

RS4-36

## CULTURE STUDIES IN THE GENERA PLEO- SPORA, CLATHROSPORA, AND LEPTO- SPHAERIA. II

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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?

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(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.

#### STUDIES OF PERITHECIAL STAGES

A primary purpose of the culture work reported here 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 substrata. 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 impor-

tance 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 among 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 (Cavara and Mollica, 1907); on salep agar (Klebahn, 1918); on malt agar and pea agar (Groves and Skolko, 1944); and on dung agar and on straw (Hughes, 1945). Cocchi (1931) states that he obtained mature perithecia of *P. herbarum* on pieces of carrot, on his agar No. 5 (mineral salts as nutrients), and on Dox's medium solidified.

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 by the array of substrata listed, Leonian's agar (Leonian, 1924) 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, 2% malt agar and filter paper cones moistened with Leonian's medium also were employed.

Attempts at inducing perithecial formation were made with cultures of all isolates listed previously<sup>2</sup> which grew in culture. Transfers

<sup>2</sup> Lists of all collections used and of cultural results for all isolates of species studied, along with descriptive material and illustrations of the species, are presented in the first paper of these studies (Simmons, 1952).

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.*

Six of the 14 isolates studied produced mature perithecia within a year under experimental conditions. These species and the conditions under which each produced perithecia are listed. A description of the perithecial stage produced in each culture is given for each species.

#### PLEOSPORA AMBIGUA (Berl. & Bres.) Wehmeyer (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*, Collection No. 33.

Perithecia about 250  $\mu$  in diameter; with fragile walls two or three cells thick; and with radiating, filamentous, pale yellow-brown, septate hyphae about 300  $\times$  2.5  $\mu$ . 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  $\times$  9.6  $\mu$ ; 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.

Perithecia mostly 500–800  $\mu$  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  $\times$  13.2–21.1  $\mu$ ; 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.

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

#### CLATHROSPORA DIPLOSPORA (E. & E.) Wehm. (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.

Perithecia 150–450  $\mu$  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  $\times$  10.5–14.0  $\times$  8.4  $\mu$ ; septation three transverse with one longitudinal septum in one or in each of both of the central cells.

*CLATHROSPORA DIPLOSPORA* (E. & E.) Wehm. (Collection No. 33)

Twenty-one subcultures (Type I) derived from a single ascospore isolate of this species produced mature perithecia on Leonian's agar and on malt agar in test tubes. The initial period at room temperature was seven days. Numerous perithecial initials visible to the unaided eye were produced evenly scattered or 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 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.

Perithecia 175–380  $\mu$  in diameter; with relatively fragile walls of black-walled cells; and mostly with numerous simple, pointed, dark-walled, septate setae about 500–1500  $\mu$  in length and 3.5  $\mu$  in width at the base. These setae are quite distinct from other vegetative hyphae. Ascospores 24.6–43.3  $\times$  15.2–24.6  $\times$  14.4  $\mu$ ; 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 several different growth conditions, among which were the following:

1. Cultures initiated at room temperature and kept at room temperature under conditions which minimized loss of moisture; on 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 percent 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 imposed; perithecial initials were not observed at any time within test periods of nine to twelve months.

*PLEOSPORA RAINIERENSIS* Wehm. (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; perithecia produced mature spores within 36 weeks but not within 32 weeks.

Perithecia 700  $\times$  400  $\mu$ , with heavy walls, but without distinctive superficial hyphae. Ascospores 32.4–42.0  $\times$  12.0–15.5  $\mu$ ; 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 maturation 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 *P. 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 the 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 pertinence 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 presumably would not produce perithecia if the species they represent are heterothallic; (2) specific nutritional requirements of the isolates were not met by the substrata used; (3) specific environmental conditions necessary for perithecial 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, provided 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 njegusensis* (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 I). 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 II).

### 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 I) and that those values representing comparisons of samples from different perithecia of the same culture may be found readily (TABLE II).<sup>3</sup>

The length-range data for samples from the different isolates (TABLE I) give a type of information usually found in published descriptions,

<sup>3</sup> In TABLES I and II the following symbols have been used in order to simplify and to standardize the method of reference to the individual perithecia and to the comparisons of different perithecia: 23, 32, 33 (cultures derived respectively from *Pleospora njejusensis*, *Clathrospora diplospora* of "normal" ascospore size, and *C. diplospora* of "large" ascospore size); 1, 2, 3, 4 (numbers assigned to individual perithecia studied); R (range of length in ocular micrometer divisions);  $R\mu$  (range of length in microns);  $m$  (mean length in ocular micrometer divisions);  $\sigma$  (standard deviation of length). In TABLE II values of 2.0 or more indicate that there exists a significant difference between mean length values of ascospore samples compared.

i.e., the observed limits of length of ascospores. For each isolate the length ranges 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 exists. These length limits, however, do not reveal the pattern of variation in the different samples.

The mean length values (TABLE I) 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

TABLE I  
RANGE OF LENGTH, MEAN LENGTH, AND STANDARD DEVIATION OF LENGTH OF ASCOSPORES FROM DIFFERENT SINGLE PERITHECIA IN CULTURES OF *Pleospora njejusensis* AND OF TWO STRAINS OF *Clathrospora diplospora*

Sample	R	$R\mu$	$m$	$\sigma$
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

samples depend on more critical statistical comparisons. The results of such comparisons for the three isolates examined are recorded in TABLE II.

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 njejusensis* in TABLE II. 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 1 against the mean length value of one sample being obtained from a random sample of spores from the second perithecium. This means, in 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 specific circumscription of this organism probably must be extended eventually to include diverse growth forms now excluded by its original description.

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 experi-

TABLE II

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*

Perithecium numbers	<i>P. njegusensis</i>	<i>C. diplospora</i> Coll. No. 32	<i>C. diplospora</i> Coll. No. 33
1-2	0.3	2.5	0.2
1-3	2.8	0.1	1.2
1-4	5.3	1.6	2.6
2-3	3.2	2.3	1.4
2-4	6.8	0.8	2.8
3-4	2.5	1.6	1.3

mental 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:  $25.7-26.9 \times 12.9-15.2 \times 8.2-9.2 \mu$

in culture:  $19.9-30.4 \times 10.5-14.0 \times 8.2 \mu$

## Collection No. 33

in nature:  $31.6-37.4 \times 16.4-18.7 \times 10.5-12.9 \mu$

in culture:  $24.6-43.3 \times 15.2-24.6 \times 14.4 \mu$

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. 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.

## GENERAL DISCUSSION

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.

The present state of affairs in the systematics of *Pleospora* and *Clathrospora* is outlined and enlarged upon by Wehmeyer (1946, 1948, and 1949-1953) 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 Weh-

meyer'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 nejgusensis*, 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. nejgusensis* 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 is decidedly limited. In addition, the size ranges of ascospores of the two fungi in nature have not been observed to overlap, and the size ranges of spores from culture do 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 (Simmons, 1952) and of the present 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, whereas 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 whereas, 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 being 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.

#### SUMMARY

1. Statistical analyses of ascospore lengths in samples derived from different perithecia of a single culture each of *Pleospora nejgusensis*, of *Clathrospora diplospora* ("normal" ascospore size), and of a species referred to as a "large-spored variety" of *C. diplospora* indicated that ascospores of *P. nejgusensis* 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.
2. 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*.
3. 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.
4. The number of mature perithecia produced by individual isolates on Léonian's agar often was characteristic for a species.
5. The cultural characters of separate isolates of single species often were different:
  - a. *Pleospora ambigua*: one isolate produced mature perithecia in culture; two other isolates did not.

b. *Pleospora njegusensis*: 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 were 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.

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