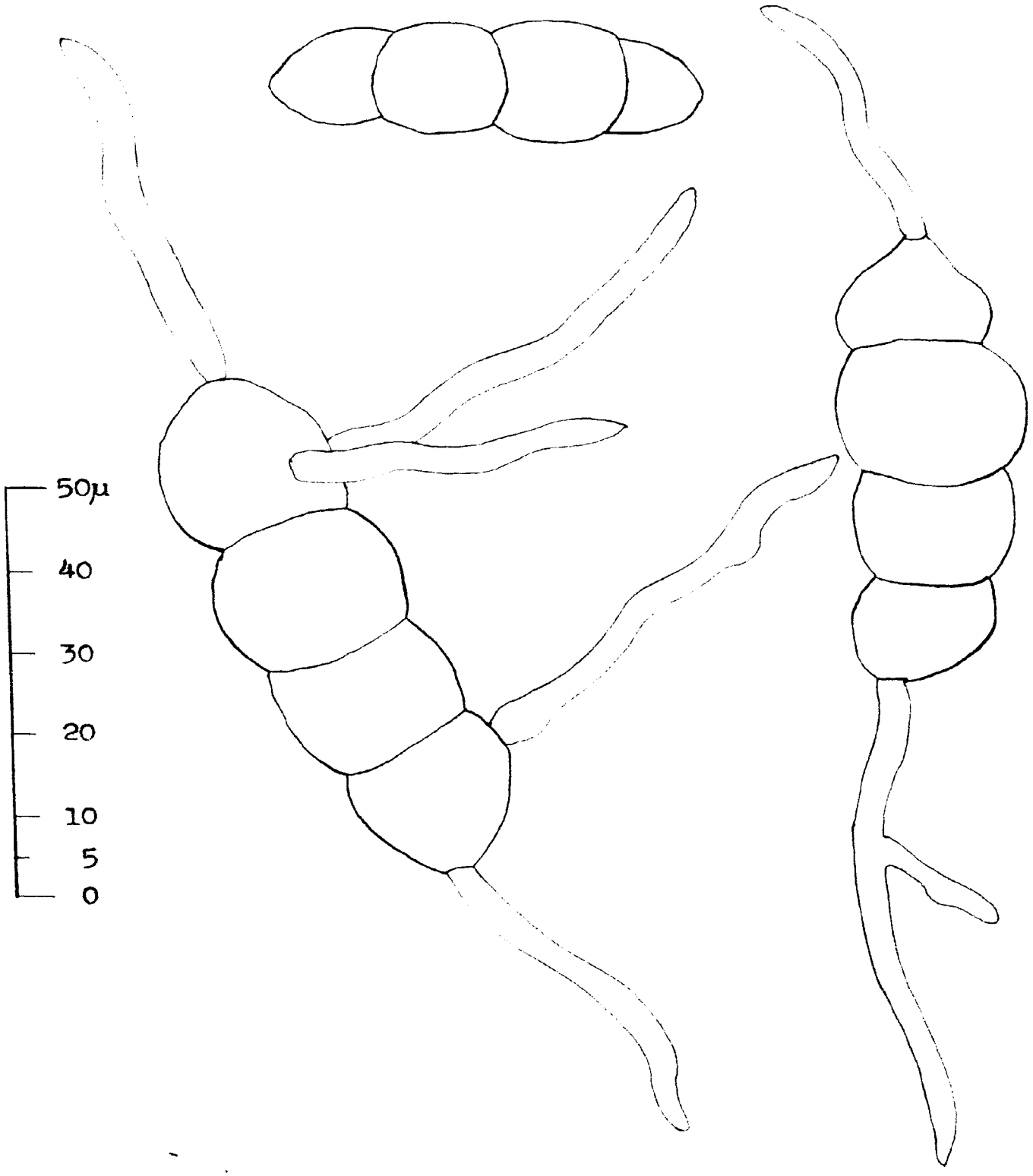


FIGURE 10

ASCOSPORES OF LEPTOSPHAERIA HETEROSPORA

was not apparent in these cultures when viewed from above, but such zonation was distinct in the subsurface mycelium. Conidia were produced abundantly. On the same medium at the low temperature colonies of a moderate amount of white mycelium about 8 mm. in diameter were produced within 10 days. A moderate amount of aerial mycelium was present in these latter cultures; zonation was not evident. On oatmeal agar within 10 days at room temperature cultures produced dense, olive-black colonies about 7 cm. in diameter with a densely matted, gray-green to black aerial mycelium with small (3--7 mm. in diameter), superficial patches of white mycelium. Conidia were produced abundantly under these conditions and zonation was marked. On oatmeal agar at the low temperature colonies of a moderate amount of white mycelium about 8 mm. in diameter were produced within 10 days. A moderate amount of aerial mycelium was present in these cultures, but zonation was not evident. On mineral-peptone agar and on mineral agar colonies of scant, white to whitish-tan mycelium about 1.5 cm. in diameter and with or lacking a small amount of aerial mycelium were produced at room temperature within 10 days. A few conidia were found in the centers of cultures produced under these conditions; zonation was faint. On the same media at the low temperature for 10 days colonies of scant, white mycelium about 11 mm. in diameter were produced. A minute amount of aerial mycelium was

present in these cultures, but zonation was not evident. Perithecial primordia were not found in any of the cultures of this species within a period of 12 months.

II. DISCUSSION

The characters of the different isolates cultured, such as color, diameter of colony in relation to age of culture, substratum most favorable for maximum growth, production of aerial mycelium, production of conidia, and production of perithecia, were found to be sufficiently distinct to permit the recognition of the individual strains. In some species, such as Clathrospora rainierensis, which produces a pinkish-white mycelium, and C. permunda, which produces surface colonies only a few millimeters in diameter within the space of several months, the distinctive cultural characters were obvious. The distinguishing characters were less obvious in C. Elynae, C. diplospora (Coll. No. 33, growth Type II), and Leptosphaeria heterospora, all of which produce abundant dark mycelium and conidia. In the course of examining numerous subcultures of these isolates, however, it was observed that their types of colonial growth on certain artificial media are sufficiently diverse to allow their separation on that basis. Specifically, C. Elynae produces on Leonian's agar, within 10 days at room temperature, heavy, greenish-black colonies with an aerial mycelium that is

scant, whitish, and cottony. Concentric zonation in the surface mycelium is marked. C. diplospora produces under the same conditions heavy, greenish-gray colonies with a dense, white, cottony aerial mycelium. Concentric zonation in the surface mycelium is only moderately marked. L. heterospora produces under the same conditions heavy, gray-green to greenish-black colonies with a copious gray-green to dark olive, cottony aerial mycelium. Concentric zonation is apparent in these cultures only in the subsurface mycelium.

Pleospora infectoria, which produces conidia of the same form-species as the previous three species (Alternaria tenuis auct.), is readily distinguished from them in culture because of its characteristic non-aerial, non-conidial type of growth on Leonian's agar. On this medium the three contrasted species produce abundant aerial mycelium and large numbers of conidia.

Those isolates which produce mature ascospores in culture (P. ambigua, P. njegusensis, P. trichostoma, C. diplospora, and C. rainierensis) can be distinguished, of course, by the ascospore characters. In addition, the number of mature perithecia produced by individual isolates on Leonian's agar often is characteristic of the isolates studied. C. rainierensis, in Petri dish cultures within nine months, produces very few mature perithecia (never more than four observed in any one culture) and very few perithecial

primordia. C. diplospora (Coll. No. 32), however, produces very numerous perithecial primordia under the same conditions, although only a few of these become mature within the same length of time. P. ambigua produces relatively few perithecial primordia, most of which, however, reach maturity. The remaining isolates produce relatively numerous mature perithecia.

Comparison of different isolates of single species permits pertinent though limited observations. In this connection it should be noted that "mass"-isolates of spores of some fungi studied often became established successfully in culture when single-spore isolates of the same fungi failed to become established. For this reason comparisons of mass-isolates from one collection with single-spore isolates from a similar collection must be interpreted with this fact in mind.

Pleospora ambigua is represented by cultures derived from ascospores of three different collections (Collections Nos. 2, 3, and 5). The isolate from Coll. No. 2 (single-spore isolate) did not produce perithecia in culture; likewise, the isolate from Coll. No. 5 (isolate derived from two or more ascospores from a single perithecium) failed to produce perithecia in culture. However, cultures of another isolate from two or more spores from the same perithecium (Coll. No. 3) did produce perithecia in culture. Such

evidence raises the question of the possibility of heterothallism being a factor in the production of perithecia in isolates of this species. It would seem possible that the isolate from spores of Coll. No. 3 developed from ascospores of genetically different mating potentials and, hence, produced perithecia, whereas the isolate from spores of Coll. No. 5 developed from ascospores of genetically similar mating potential and, hence, did not produce perithecia. The solution of this problem must be based on further experiments involving a large number of single-spore isolates.

Pleospora njegusensis is represented by cultures derived from ascospores from two different collections (Collections Nos. 23 and 24). Both single-spore isolates and isolates from several germinated spores of Coll. No. 23 produced mature perithecia in culture. Isolates derived from more than one ascospore from a single perithecium of Coll. No. 24 did not produce perithecia. Heterothallism is ruled out as a factor in production of fruiting bodies in the strain of P. njegusensis derived from Coll. No. 23. On this basis it would be logical to expect that isolates from Coll. No. 24 also would produce perithecia. Since this did not occur, the question is raised of the possibility that there are at least two different genetic strains of the species in the two morphologically similar collections. The fungus in Coll. No. 23 may represent a homothallic strain or, possibly,

may represent two strains whose characters of production and non-production of perithecia segregate during the formation of ascospores. On the other hand, the fungus in Coll. No. 24 may reflect a heterothallic condition or, possibly, may represent a condition characterized by the presence of two strains which are perithecial and non-perithecial respectively when grown separately. In any case, this question can be solved only by future intensive experimental work.

Pleospora trichostoma is represented by cultures derived only from one specimen (Collection No. 29). Isolates of single spores and of single asci were made. Although two distinct growth types were produced in different single-spore strains, no differences in the type or quantity of perithecial production was found in any of the cultures. Such evidence, in the light of the present limited knowledge of the genetics of these organisms, rules out heterothallism as a factor in perithecial initiation in this species.

Clathrospora diplospora is represented by cultures derived from two different specimens (Collections Nos. 32 and 33). The isolate of this fungus from Coll. No. 32 is based on mycelia from two or more ascospores from a single perithecium; isolates from perithecia in Coll. No. 33 are based on single ascospores. Perithecia were produced both by isolates from more than one spore and by isolates from single spores. In the second instance heterothallism appears

to be ruled out as a necessary factor in perithecial production. Isolates from different single spores of Coll. No. 33 reveal the presence of at least two types of growth strains in this species. Specifically, one strain (Type I) produced perithecia only; conidia were never found. The other strain (Type II) produced conidia only; perithecia were never found. The presence of a similar situation has been established experimentally in Glomerella cingulata, in which predominantly perithecial (but not necessarily non-conidial) strains and conidial strains (always non-perithecial) have been isolated, each from single ascospores (Lucas et al., 1944; Chilton et al., 1945). In the Type II strain of C. diplospora it would be practically impossible to establish the connection between this conidial strain and its perfect stage on the basis of perithecial production in the types of cultures used here. If a collection of this imperfect stage were obtained in nature, it could not be identified with its specific perithecial phase unless other physiologic or morphologic characters can be discovered for it.

CHAPTER V

STUDIES OF THE CONIDIAL STAGES

Single ascospores of each of four of the species studied produced growths with conidial stages in pure culture. All of these species (*Pleospora infectoria*, Coll. No. 21; *Clathrospora diplospora*, Coll. No. 33; *Clathrospora Elynae*, Coll. No. 34; and *Leptosphaeria heterospora*, Coll. No. 43) yielded stages referable to the form-genus *Alternaria*. Because the colonial and conidial morphology of isolates of *C. diplospora*, *C. Elynae*, and *L. heterospora* were so similar, an experiment was devised to determine whether growth under different conditions would cause significant variation in conidial size and thus furnish a means for distinguishing the three species by their conidial stages.

Data were obtained from two different types of experiments, as follows: (I) cultures of the three isolates were established on plant stems and observations were recorded on characters usually included in form-species descriptions; (II) cultures were established on four different media and data sufficient for statistical calculations were obtained on conidium morphology.

I. STUDY OF CULTURES ON PLANT STEMS

A. Materials and Methods

Origin of conidial material. The origins of the individual conidial strains have been noted in connection with the discussions of their perithecial stages (Culture Studies: General Data). In brief, each Alternaria strain was derived from a single ascospore of its perfect stage. For the purposes of this study one conidial strain was selected at random from those available for each perfect stage.

Culture substratum used. In this initial attempt at establishing the form-species identities of the isolates, cultures were initiated on dead, sterile stems of Chrysanthemum sp. which were moistened with distilled water. Such a substratum was used for the purpose of approximating conditions which might be found in nature.

Culture methods and procedure. Conidia for starting subcultures were obtained from the original isolates on Leonian's agar. Inoculations on new substrata were made from suspensions of conidia in sterile distilled water. Using a wire loop, a single drop of such a suspension was introduced into each test-tube containing stems. All cultures were maintained at room temperature of 22°--26° C. in diffuse light during daylight hours. Two identical series

were maintained.

Cultures of the three different Alternaria isolates were examined on the tenth day after inoculation and descriptions of the material observed were written. These descriptions follow in outline the procedure in general use in the literature descriptive of such fungi.

B. Experimental Results

Descriptions of conidial stages.

Clathrospora diplospora (E. & E.) Wehm. in press (conidial stage; Figure 11)

Hyphae hyaline to pale olive to olive to dark olive-brown, septate, branching, usually 3.6--4.8 μ wide.

Conidiophores medium to dark olive, septate, mostly 21.8--66.6 x 3.6--4.9 μ but often bearing conidia when no longer than 2.4 μ, usually erect, simple and straight with a single scar at apex or, rarely, branched and geniculate with a scar at the apex of each branch and at each bend, swollen slightly at apex, borne separately on aerial hyphae, appearing separately on surface of plant substratum or frequently clustered in groups of two or more.

Conidia smooth or verrucose in the same chain or even on the same spore (upper portion smooth, lower portion verrucose), surface topography not definitely correlated with

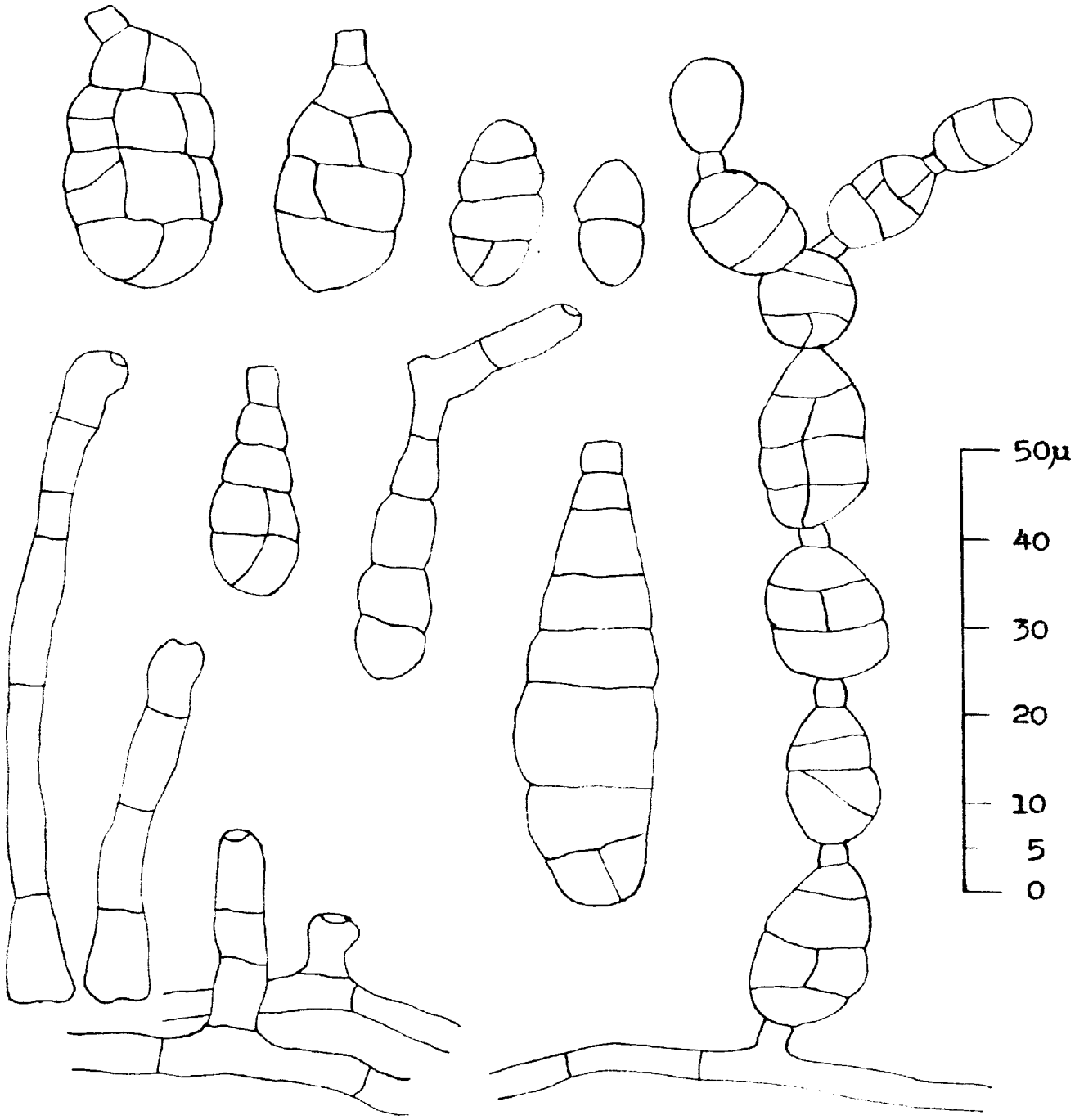


FIGURE 11

CONIDIA AND CONIDIOPHORES OF CLATHROSPORA DIPLOSPORA (COLLECTION NO. 33)

relative age (i. e., with position of individual spore in the chain); in shape[4] beakless oval, short or long oval, short or long cone, or cylindrical; 10.9--55.7 x 6.1--14.5 μ; hyaline to pale olive to medium olive to olive-brown to dark olive-brown; septation 1--8 transverse and 0--6 longitudinal; beak present or, rarely, absent, transition from body of spore to beak gradual or abrupt, appearing as a scarcely differentiated apical cell (but usually lighter in color than cells of the spore body) or as a distinctly elongated, simple or branched, straight or geniculate rostrum, 2.4--6.0 (--21.8) x 2.4--4.8 μ, concolorous with or slightly lighter than the body cells; in chains of 5--10 (--20); chains simple or branched.

Cultural (on dead, sterile stems of Chrysanthemum): aerial mycelium loose cottony to compact cottony, abundant, light gray to gray-green to olive.

Clathrospora Elynae Rab. (conidial stage; Figure 12)

Hyphae hyaline to medium olive, septate, seldom deviating from 3.6 μ in width.

Conidiophores olive-brown, septate, mostly 36.2--55.7 x 4.2 μ but often bearing conidia when as short as 2.4 μ, erect to inclined, not seen to branch or to become

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[4] Descriptive terms applied to shapes of conidia are based on those defined by Neergaard (1945).

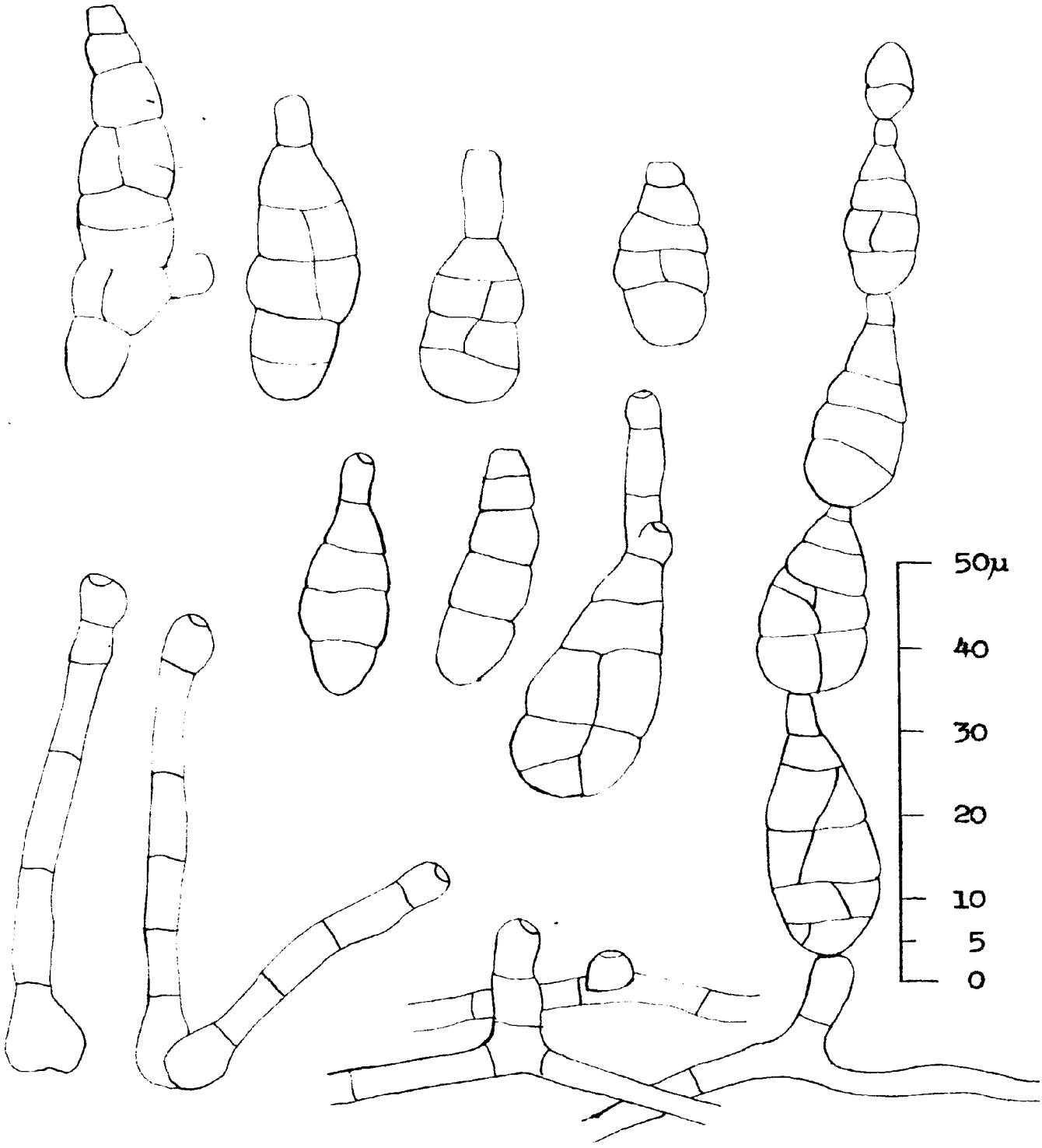


FIGURE 12

CONIDIA AND CONIDIOPHORES OF CLATHROSPORA ELYNAE

geniculate, with terminal scar, slightly swollen at apex, decidedly swollen at base, usually solitary on hyphae but occasionally appearing as paired or bunched on surface of substratum.

Conidia usually verrucose, but smooth when young; in shape beakless oval, short or long oval, short or long cone, or, rarely, cylindrical; 9.7--36.3 x 6.1--12.1 μ; hyaline to medium olive to dark olive-brown; septation 1--6 transverse and 0--4 longitudinal; beak (when present) simple or, rarely, geniculate with a scar at apex and at each bend, short (appearing scarcely differentiated from body cells except by lighter color) or elongate, 1.2--18.2 x 2.4--3.6 μ; in chains of 5--10 or frequently more; chains usually simple but occasionally branching.

Cultural (on dead, sterile stems of Chrysanthemum): aerial mycelium light gray to dark olive, loose cottony.

Leptosphaeria heterospora (de Not.) Niessl (conidial stage; Figure 13)

Hyphae hyaline to light green to dark olive, septate, branching, 2.4--3.6 μ wide.

Conidiophores medium olive, septate, up to 54.5 x 4.8 μ in size, erect or inclined, not seen to branch but some geniculate, scars at apex and at bends, not noticeably swollen at apex, borne singly on hyphae but appearing

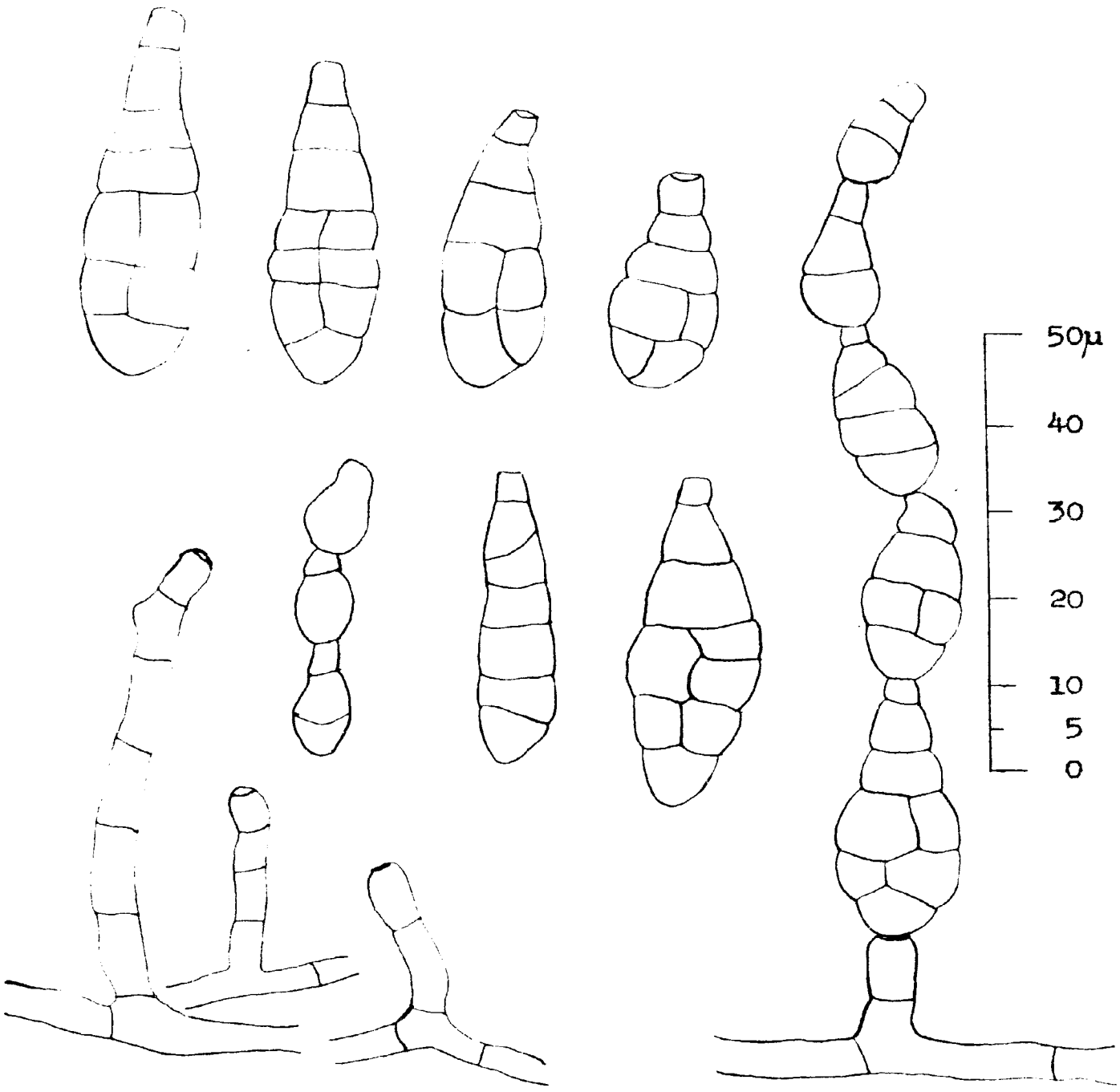


FIGURE 13

CONIDIA AND CONIDIOPHORES OF LEPTOSPHAERIA HETEROSPORA

clustered on substratum.

Conidia smooth or verrucose; in shape short or long oval, short or long cone, or cylindrical; 10.9--31.5 x 6.1--10.9 μ; hyaline to medium olive to dark olive; septation 1--6 transverse and 0--3 longitudinal; beak present as scarcely differentiated apical cell or as elongate, septate rostrum, simple, sometimes geniculate, with scars at apex and at bends, tapering from body of spore to rostrum or with abrupt transition, 1.2--20.6 x 2.4--4.2 μ; in chains of 3--10 or more; chains simple or branched.

Cultural (on dead, sterile stems of Chrysanthemum): aerial mycelium loose cottony, light to dark gray.

C. Discussion

Identification of these individual isolates of Alternaria on the basis of the descriptions given and with the use of the modern taxonomic treatment of the form-genus by Neergaard (1945) necessitates their placement under the binomial Alternaria tenuis auct. sensu str. Clathrospora diplospora, C. Elynae, and Leptosphaeria heterospora cannot be distinguished in their conidial stages by the use of ordinary morphologic criteria. Although it is evident that the isolates of these species are genetically distinct, they must be regarded as members of the form-species Alternaria tenuis. It is apparent from this experiment that form-species

as described in the literature may represent the imperfect stages of a number of species, either in one genus or distributed in closely related genera. It follows, then, that the perfect-stage binomial cannot logically be applied to a single form-species on the basis of a single connection of a perfect and a conidial stage.

II. STUDY OF CULTURES ON ARTIFICIAL MEDIA WITH A STATISTICAL ANALYSIS OF CONIDIAL LENGTH

A. Materials and Methods

Origin of conidial material. Each Alternaria strain was derived from a single ascospore of its perfect stage, as indicated previously in Culture Studies: General Data and in Studies of the Conidial Stages (I. Study of Cultures on Plant Stems).

Culture substrata used. Cultures used as the sources of data for statistical studies were carried on four different media, each medium being used at an acid pH and at a basic pH. These media were Leonian's agar (pH 4.9 and pH 7.7), oatmeal agar (pH 5.7 and pH 7.7), mineral-peptone agar (pH 5.1 and pH 7.7), and mineral agar (pH 5.2 and pH 7.7). These media are defined in a preceding chapter under List of Media and Plant Substrata Used. It should be noted that three of the media (Leonian's agar, mineral-peptone

agar, and mineral agar) comprise a series which is decreasingly complex in composition. Such a series of substrata was used in an attempt to discover and to define, as nearly as possible, culture conditions under which the different isolates could be distinguished or under which variation within single isolates would occur.

Culture methods used. Conidia for starting subcultures were obtained from the original isolates on Leonian's agar. Inoculations on new media in each series were made from suspensions of conidia in sterile distilled water. Using a wire loop, a single drop of such a suspension was placed in the center of each Petri dish of artificial medium. All cultures were maintained under the same external conditions -- room temperature of 22°--26° C. and diffuse light during daylight hours. Two identical series were maintained.

Statistical procedure and methods. Previous observations indicated that under the best conditions for growth some of the colonies would extend to the side-walls of the Petri dishes within a growth-period of ten days. For this reason nine days was fixed as the maximum period within which data could be taken from all cultures before mechanical interference with radial growth occurred. In order to have comparative data referable to colony age, the nine-day period was divided arbitrarily into three successive intervals of three days each, data being obtained from each culture at the

ends of the three-, six-, and nine-day periods.

The sampling method consisted of dragging a sterile wire-loop across the surface of the mycelium in a radius of a colony. (Succeeding samples from a colony were not taken from the same radius.) The spores obtained were mounted in Amman's mounting fluid.[5] Using a mechanical stage, the mount was moved laterally across the microscope field. Data were recorded on all conidia encountered up to a total of 50 spores per mount (except in the few cases when this number of spores could not be found in a single mount). The data consisted of measurements in ocular micrometer divisions (to the nearest division) of length, excluding the beak, and of width of the spores. These measurements were transposed into micron values for reference purposes and the length ranges in microns are listed in subsequent Tables. However, all calculations are based on the original data expressed in ocular micrometer divisions.[6]

B. Experimental Results

Preliminary calculations on width data revealed that

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[5] Although this mounting medium is known to cause shrinkage of some fungus elements, such distortion was not seen to occur in the mounts used within the time of observation.

[6] For the purposes of record, in this series of measurements one ocular micrometer division equals 1.17 μ.

the degree of variation of this character within individual species and between different species was so small that neither intraspecific nor interspecific differences could be established. Calculations on length-width ratios were not sufficiently different from similar calculations on length data alone to warrant their use. For these reasons statistical calculations were carried out only on spore-length data, particularly since this character offered a decidedly greater range of values.

Making use of standard statistical formulae, the following values were determined from the length data of each sample: range of length, mean length, and standard deviation of length (Tables II and III). The mean length values and the standard deviation of length values then were used in comparing data obtained from different samples by employing them in a standard ratio designed as a test for significance of difference between means (Tables IV, V, VI, and VII).

C. Discussion of Tables

Values derived from the data are arranged in the different Tables in such a manner that those values representing individual cultures under specific conditions may be read directly (Tables II and III) and that those values representing comparisons of cultures of the same or of different isolates under comparable conditions may be found readily

(Tables IV, V, VI, and VII). In Tables IV--VII values of 2.0 or more (underlined in Tables) indicate that there exists a significant difference between mean length values under the conditions noted.

In order to simplify and to standardize the method of reference to the individual samples and to the comparison of two different samples, the following symbols have been used in the Tables:

33 -- culture derived from Clathrospora diplospora
34 -- culture derived from Clathrospora Elynae
43 -- culture derived from Leptosphaeria heterospora

3 -- age of culture three days
6 -- age of culture six days
9 -- age of culture nine days

L -- Leonian's agar as substratum
O -- oatmeal agar as substratum
P -- mineral-peptone agar as substratum
M -- mineral agar as substratum

a -- pH of medium in the acid range
b -- pH of medium in the basic range

R -- range of length in ocular micrometer divisions
Rμ -- range of length in microns
m -- mean length in ocular micrometer divisions
σ -- standard deviation of length

----- -- values not determined because of the absence or extreme scarcity of conidia in the culture

Examples: In Table II "33-L-3-a" under "R" locates the values indicating the range of length (in micrometer divisions) of conidia in a sample taken from a culture of C. diplospora three days old on Leonian's agar of acid pH. In Table IV "L-O-a" under "3" of C. diplospora locates the value

indicating the degree of significance of the difference between the mean length of conidia from a culture of C. diplospora three days old on Leonian's agar of acid pH and the mean length of conidia from a culture of C. diplospora three days old on oatmeal agar of acid pH.

Tables II and III summarize the actual range of measurement, the mean length, and the standard deviation of the mean length of samples of the three isolates on the four agars used, at three different ages, and at the acid (Table II) and the basic (Table III) reactions used. In Tables II and III the spore-length ranges of the different samples show a great degree of overlapping between and within individual isolates. No general statement can be made on species variation or differentiation on the basis of these length extremes only. Similarly, the mean length and standard deviation of length values do not fall into a pattern definite enough for the formulation of an unqualified conclusion. The most obvious generalities to be noted in these Tables are (1) the mean length and the standard deviation of length are, in most cases, greatest at the end of the first three-day growth period, and (2) mean lengths and standard deviations of mean lengths show a general pattern of decrease in value in samples from the more complex to the less complex media.

Tables IV, V, and VI list values from which

TABLE II

RANGE OF LENGTH, MEAN LENGTH, AND STANDARD DEVIATION OF LENGTH OF CONIDIA SAMPLES FROM ALTERNARIA CULTURES OF DIFFERENT SPECIES ORIGIN, OF DIFFERENT AGES, AND PRODUCED ON DIFFERENT MEDIA WITH pH'S IN THE ACID RANGE

Sample	R	Rμ	m	σ
33-L-3-a	10--41	11.70--47.97	25.04	6.64
-6-	9--27	10.53--31.59	17.78	4.86
-9-	8--35	9.36--40.95	21.74	5.39
33-O-3-a	5--43	5.85--50.31	22.30	8.55
-6-	10--40	11.70--46.80	22.58	7.36
-9-	11--30	12.87--35.10	19.70	4.85
33-P-3-a	9--38	10.53--44.46	18.56	7.02
-6-	10--30	11.70--35.10	16.84	4.24
-9-	10--27	11.70--31.59	16.34	3.21
33-M-3-a	7--41	8.19--47.97	17.08	6.73
-6-	6--28	7.02--32.76	15.32	5.62
-9-	10--30	11.70--35.10	16.12	3.85
34-L-3-a	8--32	9.36--37.44	19.64	6.37
-6-	10--35	11.70--40.95	18.76	5.87
-9-	8--25	9.36--29.25	15.12	3.52
34-O-3-a	13--30	15.21--35.10	20.56	4.88
-6-	8--23	9.36--26.91	15.20	3.75
-9-	8--25	9.36--29.25	15.04	3.85
34-P-3-a	------	----------	-----	----
-6-	------	----------	-----	----
-9-	10--20	11.70--23.40	14.16	2.36
34-M-3-a	------	----------	-----	----
-6-	------	----------	-----	----
-9-	8--18	9.36--21.06	11.72	2.47
43-L-3-a	8--37	9.36--43.29	21.96	6.11
-6-	9--37	10.53--43.29	20.68	6.31
-9-	11--38	12.87--44.46	21.14	5.91
43-O-3-a	9--35	10.53--40.95	19.48	5.80
-6-	9--31	10.53--36.27	18.30	5.32
-9-	11--26	12.87--30.42	18.50	4.00
43-P-3-a	10--25	11.70--29.25	17.26	3.91
-6-	9--26	10.53--30.42	16.00	4.10
-9-	10--26	11.70--30.42	15.54	3.85
43-M-3-a	7--28	8.19--32.76	15.90	5.28
-6-	8--22	9.36--25.74	15.14	3.02
-9-	9--25	10.53--29.25	14.38	4.40

TABLE III

RANGE OF LENGTH, MEAN LENGTH, AND STANDARD DEVIATION OF LENGTH OF CONIDIA SAMPLES FROM ALTERNARIA CULTURES OF DIFFERENT SPECIES ORIGIN, OF DIFFERENT AGES, AND PRODUCED ON DIFFERENT MEDIA WITH pH'S IN THE BASIC RANGE

Sample	R	Rμ	m	σ
33-L-3-b	11--36	12.87--42.12	22.60	5.07
-6-	10--40	11.70--46.80	19.86	6.32
-9-	11--50	12.87--58.50	22.68	7.66
33-O-3-b	11--36	12.87--42.12	20.36	5.56
-6-	11--29	12.87--33.93	20.26	4.35
-9-	11--32	12.87--37.44	18.40	4.61
33-P-3-b	12--30	14.04--35.10	17.80	5.06
-6-	10--33	11.70--38.61	19.32	5.45
-9-	11--37	12.87--43.29	19.84	7.20
33-M-3-b	9--32	10.53--37.44	18.16	4.29
-6-	10--28	11.70--32.76	16.78	4.10
-9-	9--29	10.53--33.93	17.40	4.47
34-L-3-b	6--33	7.02--38.61	18.14	5.20
-6-	7--23	8.19--26.91	14.96	3.67
-9-	10--29	11.70--33.93	16.08	3.68
34-O-3-b	8--27	9.36--31.59	15.74	4.48
-6-	10--23	11.70--26.91	15.54	3.08
-9-	10--26	11.70--30.42	15.24	3.52
34-P-3-b	7--28	8.19--32.76	14.96	4.49
-6-	9--28	10.53--32.76	13.88	4.16
-9-	9--28	10.53--32.76	13.46	3.94
34-M-3-b	7--26	8.19--30.42	15.28	4.35
-6-	9--26	10.53--30.42	15.74	4.18
-9-	8--20	9.36--23.40	12.32	2.94
43-L-3-b	7--36	8.19--42.12	21.72	6.18
-6-	10--29	11.70--33.93	19.68	5.34
-9-	9--35	10.53--40.95	18.94	5.48
43-O-3-b	8--30	9.36--35.10	18.54	5.10
-6-	10--26	11.70--30.42	17.50	4.33
-9-	10--28	11.70--32.76	16.98	4.40
43-P-3-b	11--28	12.87--32.76	17.00	3.88
-6-	8--30	9.36--35.10	16.52	4.42
-9-	9--27	10.53--31.59	16.58	4.46
43-M-3-b	10--30	11.70--35.10	15.72	3.91
-6-	8--22	9.36--25.74	13.44	3.94
-9-	9--28	10.53--32.76	15.44	3.89

observations may be drawn on the effect upon individual isolates of differences in media, of differences in pH, and of differences in age of culture.

Individual isolates of a species show striking differences when mean lengths of conidia from different media are compared (Table IV). In this respect L. heterospora is the most notable of the three species studied. Here significant differences in conidial lengths are present between samples from all pairs of media in the acid range excepting the mineral-peptone agar--mineral agar series, i. e., in 83 per cent of compared pairs. Similar differences are noted in the basic pH range, in which 67 per cent of pairs of media compared yield samples significantly different. Since all media in the acid range do not have the same pH, it might be argued that the definite differences in mean lengths are due to the reaction differences of the media. This factor, however, appears to have little effect on mean lengths, as can be judged from specific pH comparisons in Table V. The comparisons in Table V cover a much greater range of pH value than is found between any two different media of the acid range. Consequently, it can be concluded that the large number of significant differences in mean length noted in Table IV, particularly for C. diplospora and L. heterospora, reflects the nutritive differences of the media rather than the pH differences. In this connection it should be noted

TABLE IV

DEGREE OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN MEAN LENGTHS OF CONIDIA SAMPLES FROM CULTURES OF THE SAME AGES AND SIMILAR pH'S, BUT PRODUCED ON DIFFERENT MEDIA

	Clathrospora diplospora			Clathrospora Elynae			Leptosphaeria heterospora		
	3	6	9	3	6	9	3	6	9
L-O-a	1.8	3.8	2.0	0.7	3.2	0.1	2.1	2.0	2.6
L-P-a	4.7	1.0	6.1	---	---	1.4	4.6	4.4	5.6
L-M-a	5.9	2.3	5.0	---	---	4.7	5.3	5.5	5.5
O-P-a	2.4	4.8	4.1	---	---	1.2	2.2	2.4	3.7
O-M-a	3.4	5.5	4.1	---	---	4.5	3.2	3.5	4.0
P-M-a	1.1	1.5	0.3	---	---	3.5	1.5	1.2	1.4
L-O-b	2.1	0.4	3.4	2.5	0.9	1.2	2.8	2.2	2.0
L-P-b	4.8	0.5	1.9	3.3	1.4	3.4	4.6	3.2	2.4
L-M-b	4.7	2.9	4.2	3.0	1.0	5.6	5.8	6.6	3.7
O-P-b	2.4	1.0	1.2	0.9	2.3	2.4	1.7	1.1	0.5
O-M-b	2.3	4.1	1.1	0.5	0.3	4.5	3.1	4.0	1.9
P-M-b	0.4	2.6	2.0	0.4	2.2	1.6	1.6	3.7	1.4

that in at least two-thirds of the media-comparisons for C. diplospora and for L. heterospora and in as many as one-half of similar comparisons for C. Elynae, significant differences in mean conidium length are found. The chemical constitution of the substratum clearly has a decided effect upon the length of conidia of these isolates under specified conditions.

For any one isolate there are relatively few instances in which contrasting pH's influenced the production of significantly different mean-length values for samples (Table V). The conclusion that the pH of the medium within the ranges used does not have a decided differential effect upon mean conidial length is borne out by the listed results of pH comparisons.

For the most part, age differences in colonies do not result in significant differences in conidial mean lengths, insofar as the three-, six-, nine-day sampling series can reveal such differences (Table VI). An exception to this generality is found in comparisons made with samples from Leonian's agar. Colonies of C. diplospora and of C. Elynae grown on this medium yielded samples in at least two-thirds of comparable pairs of ages which exhibited significant differences in mean spore length. Such differences are found in a few comparisons from other media, but, as stated, significant differences in mean length usually are not correlated

TABLE V

DEGREE OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN MEAN LENGTHS OF CONIDIA SAMPLES FROM CULTURES OF THE SAME AGES PRODUCED ON MEDIA DIFFERING ONLY IN pH

	Clathrospora diplospora			Clathrospora Elynae			Leptosphaeria heterospora		
	3	6	9	3	6	9	3	6	9
L-a-b	2.1	1.8	0.7	1.3	3.9	1.3	0.2	0.9	1.9
O-a-b	1.3	1.9	1.4	4.1	0.4	0.3	0.9	0.8	1.8
P-a-b	0.6	2.5	3.1	---	---	1.0	0.3	0.6	1.3
M-a-b	1.0	1.5	1.5	---	---	0.9	0.2	2.4	1.3

TABLE VI

DEGREE OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN MEAN LENGTHS OF CONIDIA SAMPLES FROM CULTURES PRODUCED ON THE SAME MEDIA BUT OF DIFFERENT AGES

	Leonian		Oatmeal		Mineral peptone		Mineral	
	a	b	a	b	a	b	a	b
33-3-6	6.3	2.4	0.2	0.1	1.5	1.5	1.4	1.6
-3-9	2.7	0.1	1.9	1.9	2.0	1.6	0.9	0.9
-6-9	3.6	2.0	2.3	2.1	0.7	0.4	0.8	0.7
34-3-6	0.7	3.5	4.3	0.3	---	1.2	---	0.5
-3-9	4.4	2.3	4.9	0.6	---	1.8	---	4.0
-6-9	11.9	1.5	0.2	0.5	---	0.5	---	4.7
43-3-6	1.0	1.8	1.1	1.1	1.6	0.6	0.8	2.9
-3-9	0.7	2.4	1.0	1.6	2.2	0.5	1.6	0.4
-6-9	0.4	0.7	0.2	0.6	0.6	0.1	1.0	2.6

with age differences.

The most important overall conclusion to be drawn from the data presented in the different Tables is that under certain conditions measurements of conidia can be used to differentiate pure strains of species in these two genera. Evidence for or against the use of statistical data for differentiating these isolates may be indicated as follows:

1. If the isolates can be separated by these means, then, in a statistical comparison of conidia of two isolates grown in a parallel manner under a number of different conditions, significant mean-value differences should be found under at least some of these conditions.

For the isolates of species used these differences are evident from data in Table VII. In comparing C. diplospora and C. Elynae (33-34) it is seen that significant differences exist between mean lengths of conidial samples in 17 out of the 20 possible conditions of comparison. In a similar comparison of C. Elynae and L. heterospora (34-43) it is observed that significant differences exist in 16 out of 20 conditions of comparison. With C. diplospora and L. heterospora (33-43) significant differences exist in 11 only of the 24 possible conditions of comparison observed.

It follows from these observations, then, that the Alternaria isolates of C. Elynae can be differentiated statistically from the Alternaria isolates of both C.

TABLE VII

DEGREE OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN MEAN LENGTHS OF CONIDIA SAMPLES OF DIFFERENT SPECIES FROM CULTURES OF THE SAME AGES ON THE SAME MEDIA

	Leonian		Oatmeal		Mineral peptone		Mineral	
	a	b	a	b	a	b	a	b
33-34-3	4.2	4.3	1.1	4.6	---	3.0	---	3.3
-6	0.0	4.7	5.7	6.3	---	5.6	---	1.3
-9	7.3	5.5	5.2	3.8	3.3	5.5	5.9	6.7
34-43-3	2.3	3.1	0.8	2.9	---	2.4	---	0.5
-6	1.9	5.0	2.9	2.6	---	3.1	---	2.8
-9	6.0	3.1	4.4	2.2	1.9	3.7	3.3	4.5
33-43-3	3.1	0.8	1.9	1.7	1.1	0.9	1.0	3.0
-6	2.9	0.2	3.3	3.2	1.0	2.8	0.2	4.2
-9	0.6	2.8	1.3	1.6	1.1	2.7	2.1	2.3

diplospora and L. heterospora under most of the conditions cited. This is considered significant for species recognition in this group of isolates. It also is evident from these observations that the conidial stages of C. diplospora and L. heterospora can be differentiated by statistical correlations of this type but under a smaller number of conditions. Although the latter two species are separable under only about half of the growth conditions imposed, species recognition by statistical methods definitely is possible insofar as the isolates used are concerned.

2. If isolates of these species can be differentiated statistically, certain pairs of conditions should exist between which mean lengths of conidia of one species do not show significant differences, while at the same time mean lengths of conidia of two species under the same conditions do show significant differences.

Such comparisons may be made using data from Tables IV, V, and VI in conjunction with Table VII. In comparing culture pairs of isolates of two species such as C. Elynae and C. diplospora comparable to the culture pairs of C. Elynae alone which do not show a significant difference in conidial mean length, it is found that differentiation of isolates can be established in at least 82 per cent of the comparisons. In all such comparisons of cultures of C. Elynae with those of C. diplospora and L. heterospora,

species identity of the isolates can be established at least two-thirds of the time. However, in comparisons of C. diplospora and L. heterospora species differentiation of the isolates, though possible, can be established in not more than 21 per cent of the cases. These observations are summarized in Table VIII.

III. CONCLUSIONS

In summary, it is concluded that, in the absence of sufficient data for identification of species in ordinary descriptive material of these conidial stages, statistical data based on measurements of conidia from isolates in pure culture may be of value in the recognition of species. Specifically, by the use of the culture methods and of the statistical methods indicated, the Alternaria stage of the isolate of Clathrospora Elynae can be differentiated from the similar isolates of C. diplospora and Leptosphaeria heterospora under a large percentage of culture conditions. However, such differentiation is possible in a relatively small percentage of comparisons involving conidial stages of the isolates of C. diplospora and L. heterospora only.

TABLE VIII

COMPARISON OF NUMBER OF PAIRS OF CONDITIONS UNDER WHICH ONE ISOLATE DOES NOT SHOW SIGNIFICANT DIFFERENCES WITH NUMBER OF COMPARABLE CONDITIONS UNDER WHICH TWO ISOLATES DO SHOW SIGNIFICANT DIFFERENCES

No. of pairs of conditions for one isolate which do not yield significant differences		No. of comparable conditions for two isolates which do yield significant differences		% of comparisons in which differentiation between isolates can be established
Isolate	No.	Isolates	No.	%
33	27	33--34	20	74.1
34	28	34--33	23	82.1
34	28	34--43	20	71.4
43	30	43--34	20	66.7
33	38	33--43	8	21.1
43	40	43--33	7	17.5

CHAPTER VI

STUDIES OF THE PERITHECIAL STAGES

A primary purpose of the culture work was to establish conditions under which mature perithecia of the species studied would be formed. Most investigators working with this group have handled only Pleospora herbarum, a species which apparently is capable of fruiting on a wide range of artificial media. However, little or nothing is known about methods of inducing the production of perfect fruiting structures of other similar species. Experiments were set up, therefore, which made use of physical factors which would be expected to be operative on these organisms in nature, namely, an initial growth period at "summer" temperature, further growth at "winter" temperature, ample moisture in substratum, and diffuse light.

If mature perithecia and ascospores could be produced, variability in size of the ascospores from single-spore strains of these organisms could be studied. The elucidation of this problem is of fundamental importance to the understanding of species circumscription in the group. In this connection, comparisons of ascospore size between samples derived from different single perithecia from the same

culture have been made.

These two lines of investigation are treated separately here. The work on production of perithecia indicates conditions under which perithecia of some species studied can be produced. The work on the comparison of size of ascospores is presented as an introduction to a type of investigation basic to the problem of establishing species limits in these fungi.

I. PRODUCTION OF PERITHECIA

A number of different media have been used by those who have worked with pure cultures of Pleospora. Most of these media have been based on decoctions of plant parts.

Work on P. herbarum has been carried out on apple decoction (Gibelli and Griffini, 1874); on dilute grape juice and on prune decoction (Bauke, 1877; Kohl, 1883); on thin decoctions of onion, of date, and of horse dung (Miyabe, 1889); on an unidentified nutrient medium (Brefeld, 1891); on potato, on gourd pulp, and on Mayer's medium[7] (Cavara and Mollica, 1907); on salep agar (Klebahn, 1918); on malt agar and pea agar (Groves and Skolko, 1944a); and on dung agar and on straw (Hughes, 1945). Cocchi (1931) states that he

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[7] Mayer's medium: water, 500 ml.; peptone, 6 g.; meat extract, 4 g.; sodium chloride, 1 g.; dextrose, 5 g.; and gelatine, 50 g.

obtained mature perithecia of P. herbarum on pieces of carrot, on his agar No. 5[8], and on the medium of Dox[9] solidified. He obtained what he considers to be immature perithecia of this species on solidified carrot decoction, on 0.5 per cent asparagine agar with or without glucose, on liquid or solidified Dunham's medium[10], on potato-glucose agar (alkaline or acid), on liquid medium No. 5, on liquid medium of Dox, on pieces of potato, and on 1 per cent asparagine solution.

Diedicke (1902) studied cultures of P. trichostoma on a prune decoction medium solidified by gelatine; Halsted (1893) identified the substratum for growing P. Tropaeoli simply as slant agar tubes.

A. Materials and Methods

Since the definition of a nutrient medium necessary for the production of perithecia in Pleospora is not supplied

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[8] Cocchi's Medium No. 5: water, 500 ml.; ammonium phosphate, 0.2 g.; potassium chloride, 0.05 g.; magnesium sulfate, 0.07 g.; calcium oxide, 0.005 g.; and with or without agar, 7.5 g.

[9] Medium of Dox: water, 1000 ml.; magnesium sulfate, 0.5 g.; potassium acid phosphate, 1.0 g.; potassium chloride, 0.5 g.; iron sulfate, 0.01 g.; and with or without agar, 15.0 g.

[10] Medium of Dunham: water, 100 ml.; peptone, 1.0 g.; sodium chloride, 0.5 g.; and with or without agar, 1.5 g.

by the array of substrata listed, Leonian's agar was used for the most part in the presently described attempts at inducing the production of perithecia. This medium, although not completely synthetic in composition, can be standardized sufficiently well to permit its duplication in any laboratory.

In a few instances, noted below, Ellis' synthetic medium as well as 2 per cent malt agar and filter paper cones moistened with Leonian's medium also were employed.

These attempts at inducing perithecial formation were made with cultures of all isolates listed previously which grew in culture. Transfers of fungus material (either conidia or aerial mycelium) from the original isolates were made to the solidified media in Petri dishes and in test tubes.

Individual cultures were allowed to remain at room temperature (22°--26° C.) until colonies had become established. Transfer of cultures then was made to a refrigerator room which remained at 5° C. and which received diffuse light during daylight hours. (Data relative to the influence of light or of the absence of light on perithecial production were not obtained.) Cultures were examined frequently for indications of the formation of perithecial initials and, later, for the presence of mature asci in perithecia which appeared.

B. Experimental Results

Of the 14 isolates treated, six produced mature perithecia within a year under experimental conditions. These species and the conditions under which each produced perithecia are listed.

Pleospora ambigua (Berl. & Bres.) Wehm. (Collection No. 3)

Two subcultures of the gross isolate of this species produced mature perithecia on Leonian's agar in Petri dishes. The initial period at room temperature was 20 days. Only a few perithecia containing mature ascospores were produced within 36 weeks. The greatest number of ascospores found in any one perithecium was 60. Asci themselves were not found, so the explanation of this number of spores in a perithecium of a species which normally produces 8-spored asci is not immediately apparent. Two explanations are possible: (1) some spores in one or more asci failed to develop, or (2) the nuclear complement of a single ascus became divided among only four ascospores instead of among the usual eight. The occurrence of 4-spored asci was common in cultures of Clathrospora diplospora, Coll. No. 33.

The following is a description of the perithecial stage produced in culture:

Perithecia about 250 μ in diameter; with fragile walls

two or three cells thick; and with radiating, filamentous, pale yellow-brown, septate hyphae about 300 x 2.5 μ. These hyphae are attached to the wall cells of the perithecia and are distinct from the vegetative hyphae of indeterminate length and of lighter color. Ascospores 20.4--22.8 x 9.6 μ; septation 5--7 transverse with one series of longitudinal septa.

Cultures of Collection No. 5 of this species were treated in the same manner but did not produce perithecial initials.

Pleospora njegusensis Bub. (Collection No. 23)

Five subcultures of a single-spore isolate and 11 subcultures of a single-ascus isolate of this species produced mature perithecia on Leonian's agar in Petri dishes and in test tubes. The initial period at room temperature was 15 days. A large number of mature perithecia containing mature asci were produced within 16 weeks. The perithecia examined contained numerous asci with mature spores.

The following is a description of the perithecial stage produced in culture:

Perithecia mostly 500--800 μ in diameter, with heavy walls, and with a few hyphae originating in the wall cells of the upper part of the perithecium. These hyphae are not distinct in any character other than position from other

hyphae present. Ascospores 28.8--46.8 x 13.2--21.1 μ; septation 7--9 transverse and two to three series longitudinal with additional longitudinal septa in individual cells.

Pleospora trichostoma (Fr.) Ces. & de Not. (Collection No. 29)

Two subcultures of single-ascus isolates and four subcultures of single-ascospore isolates of both Type I and Type II development produced perithecia on filter paper cones moistened with Leonian's medium. The initial period at room temperature was 10 days. Only a few large perithecia containing mature asci were produced within 32 weeks.

Similar isolates produced perithecia on Leonian's agar in test tubes. The initial period at room temperature was 10 days. Within 16 weeks large perithecial initials without ascus differentiation were observed. Within 20 weeks mature perithecia with numerous asci, a few of which contained mature ascospores, were produced.

The following is a description of the perithecial stage produced in culture:

Perithecia 200 μ to 1 mm. in diameter, with heavy walls of opaque cells, and without distinctive hyphae originating in wall cells. Ascospores 45.6--50.4 x 18.0--19.2 μ; septation three transverse with one longitudinal septum in one or in each of both of the central cells.

Clathrospora diplospora (E. & E.) Wehm. in press (Collection No. 32)

Two subcultures of a gross isolate of ascospores from this specimen produced mature perithecia on Leonian's agar in test tubes. The initial period at room temperature was 14 days. Numerous small and a few relatively large perithecia containing many mature asci were produced by the end of 16 weeks.

Similarly treated cultures on Leonian's agar in Petri dishes produced well-defined perithecial initials, but asci were not found under these conditions within 32 weeks.

The following is a description of the perithecial stage produced in culture:

Perithecia 150--450 μ in diameter, with relatively fragile walls two or three cells in thickness, and mostly with numerous blunt or pointed, thick-walled, septate setae, but setae completely lacking on some perithecia. Ascospores 19.9--30.4 x 10.5--14.0 x 8.4 μ; septation three transverse with one longitudinal septum in one or in each of both of the central cells.

Clathrospora diplospora (E. & E.) Wehm. in press (Collection No. 33)

Twenty-one subcultures (Type I) derived from a single

ascospore isolate of this species produced mature perithecia on Leonian's agar, on 2 per cent malt agar, and on Ellis' synthetic medium in test tubes. The initial period at room temperature was seven days. Numerous perithecial initials visible to the unaided eye were produced evenly scattered to crowded on and within the substrata within four weeks; perithecia containing mature ascospores were found in the same cultures only after about 16 weeks. Unruptured asci containing only four mature ascospores and without evidence of other immature spores were not uncommon.

Similar cultures retained at room temperature under conditions which minimized loss of moisture did not produce perithecial initials within the space of eight weeks.

Three series of subcultures of this isolate on Leonian's agar were allowed different initial periods of growth at room temperature, namely, one day, seven days, and 14 days, after which they were removed to the cold room. Completely formed ascospores were found in cultures given an initial period of seven days fully a week before ascospores of similar maturity were found in cultures of either one day or 14 days of initial room-temperature growth.

The following is a description of the perithecial stage produced in culture:

Perithecia 175--380 μ in diameter; with relatively fragile walls of black-walled cells; and mostly with numerous

simple, pointed, dark-walled, septate setae about 500--1500 μ in length and 3.5 μ in width at the base. These setae are quite distinct from other vegetative hyphae. Ascospores 24.6--43.3 x 15.2--24.6 x 14.4 μ; septation three transverse with one longitudinal septum in each of the two central cells.

Cultures of the Type II isolate of this specimen were subjected to numerous different growth conditions. The following listed methods of treatment were imposed upon these cultures:

1. Cultures initiated at room temperature and kept at room temperature under conditions which minimized loss of moisture (Leonian's agar).

2. Cultures initiated at room temperature but removed to the cold room after seven days.

a. on Leonian's agar.

b. on a series of Leonian's agars in which the nutrient contents were reduced to 5, 10, 25, and 50 per cent of the standard amounts.

3. Cultures initiated at room temperature but removed to temperature of cold room after one, seven, and 14 days (Leonian's agar).

4. Cultures initiated at room temperature for one day; removed to a temperature below 0° C. for periods of one, seven, and 14 days; then returned in part to a moist

chamber at room temperature and in part to the cold room (filter paper cones moistened with Leonian's medium).

5. Cultures initiated at room temperature for two days; removed to temperature below 0° C. for two days; then alternated between the two temperatures in 2-day periods for 14 days; finally placed in refrigerator room (filter paper cones moistened with Leonian's medium).

These cultures produced only conidial stages under all conditions tried; perithecial initials were not observed at any time within test periods of nine to twelve months.

Clathrospora rainierensis Wehm. inedit. (Collection No. 37)

One subculture of a single-ascus isolate and four subcultures of single-ascospore isolates of this specimen produced perithecia on Leonian's agar in Petri dishes. The initial period at room temperature was 20 days. Only a few perithecia (two--four) were formed in any one culture; all perithecia produced mature spores within 36 weeks but not within 32 weeks.

The following is a description of the perithecial stage produced in culture:

Perithecia 700 x 400 μ, with heavy walls, but without distinctive superficial hyphae. Ascospores 32.4--42.0 x 12.0--15.5 μ; septation seven transverse with one or two series of longitudinal septa and occasional additional

longitudinal septa in individual cells.

C. Discussion

The media used here as substrata for production of perithecia are not cited as being specific for perithecial formation by the species studied. Data presented indicate the probability that physical factors of the cultural conditions have positive influence upon the production of mature fruiting structures. Specifically, in this connection, must be mentioned the influence of low temperature, the initial period of growth at room temperature, and the time necessary for the maturation of cultures of the different isolates. A ready example illustrating the influence of these factors is found in the experiments with Clathrospora diplospora (Collection No. 33, Type I). Similar subcultures of the isolate were subjected to a low temperature and to room temperature during the growth period; fruiting structures were produced at low temperature within four weeks but were not produced at room temperature within eight weeks. Other series of cultures allowed initial room-temperature growth periods of one, seven, and 14 days exhibited a maturation differential, in that mature perithecia appeared in cultures allowed seven days before mature structures appeared in the other cultures.

The length of time required for production and matura-

tion of perithecia by isolates of the different species ranged from 16 to 36 weeks. Pleospora njegusensis and Clathrospora diplospora required only 16 weeks at low temperature for maturation. P. trichostoma required only 20 weeks when grown on Leonian's agar in test tubes but needed 32 weeks when grown on filter paper moistened with Leonian's medium. A length of time of about 36 weeks was required both by P. ambigua and by C. rainierensis before maturity was reached.

An explanation of the influence of these physical factors of low temperature, time, and initial treatment is not available from the data at hand. One possible explanation involves the probability that so-called staling products resulting from physiologic activities of the mycelium collect in the medium or in the mycelium and, at a critical point in development, affect the initiation of fruiting. Another possibility rests on the fact that the rate of gaseous exchange in the medium is reduced at low temperatures, thus limiting the availability of atmospheric oxygen to the organism and possibly bringing about physiologic conditions in the mycelium that would favor perithecial formation. These suggestions and others of equally uncertain value depend upon future investigation for clarification.

Judging from the results of these experiments, it is certain that the artificial conditions imposed upon the cultures did not prohibit the formation of perithecia by some

of the isolates of the species used but, in fact, may have promoted such development. It is not certain, however, whether or not similar conditions actually inhibited the production of perithecia by isolates of other species cultured. There are several possible reasons for non-production of perithecia by the latter isolates: (1) five of the isolates were from single spores and would not, therefore, produce perithecia if they are heterothallic; (2) specific nutritional requirements of the isolates were not met by the substrata used; (3) specific environmental conditions necessary for perithecium production were not provided. It has been established that the length of time for which some cultures are allowed to remain at room temperature before being transferred to a low temperature has a positive effect upon the time of maturation of perithecial structures. Finally, it has been demonstrated in the case of Clathrospora diplospora that a period of growth under low temperatures is necessary for the production of perithecial initials.

II. STATISTICAL ANALYSIS OF ASCOSPORE LENGTH

The determination of the range of size of ascospores has always been considered to be of primary importance in taxonomic circumscription of species of the Pleosporaceae. Usually the size-range indicated in the description of type material is accepted as the spore size-limit of the species,

and only after studying numerous collections of what he considers to be one species does an investigator feel justified in emending a description. In practice, a worker, rather than change the established concept of a species, often announces new specific or varietal names for collections which vary from the type in ascospore dimensions, although other character differences may not be found. Such separation may or may not be valid, but the only certain way of finding out is by determining the variability in spore size of material of unquestionable species identity.

It cannot always be known with certainty that several similar specimens collected in different localities or on different substrata actually belong to the same species. For this reason any method of obtaining material from a single source sufficient for determining the variability of the organism under a large number of environmental conditions is desirable. The method most nearly meeting these requirements is that of pure-culture work with single-ascospore strains. By this means, providing the organism can be induced to fruit in culture, data can be obtained on a single organism under any desired number of conditions without fear of confusing characters of one fungus with those of another.

The following brief study is intended to show the variability of ascospore length within and between samples obtained from individual perithecia which were derived from

a single ascospore or which were derived, in one case, from spores from a single perithecium.

A. Materials and Methods

Isolates derived from ascospores of three different collections produced enough perithecia with sufficient numbers of mature ascospores that statistical methods of comparison could be used. The cultures of Pleospora niegusensis (Collection No. 23) and of Clathrospora diplospora (Collection No. 33) were derived from single ascospores; the culture of C. diplospora (Collection No. 32) was derived from at least two ascospores from a single perithecium. These cultures were maintained on Leonian's agar under growth conditions listed previously and were sampled for the present purpose when numerous mature perithecia had been produced.

Each sample of ascospores was obtained by isolating and crushing a single mature perithecium in a drop of Amman's mounting fluid on a glass slide. By means of a mechanical stage the preparation was moved laterally across the field of the microscope and length data were recorded on all mature ascospores appearing in the field up to a total of 50 spores per perithecium. (A spore was considered to be mature if it had the minimum number of septa present which its generally accepted specific description indicated.) Data were recorded in terms of ocular micrometer divisions and statistical

calculations were made on the data expressed in these units. Samples were obtained from four different perithecia of each of the isolates.

B. Experimental Results

Making use of standard statistical formulae, the following values were determined from the length data of each sample: range of length, mean length, and standard deviation of mean length (Table IX). The mean length values and the standard deviation of mean length values then were used in comparing data obtained from different samples by employing them in a standard ratio designed as a test for significance of difference between means (Table X).

C. Discussion of Tables

Values derived from the data are arranged in the Tables in such a manner that those values representing samples from individual perithecia may be read directly (Table IX) and that those values representing comparisons of samples from different perithecia of the same culture may be found readily (Table X). In Table X values of 2,0 or more (underlined in the Table) indicate that there exists a significant difference between mean length values of samples from the perithecia indicated.

In order to simplify and to standardize the method of

reference to the individual samples and to the comparison of two different samples, the following symbols have been used in the Tables:

23 -- culture derived from Pleospora njegusensis (Collection No. 23)
32 -- culture derived from Clathrospora diplospora (Collection No. 32)
33 -- culture derived from Clathrospora diplospora (Collection No. 33)

1 -- ascospore sample from perithecium No. 1
2 -- ascospore sample from perithecium No. 2
3 -- ascospore sample from perithecium No. 3
4 -- ascospore sample from perithecium No. 4

Per. No. -- numbers assigned to perithecia whose ascospores are being compared

R -- range of length in ocular micrometer divisions
Rμ -- range of length in microns
m -- mean length in ocular micrometer divisions
σ -- standard deviation of mean length

Examples: In Table IX "19-1" under "R" locates the values indicating the range of length (in micrometer divisions) of ascospores in a sample from perithecium No. 1 of the P. njegusensis culture. In Table X "1-2" under P. njegusensis locates the value indicating the degree of significance of the difference between the mean length of ascospores from perithecium No. 1 of P. njegusensis and the mean length of ascospores from perithecium No. 2 of the same culture.

The length-range data for samples from the different isolates (Table IX) give a type of information usually found in published descriptions, i. e., the observed limits of length of ascospores. For each isolate the length ranges

TABLE IX

RANGE OF LENGTH, MEAN LENGTH, AND STANDARD DEVIATION OF LENGTH OF ASCOSPORES FROM DIFFERENT SINGLE PERITHECIA IN CULTURES OF *PLEOSPORA NJEGUSENSIS* AND OF TWO STRAINS OF *CLATHROSPORA DIPLOSPORA*

Sample	R	Rμ	m	σ
23-1	27--40	31.59--46.80	32.90	3.47
-2	29--40	33.93--46.80	32.74	2.34
-3	27--40	31.59--46.80	31.18	2.61
-4	27--33	31.59--38.61	30.14	1.33
32-1	19--26	22.23--30.42	20.60	1.40
-2	18--23	21.06--26.91	19.96	1.23
-3	18--23	21.06--26.91	20.58	1.43
-4	17--23	19.89--26.91	20.16	1.28
33-1	21--37	24.57--43.29	30.20	2.79
-2	24--35	28.08--40.95	30.32	2.87
-3	25--37	29.25--43.29	29.50	2.89
-4	23--35	26.91--40.95	28.78	2.68

of spores from the different perithecia are in close enough agreement that few investigators would consider it probable that any significant difference between samples actually existed. These length limits, however, do not reveal the pattern of variation in the different samples.

The mean length values (Table IX) present information which offers a better means of comparison between samples. These mean values reveal that the patterns of variation in samples having identical size ranges are not necessarily the same. But conclusions concerning the significance of differences in mean length values of similar ascospore samples depend on more critical statistical comparisons. The results of such comparisons for the three isolates examined are recorded in Table X.

The most remarkable results of comparisons of ascospore samples from different perithecia of a single pure culture are apparent in the values listed for Pleospora niegusensis in Table X. In five of the six compared pairs of samples, significant differences in mean length are found between perithecia produced in a single culture of a single-spore isolate. The statistical interpretation of any one of these five comparisons is that, within the limits of random sampling, the chances are 19 to one against the mean length value of one sample being obtained from a random sample of spores from the second perithecium. This means, in

TABLE X

DEGREE OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN MEAN LENGTHS OF ASCOSPORES FROM DIFFERENT SINGLE PERITHECIA IN CULTURES OF *PLEOSPORA NJEGUSENSIS* AND OF TWO STRAINS OF *CLATHROSPORA DIPLOSPORA*[11]

Per. No.	*P. njegusensis*	Per. No.	*C. diplospora* No. 32
1-2	0.3	1-2	2.5
1-3	2.8	1-3	0.1
1-4	5.3	1-4	1.6
2-3	3.2	2-3	2.3
2-4	6.8	2-4	0.8
3-4	2.5	3-4	1.6

Per. No.	*C. diplospora* No. 33
1-2	0.2
1-3	1.2
1-4	2.6
2-3	1.4
2-4	2.8
3-4	1.3

[11] Each comparison is between a pair of perithecia from a culture of a single isolate, not between perithecia of different species or different isolates of a species.

application, that it probably would be possible to examine a relatively large number of perithecia of P. njegusensis and find numerous different patterns of variation in ascospore size. If such a variety of spore-length patterns can be found in one pure-culture derived from a single ascospore, it seems reasonable that a worker could expect to find even greater ranges of spore variation and more numerous different length patterns in material grown on different artificial media or collected in nature.

The comparisons of data from samples of the two different isolates of Clathrospora diplospora yield values which indicate that, for the most part, these isolates vary within their own well-defined limits and in a relatively small number of length-variation patterns under the experimental conditions employed. In one-third of the comparisons for each of these isolates significant mean differences between samples from different perithecia were found. It would be expected that more pronounced variation would be found in material from different substrata. However, judging from the length ranges and the mean-length values of these two different isolates, it appears highly improbable that they are genetically the same. A comparison of ascospore size between samples from perithecia in the original collections and from perithecia produced in culture is indicated here:

Collection No. 32

in nature: 25.7--26.9 x 12.9--15.2 x 8.2--9.4 μ

in culture: 19.9--30.4 x 10.5--14.0 x 8.2 μ

Collection No. 33

in nature: 31.6--37.4 x 16.4--18.7 x 10.5--12.9 μ

in culture: 24.6--43.3 x 15.2--24.6 x 14.4 μ

Although the shape and septation of ascospores of the two fungi are the same, the size ranges of the spores from nature do not overlap and the size ranges of spores from cultures do not overlap significantly. This comparison reveals that the two "strains" of C. diplospora studied must actually be considered as separate species.

III. CONCLUSIONS

The conclusions concerning culture methods for production of perithecia by the isolates used must necessarily be general. Conditions of culture (including substratum, growth at low temperature, growth at room temperature before removal to low temperature, and length of time required for development) are cited for the production of perithecia by isolates of several species. These conditions are not indicated as being critical for perithecial production but are presented as means by which mature fruiting structures of the isolates studied may be obtained for use in morphologic

and cytologic studies.

The major conclusion derived from statistical comparisons of ascospore samples of Pleospora njegusensis is that this species exhibits several distinct patterns of spore-length variation and that it could be expected to show even greater variation in nature than is found in the culture studied. The specific circumscription of this organism probably must be extended eventually to include diverse growth forms now excluded by its description.

The patterns of variation of the two isolates referred to as Clathrospora diplospora appear to be less numerous than those of P. njegusensis under the experimental conditions involved. However, examination of perithecial material obtained on different substrata may necessitate extension of the spore-length circumscription of the species as described, but it is considered unlikely that such an extension based on pure cultures of the small-spored isolate would include the spore range of the large-spored isolate. On the basis of the cultural and statistical data presented here, the two "strains" of C. diplospora studied must be considered as separate species.

CHAPTER VII

GENERAL DISCUSSION AND SUMMARY

The present investigation has shown that experimental studies of the inherent variability of species in the Pleosporaceae must be accomplished before accurate species circumscriptions can be established. For this reason the observations herein noted place emphasis upon a better understanding of the individual species rather than upon their identification.

Since variation in spore length has been used extensively in the definition of species of Alternaria and of the Pleosporaceae, the constancy of the length ranges of spores of these species has been investigated. The view maintained in this study should be contrasted to that in recent taxonomic investigations on these groups.

The results of previous investigations on conidial morphology of certain fungi have been used, in conjunction with culture characters, as bases for extending the descriptions of species to include increasingly diverse forms. This interpretation has resulted in the necessity for reducing numbers of binomials to synonymy in the Fungi Imperfecti.

In one recent instance the circumscription of Alter-

naria tenuis auct. is such that specimens exhibiting similar spore and conidiophore morphology are included although isolates from these collections exhibit quite distinct differences when cultural characters are compared (Neergaard, 1945). (To be noted particularly in this connection are Neergaard's statements concerning the appearance of colonies in pure culture on artificial media and also the plant substratum specificity or preferences of the individual isolates.) Here the implication is that we are concerned with a number of naturally related forms differing in slight detail.

Groves and Skolko (1944b), who also studied numerous isolates of Alternaria tenuis, recognized different patterns of variation in spore size in different strains of this form-species. On the basis of their observations on several variable spore characters, however, they concluded that "the only workable method of handling these forms was to group them all together and consider them as strains or morphotypes of one species." Their concept resulted in the synthesis with Alternaria tenuis "of a great many Alternaria species including A. fasciculata (E. & M.) Jones & Grout, A. humicola Oud., A. maritima Sutherland, and many other species that have been described chiefly on the basis of the host."

In other recent work Snyder and Hansen (1940, 1941, and 1945), in their taxonomic treatment of sections of the form-genus Fusarium, have grouped under 10 binomials the

multitude of Fusaria previously described. In addition, these authors have placed under eight binomials at least 22 described ascomycetous forms reported to be ontogenetically related to the Fusaria. Their taxonomic treatment of the perithecial stages apparently was based not on the systematic merits of the perfect stages themselves but primarily on the basis of the form-species delimitations of their conidial counterparts. The taxonomic changes suggested by these authors are based to a great degree on data from culture work and are intended to increase the usability of the Fusarium taxonomy. However, in no case do they arrive at the definition of a natural species in the group.

Such procedures as these in taxonomic work must be understood for what they are, namely, methods by which populations circumscribed on the basis of morphologic characters may be assigned binomials. The distinction between the concepts of the form-genus and of the form-species, as applied to the Fungi Imperfecti, and the concepts of the genus and of the species, as usually applied to perfect stages, must be carefully maintained.

In none of the studies reviewed above and in no other definitive work encountered on species of these form-genera has the conidial material for comparisons been derived directly from known ascomycetous stages. The point is granted that it is not practical and often, in fact, it may not be

possible to connect perfect stages with known conidial stages. This is borne out well by the fact that many distinct form-species of Alternaria are described for which no perfect stages are known. A case in point in the present study is that of A. tenuis which was the only stage produced in cultures by certain ascospore isolates of Clathrospora diplospora, C. Elynae, and Leptosphaeria heterospora. However, it is not granted that previous taxonomic work on this and similar form-genera presents more than a more or less usable means of identifying form-species encountered. In no known instance in the literature are the individual isolates considered as separate genetic entities if they correspond well with an existing description based on morphology. In fact, the close correspondence of an isolate with an established description usually is accepted as a means of strengthening the concept of the form-species described.

If the definition of a given form-species becomes so concrete that it is accepted in the sense of a species instead of merely a portion of a life cycle, objections must be raised. This increasingly common practice of treating morphologically similar populations of the Fungi Imperfecti as genetically similar or identical species is based on false premises, in that such similar populations need not necessarily have any great degree of genetic similarity. This point has been illustrated here by the work presented on

three different isolates of the form-species Alternaria tenuis, each isolate having been derived from an ascospore of a different "perfect" species. In this instance the overall morphology of many subcultures and of spores of the three different species remained within the modern descriptive circumscription of A. tenuis, but the fact of the diverse origins of the isolates upholds the view that this binomial must be considered as a name given for the imperfect stages of many morphologically similar but genetically different organisms.

The statistical work presented here on the Alternaria stages of Clathrospora diplospora, C. Elynae, and Leptosphaeria heterospora is intended to be descriptive of these fungi rather than diagnostic. These three strains of the imperfect stages can, in fact, be distinguished from each other by statistical methods when grown under certain environmental conditions. The culture and statistical techniques outlined could be applied to differentiation of individual strains of the same or of different conidial stages. It is not illogical to expect, however, that equally as definite statistical differences would be obtained between conidial samples from different ascospore isolates of one species under some environmental conditions. The total conidial variability of one species, as reflected by variations observed in numerous isolates of the organism, would not neces-

sarily be distinct from the total conidial variability of another species. In consequence, studies of the total variation of conidial stages are not expected to be primarily diagnostic in value.

For critical work on specific imperfect stages, definite refinements of the present descriptive methods are desirable. It is suggested that such revisions in descriptive technique might be guided along lines already well-established in bacteriology in the identification procedures based on differences in cultural characters of morphologically similar organisms. As has been stated, the statistical work on the form-species of Alternaria is intended to be descriptive of the variability of the genetically distinct isolates. It is not anticipated that any taxonomic changes will be derived from such limited observations. On the other hand, it is asserted with conviction that the form-species of the Fungi Imperfecti (in particular, Alternaria tenuis) must be treated in both theoretical and applied fields of mycology as synthetic groupings until individual exceptions can be proved.

The present state of affairs in the systematics of Pleospora and Clathrospora is outlined and enlarged upon by Wehmeyer (1946, 1948, and 1949) in his studies of these genera and of the closely related genus Leptosphaeria. From his observations on numerous collections of Pleospora, it is

his opinion (1948) that, "although occasional collections or groups of collections stand out as distinct, the great majority fall into larger groupings showing...overlapping variations...for any one of a number of important characters." He maintains that the septation of mature ascospores is the least variable character of species in this group of fungi and uses this character, in correlation with ascospore form, size, and color and with data on perithecia, asci, and habitat, in delimiting species or species-complexes. Because of the high degree of intergradation in characters of different collections, Wehmeyer has concluded that the best way at present of handling series of Pleospora forms which are morphologically intergradient is to set certain limits of variation more or less arbitrarily and to refer to each group either by a commonly accepted binomial or as a "species-complex" (e. g., Pleospora vagans complex). He admits frankly that such separations are conservative and that "further study of larger numbers of collections may show other characters which may be used to segregate smaller groups as species or varieties, or may advise the union of groups now supposed to be good species" (1946).

It is interesting but not surprising that statistical work described here for lengths of ascospores from pure cultures bears out rather well Wehmeyer's conservative taxonomic procedure based completely on material collected from

natural habitats. The statistical results recorded for one subculture of a single-spore isolate of Pleospora njegusensis, for instance, indicate that ascospores of this species vary in numerous different length-patterns, and it is conceivable that, had a collector found any two different patterns in nature or had they been described individually from nature, two different "varieties" or even "species" might have been proposed. With the knowledge that the spores of P. njegusensis are significantly variable, even in pure culture, it would seem ill-advised to consider describing the morphologic variants of the species as entities at any taxonomic level. This conclusion may be considered as strengthening the view of Wehmeyer that many collections of Pleospora with intergrading morphology are best handled at present in relatively large taxonomic blocks.

The statistical results recorded for the isolates of the two "different" spore-forms of Clathrospora diplospora might be considered as supporting Wehmeyer's view that "occasional collections or groups of collections stand out as distinct" (1948), since within the culture examined for each of these isolates the variation in length patterns of ascospores was decidedly limited. In addition, the size ranges of ascospores of the two fungi in nature were not observed to overlap, and the size ranges of spores from culture did not overlap significantly. For these reasons the isolates

studied are considered to represent two distinct species of Clathrospora.

Conclusions drawn from the statistical data in conjunction with the facts known about the origins of the isolates might appear to be contradictory when results of the conidial work and of the ascospore work are compared. These conclusions assert, on the basis of similar types of observations, that, in one instance, the form-species of Alternaria may contain several different organisms, while, in the other instance, a species circumscription in Pleospora or in Clathrospora may represent only a portion of the variation potential of one organism. These data and conclusions, however, actually reflect the present-day situation in the taxonomy of the two groups, i. e., individual form-species of some Fungi Imperfecti have been used as receptacles for morphologically similar but genetically distinct organisms while, in the Pleosporaceae, several portions of the natural population of a single genetic entity may have been circumscribed as different "species" without an understanding of the inherent variability of the organism.

It is interesting to speculate on the possibility of setting up a system by which the variability of a specific organism could be determined and, in consequence, by which its identity in a natural system of classification could be defined. It is believed that such an ideal method cannot be

accomplished alone by examination of material discovered in nature; the unknown factors of environmental influence are too numerous. On the other hand, it is not maintained that culture work under controlled but artificial conditions can yield the final answer. A plan aimed at a realistic investigation of a species must incorporate observations both from nature and from culture with the foundations of the system based on experimental procedures which can be defined and controlled. More specifically, the type of variation usually discussed by taxonomists in connection with the characters of a natural population cannot be admitted in an attempt to establish a sound basis for species circumscription, since such variation in a "species" is assumed on the basis of similarity in morphology of different portions of the population. It is suggested that variation established in cultures of an organism derived from a known source may be used as a basis for interpreting the variation in populations found occurring naturally.

Conclusions and data derived from the experimental studies may be summarized as follows:

1. Isolations were attempted with ascospores from perithecia of 43 collections of species of Pleospora, Clathrospora, and Leptosphaeria.

a. Three of 12 isolates which became established in culture (in addition to cultures of Pleospora infectoria

received from another source) produced conidia in culture. These were Clathrospora diplospora, C. Elynae, and Leptosphaeria heterospora.

b. Six other isolates of the 12 established in culture produced mature perithecia. These were Pleospora ambigua, P. njegusensis, P. trichostoma, Clathrospora diplospora, an isolate of a species identified as a "large-spored variety" of C. diplospora, and C. rainierensis.

c. In no case was it observed that a conidial strain also produced perithecia or that a perithecial strain also produced a conidial stage in culture.

2. The characters of the different isolates in culture were sufficiently distinct to permit the recognition of the individual species insofar as the isolates used were concerned.

3. Identification of the conidial stages of Clathrospora diplospora, of C. Elynae, and of Leptosphaeria heterospora by means of commonly accepted descriptions necessitated their placement in the form-species Alternaria tenuis.

4. Statistical analyses of conidial lengths observed in strains of Clathrospora diplospora, of C. Elynae, and of Leptosphaeria heterospora indicated that, under certain environmental conditions, the strains studied of each of the species could be differentiated from each of the other two.

5. Statistical analyses of ascospore lengths in

samples derived from different perithecia of a single culture each of Pleospora njegusensis, of Clathrospora diplospora ("normal" ascospore size), and of a species referred to as a "large-spored cariety" of C. diplospora indicated that ascospores of P. njegusensis vary in numerous different patterns of length and that ascospores of each of the isolates of "C. diplospora" vary in a decidedly smaller number of different length-patterns.

6. Ascospore-size data and statistical data on ascospore length indicated that the two fungi referred to as different strains of Clathrospora diplospora are two distinct species of Clathrospora.

7. Production of mature perithecia by different strains of Pleospora and Clathrospora appeared to be positively influenced by the physical factors of room temperature during the initial period of growth, of low temperature during the remainder of the growth period, and of the total growth time.

8. The number of mature perithecia produced by individual isolates on Leonian's agar often was characteristic for a species.

9. The cultural characters of separate isolates of single "perfect" species often were different:

a. Pleospora ambigua: one isolate produced mature perithecia in culture; two other isolates did not.

b. Pleospora niegusensis: the isolate from ascospores of one collection produced mature perithecia in culture; a similar isolate from a second collection did not.

c. Pleospora trichostoma: two distinct growth types were produced by different single-spore strains; however, no differences in the type or quantity of perithecial production was found in any of the cultures.

d. Clathrospora diplospora: strains derived from ascospores from two different collections produced mature perithecia in culture; a third strain derived from ascospores from the same collection as one of the perithecial strains produced only conidia in culture.

10. Nutrient differences in media upon which the Alternaria strains were grown resulted in a greater percentage of statistical differences in comparisons among the strains than did pH or age differences.

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