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THE BEET WATER MOLD AND SEVERAL RELATED ROOT PARASITES[1]

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APHANOMYCES COCHLIOIDES DRECHSLER

INTRODUCTION AND HISTORY

The occurrence of a species of Aphanomyces as one of the agents causing root rot of sugar beets (*Beta vulgaris* L.) was reported in a brief paper published by Peters (*24*)[2] in 1906. According to this account, examination of a large number of specimens affected with "Wurzelbrand" and obtained from all parts of Germany had revealed a member of that genus as one of the three principal parasites concerned with the disease, the others being *Pythium debaryanum* Hesse and *Phoma betae* Frank. Because of the absence of protuberances from the oogonial wall and a certain degree of similarity in the appearance and sizes of the female organs, the fungus was identified as *Aphanomyces laevis* De Bary. Its isolation and subsequent cultivation on artificial media had been accomplished in the way that had proved successful with *P. debaryanum*. Its pathogenicity had been demonstrated experimentally by the destruction of seedlings grown on soil to which the fungus had been added.

A longer paper (*25*) published in 1911 gave a more complete account of the three principal beet parasites mentioned and included a more detailed description of the water mold in question. In spite of certain departures from De Bary's somewhat meager original description (*2*), Peters continued to regard the form from sugar beets as identical with *Aphanomyces laevis*. As a result, the latter binomial has been cited rather frequently in the literature of various European countries among the names of parasites held responsible for the damping off and root rot of beets. It is not apparent that the fungus was again studied morphologically, nor is there any clear indication that it has subsequently been available in pure culture for such study, except for Edson's record (*12*) of an isolation made from material collected in the experimental plots at Dahlem, Germany, but unfortunately lost before any use could be made of it.

In 1913 Edson (*11*) reported *Aphanomyces laevis* as one of the causal agents responsible for the damping off and root rot of sugar beets in the United States. Later (*13*), however, he discovered that the American fungus that formed the basis of the report was not identical with the European beet parasite referred to De Bary's

[1] Received for publication Oct. 30, 1928; issued April 1929.
[2] Reference is made by number (italic) to "Literature cited," p. 360.

binomial, the method of zoospore formation exhibited by it precluding altogether assignment to the genus Aphanomyces. With his description of the fungus as *Rheosporangium aphanidermatum* and its subsequent assignment to the genus Pythium in a larger sense (*14*), references to *A. laevis* as an active cause of disease in beets in the United States seem to come to an end. Apparently the association of any species of Aphanomyces with beet diseases had never been observed in the United States when the present studies were begun, although the seedling and root troubles caused by certain other fungi, such as *Phoma betae, Corticium vagum* B. and C. var. *solani* Burt (*Rhizoctonia solani* Kühn), and various species of Pythium designated rather indiscriminately as *P. debaryanum,* had been given considerable attention.

The discovery during more recent years of a number of saprolegniaceous forms associated with injuries to several crop plants suggested the advisability of determining anew whether the fungus observed by Peters might not, after all, be present in American beet fields. Interest in such inquiry was dictated particularly by the somewhat problematical relationship of the beet parasite to *Aphanomyces euteiches* Drechs. and to *A. raphani* Kendrick, which, under favorable weather conditions, are known to cause very appreciable damage to peas (*Pisum sativum* L.) and to radishes (*Raphanus sativus* L.), respectively, in regions including some areas in which the sugar beet is cultivated extensively. Accordingly, during the last week in June, 1927, the writer made isolations of presumably pathogenic forms in experimental fields near East Lansing and Saginaw, Mich. Owing to some weeks of droughty weather that had preceded, the crop, which was advanced far enough to necessitate thinning, showed relatively few plants bearing evidences of infection, although damping off was said to have occurred somewhat earlier in considerable quantity. Relatively severe damping off was observed, however, in a plot of much younger seedlings that were kept well watered by an overhead sprinkling system. From this plot, too, diseased specimens were collected and employed in the isolation of pathogenic types.

METHOD OF ISOLATION

While some of the fungi causing damping off and root rot, such as *Rhizoctonia solani, Pythium debaryanum* and *P. ultimum* Trow, are readily obtained by the usual procedure of placing portions of diseased tissue on plates of hard agar media, others fail to come to light on such treatment. Consequently, the large majority of phycomycetous parasites associated with rootlet diseases either have remained entirely unknown or have been encountered so infrequently that their actual widespread distribution has not been suspected. In most specimens all except the most freshly invaded portions of the host tissues are occupied by bacteria that are present as secondary invaders. It is a matter of common knowledge that when pieces of such tissue are planted on culture media, without any preliminary treatment other than perhaps washing or surface sterilization, the bacteria within the tissue find their way to the surface, where they immediately begin to multiply in the free water usually adhering. The fungus, on the other hand, is frequently slow to resume vegetative

growth, as evidently the resting structures (oospores and conidia) require some time for germination, and the living mycelium, when any is present, is usually reduced to a small quantity. As a result, with the bacterial growth soon completely hemming in the fungus, the vegetative growth of the latter either is not resumed or is intercepted at an early stage.

To circumvent this difficulty, the writer has adopted the practice of first placing all putrescent material suspected of harboring parasites assignable to Pythium, Phytophthora, Aphanomyces, and related genera, the isolation of which may be desired, in sterilized water. Petri dishes containing 10 to 15 c. c. of sterile water are generally used. When the affected parts are small like rootlet tips, they are used in their entirety; but when they are bulkier, like potato tubers or tomato, cucumber, or eggplant fruits, pieces of convenient size, weighing 0.5 to 1 gm., are removed. In cases of heavy bacterial contamination, especially in hot weather, the water is changed several times until it is no longer noticeably turbid. Usually within 12 hours, though sometimes not within 24 hours, extramatrical mycelium consisting of vigorous, actively growing filaments makes its appearance, its development being presumably encouraged by the leaching away of the accumulated products of bacterial origin. The efficacy of such cultivation, at least for certain purposes, is attested by its continued use by students of the water molds and allied aquatic or amphibious types even after the adoption of pure-culture methods and artificial media in mycological research generally, Peters's study of the very sugar-beet parasite under consideration providing a pertinent instance.

After adequate mycelial development has been secured, the pieces of tissue are removed, placed between sheets of filter paper where the free water is blotted off as completely as possible, and then transferred immediately to freshly poured plates of some suitable nutrient agar medium like maize-meal agar, care being taken to bring about firm contact with the substratum. Owing to the thoroughly contaminated character of the material at the beginning, aseptic precautions, except for the use of sterilized instruments in making the final transfer to the plates, and the avoidance of unnecessary sliding over the surface of the agar, are of little value. Thorough removal of the free moisture is far more important, since in its absence the accompanying bacteria are not generally capable either of rapid multiplication or of spreading over the surface of the agar in any measure sufficient to head off the extension of actively growing mycelium. To be sure, the advancing phycomycetous hyphae are even then usually accompanied by such contaminating organisms, which, especially under warm conditions, may be present in some degree at the very tips of the individual filaments. Transfers made from the original agar plates, therefore, usually require additional attention to free them of these forms, the method described by Brown (*4*) being especially useful for this purpose. While at the high summer temperatures sometimes prevailing in the United States the method would seem occasionally to lose some of its efficacy, refrigeration of the deep plain agar plates at 15° to 20° C. provides a simple expedient for adapting it for service in any season. When "spreaders"—organisms capable of rapid distribution over the surface of the agar independent of any fungus—are encountered, it is frequently advantageous

to use deep layers of nutrient media also for the original plates; or, adopting the technic used by Rands in his isolation of *Phytophthora cinnamomi* Rands (*26*), the pieces of blotted tissue may be thrust directly into tubes of nutrient agar. In either case the fungi to be isolated are enabled to develop extensively deep down in the substratum without being seriously impeded by the presence of bacteria on the surface.

The procedure here outlined, consisting essentially of first bringing about a renewal of vegetative growth and then transferring the growing mycelium without the excess of water by means of which the contaminating bacteria too often prevent its extension, has the advantage of being independent of zoospore formation so often absent or suppressed in terrestrial forms. It has proved highly effective in the isolation of types without a strong extramatrical tendency, such as *Pythium arrhenomanes* Drechs., which occurs as a parasite on maize roots (*10*). Or, as in the case of mottle necrosis of sweet potatoes (*17*, *18*) due largely to *P. ultimum*, it makes possible the ready isolation of forms not generally lacking in aggressiveness but adversely affected evidently as a result of peculiar conditions in the morbid tissue itself. Its greatest usefulness, however, lies in the readiness with which it permits the isolation of species of Phytophthora, Pythium, Aphanomyces, and related genera from putrescent host tissues. At the same time its application does not by any means prevent the discovery of other fungi that may be present in either a parasitic or a saprophytic relation.

OCCURRENCE

Thus, when the diseased beet seedlings already mentioned were employed, the usual fungus types featured in publications by Edson (*11*, *12*) and by Coons and Stewart (*7*) appeared in quantity. The mycelia of *Corticium vagum* and *Phoma betae* were obtained from a large proportion of the diseased specimens. The genus Pythium was abundantly represented with respect not only to the number of separate isolations but also to the number of species involved, nearly a dozen distinct forms being recognizable among the several scores of cultures that were retained for further examination. Of more particular interest here is the discovery in a fair proportion of individual specimens of a species of Aphanomyces, which in a brief abstract (*9*) was designated as a new species, *A. cochlioides*. Of 15 specimens collected in the field at East Lansing, Mich., 3 yielded cultures of the water mold; of 32 specimens collected near Saginaw, Mich., the same parasite appeared in 7 instances; and of 34 specimens from the plot provided with overhead irrigation, 19, or somewhat more than one-half, gave rise to cultures of the identical fungus. In some instances one or several species of Pythium were obtained from specimens yielding the water mold, and in other specimens *Corticium vagum* or *Phoma betae* were present as accompanying forms. The relative distribution of the various types of fungi associated with damping off in the comparatively dry fields on the one hand, and in the well-irrigated plot on the other, indicates that while the presence of an abundance of moisture promotes the development of such parasites generally, it encourages the development of *A. cochlioides* to a considerably greater extent than the others. A close parallelism to

the overwhelming predominance of *A. euteiches* over a similar and to a large extent identical assortment of parasites, characteristic of root rot of peas in wet seasons, is indicated.

ARTIFICIAL CULTIVATION

The saprolegniaceous parasite may be cultivated readily on nearly all kinds of artificial media usually used in laboratories. Unlike Peters and Edson, the writer experienced no difficulty in keeping cultures alive for protracted periods of time, transfers made nine months after planting yielding subcultures without exception. There is no reason to believe that the fungus perishes more readily than the several congeneric forms that have been maintained in culture continuously, although transferred only once a year. Indeed, its capacity for forming an abundance of good oospores in cultures contaminated with bacteria, as, for example, those resulting from direct plantings of diseased seedlings on maize-meal agar plates, would seem to make its survival less rather than more precarious. Contaminating bacteria, nevertheless, should be removed, because plantings made from old cultures in which oospores represent the only living elements, are usually successful only when the fungus is thrust into the small quantity of free water generally present at the bottom of the sloping surface. As the germination of the thick-walled structures usually requires several days, an admixture of bacteria ready to start multiplying at once is obviously very undesirable. Plantings made on the drier surface of the medium, where bacteria would find conditions less favorable, as a rule are unsuccessful, owing apparently to the failure of the oospores to germinate there.

In macroscopic appearance cultures of the parasite on the same medium show a high degree of uniformity. A moderate tendency toward the production of aerial mycelium is characteristic, this tendency being stronger here than in most of the congeneric forms that the writer has had occasion to study, though *Aphanomyces euteiches* with its arachnoid aerial habit shows comparable development in this respect.

MORPHOLOGY

Mycelial Characteristics

Similarity in general habit to the pea root-rot fungus is evident also when the mycelium is examined under the microscope. Branching of the hyphae (fig. 1, A, *a* and *b*) occurs at intervals of moderate length, and at angles usually not much smaller than a right angle. In addition to branches of indefinite length, there are others that remain relatively short, thus forming diverticulate spurs, the latter type being especially frequent in proximity to sexual structures. Approximately uniform diameters are maintained in filaments over long distances, often from origin nearly to the tip. Relatively delicate ramifying systems such as occur in the mycelium of *Plectospira myriandra* Drechs. as well as in the form described in the present paper as *P. gemmifera* were not produced by the beet parasite on any of the substrata that were employed.

Asexual Reproduction

The production of zoospores can be induced without difficulty by transferring young mycelia developed on suitable substrata to water

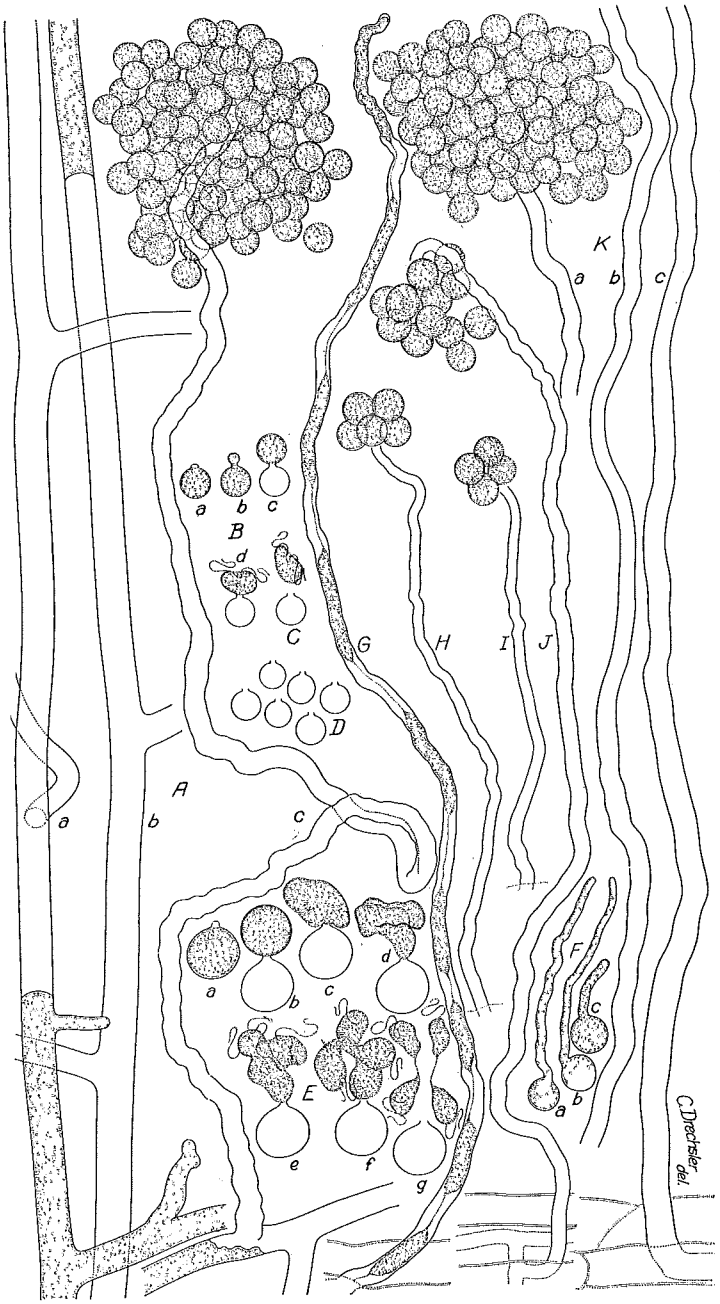

FIGURE 1.—Asexual reproduction of *Aphanomyces cochlioides*. All figures drawn with the aid of a camera lucida. ×445

(For explanatory legend see opposite page)

When beet seedlings that have damped off as a result of infection by the parasite are placed in distilled water, within a few hours extramatrical hyphae begin to appear from the cortex of the affected water-soaked parts. These hyphae on elongating generally show certain decreases in diameter from the base toward the tip, as well as prolonged series of sinuous irregularities somewhat suggestive of the modifications distinctive of conidiophores of the genus Polythrincium, and evidently similar to those occurring in the evacuation tubes of *Aphanomyces raphani*. Eventually the protoplasmic contents become divided, and the delimited protoplasts are discharged altogether after the manner characteristic of the genus Aphanomyces. Not every extramatrical element is discharged through its own apex, since in some instances the zoospores were observed passing downward into the intramatrical mycelium, to be discharged presumably through the apex of some communicating element. Nevertheless, the extramatrical elements chiefly represent evacuation tubes of sporangial units, which for the most part are concealed within the host tissue. The number of zoospores liberated may vary from a few, as in the case of the two small tubes illustrated in Figure 1, H and I, to more than 300, the latter number being associated often with tubes twice as wide (8 to 10 μ) and 10 to 20 times as long (1 to 2.5 mm.). A more nearly average condition is represented by a basal diameter of 6 to 8 μ, a length of 400 to 1,000 μ, and a number of zoospores between 100 and 200. (Fig. 1, K, *a–c*.) Since in the course of a few days a diseased seedling not more than 50 mm. in length will ordinarily permit discharge from several hundred efferent hyphae, it is obvious that the total number of spores from a single specimen is to be reckoned in tens of thousands.

The morphology of the zoosporangium can be studied to somewhat better advantage when mycelium in a suitable transparent medium like maize-meal agar is cut into pieces of convenient size and transferred to water. The sporangial units are here revealed as long mycelial tracts consisting of portions of large axial filaments often 3 to 4 mm. in length, together with extensive portions of numerous branching systems. Both axial and branching elements are delimited by septa, which may occur as the usual type of cross wall (fig. 1, A, *a–b*) or as partitions of more irregular outline (fig. 1, A, *c*). Plural evacuation tubes are usually present in the form of long extramatrical elements inserted at moderate intervals. Like those produced with the use of the natural substratum they are ordinarily more or less contorted, with extensive portions showing sinuous contours. While

EXPLANATORY LEGEND FOR FIGURE 1

A.—Portions of an evacuated sporangium developed from mycelium grown on maize-meal agar, *a* representing the basal portion of the axial element, *b* the distal portion, and *c* an evacuation tube arising from one of the lateral branches. A section of the filament exceeding 2.5 mm. in length between *a* and *b* is omitted because of lack of space.

B.—Successive stages, *a* to *d*, in the evacuation of the cyst membrane and the development of the motile zoospore C.

D.—Evacuated cyst membranes.

E.—Successive stages, *a* to *g*, in the development of four zoospores from a single abnormally large encysted structure.

F.—Germination of several zoospores, *a* to *c*, by delicate germ tubes.

G.—Evacuation tube immediately previous to discharge, produced from the stem of an infected beet seedling placed in water, the host epidermis being represented by dotted lines.

H, I.—Small evacuation tubes after the discharge of only five and four zoospores, respectively, arising from the stem of a beet seedling.

J.—A somewhat longer evacuation tube, with discharged zoospores, produced from the stem of a beet seedling.

K.—A longer evacuation tube, after discharge of more than 100 zoospores, produced from the stem of a beet seedling and drawn in successive sections, *a* to *c*.

some diminution in diameter toward the apex is usually perceptible, the pronounced attenuation regularly characteristic of the discharge tubes of *Aphanomyces euteiches* is very infrequent.

Whether beet seedlings or artificial media are employed, the degree of coherence between the encysted zoospores is not constant. At times large clusters remain together even when subjected to some mechanical disturbance, but often, too, the aggregations may be so loose as to disintegrate with great readiness. In any case the development of the motile stage normally follows a quiescent period of two or three hours and comprises the same succession of events (fig. 1, B, *a–d*, C) as was delineated for *Aphanomyces euteiches*. Indeed, the only morphological detail in which a specific difference in the structures involved can be detected is in the diameter of the papilla through which the encysted spore is evacuated. In *A. cochlioides* this dimension is noticeably smaller, being approximately equal to only one-fifth (fig. 1, D) instead of one-fourth to one-third of the diameters of the zoospores. The subsequent rounding up of the motile bodies and their germination by slender germ tubes (fig. 1, F, *a–c*) complete the cycle of asexual reproduction. It is scarcely necessary to add that the irregularities observable in congeneric forms occur also in the species under consideration; for example, incomplete discharge of zoospores and their encystment within the sporangial wall, or incomplete cleavage of the protoplasm within the sporangium with the resulting appearance of giant encysted forms. The formation of plural motile zoospores from the evacuated contents of such abnormally large structures (fig. 1, E, *a–g*) involves a belated cleavage in the naked protoplasmic mass that is not without some measure of similarity to the process of zoospore formation prevailing in the genus Pythium. In no instance was the motile stage seen to arise after the manner described by Gicklhorn (*15*) for his *A. ovidestruens*, that is, by the protoplast within the cyst envelope after an exhibition of first jerky then slowly rotating movements squeezing through a small circular hole into the surrounding water.

Sexual Reproduction

While in the tissues of beet seedlings that have newly succumbed to the attack of *Aphanomyces cochlioides* only vegetative mycelium of the parasite is present, specimens collected in later stages exhibit oogonia and oospores in greater or smaller numbers. An evidently parallel development can be observed conveniently in the course of about a week by putting freshly damped-off beet seedlings into water, the latter being renewed from time to time. With the spread of the fungus over the entire plant, the stem, roots, and leaves become progressively water-soaked in appearance and thereupon continue for several days to give rise to extramatrical filaments, which discharge liberal quantities of zoospores. Later the tissues involved become much softened in texture, flabby, and nearly transparent. Zoospore production ceases, and oogonia and oospores make their appearance here and there and finally are found scattered rather liberally through larger portions of the collapsed host. (Fig. 2.) As has been noted previously, an abundance of sex organs are produced fairly promptly also on maize-meal agar plates on which have been planted pieces of of infected tissue. The fungus growth surrounding such plantings is naturally heavily contaminated with bacteria. Since in cultures

on the same medium but free of such contamination, oogonia and oozpores are much slower in appearing, it is probable that the pro-

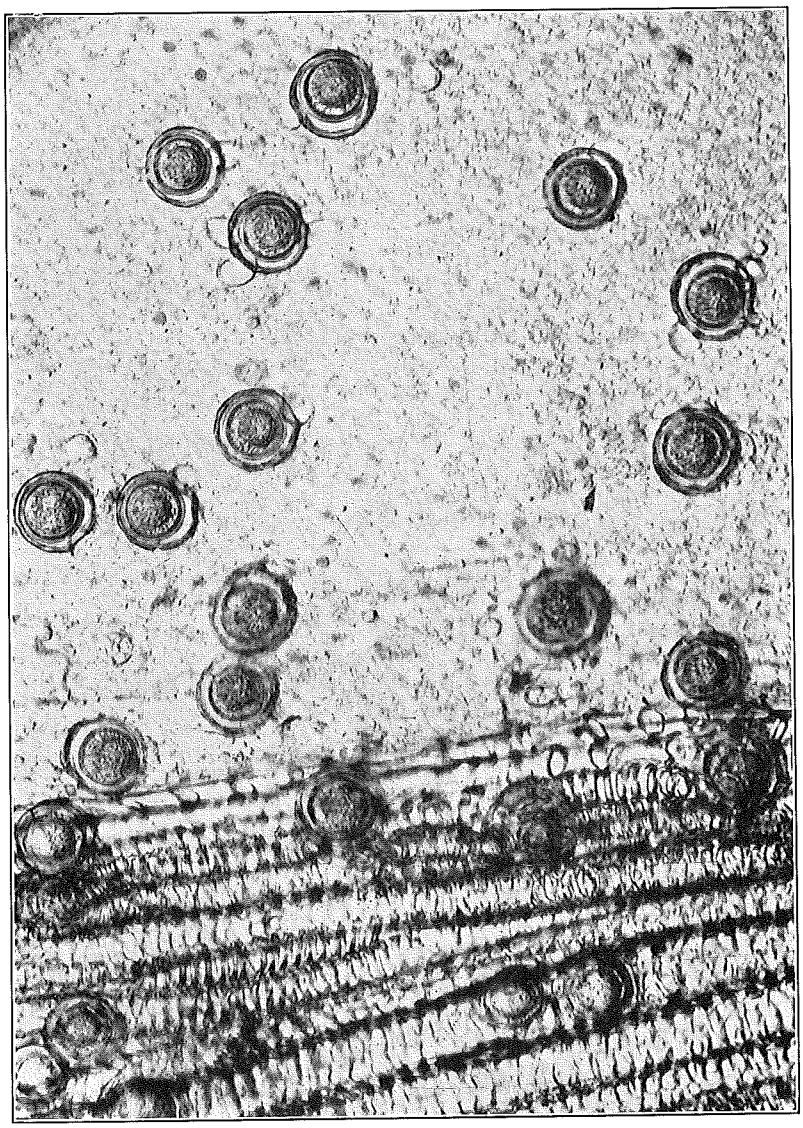

FIGURE 2.—Sexual apparatus of *Aphanomyces cochlioides* in the cortical tissue of a beet seedling which had been kept partly immersed in water for six days after it had damped off as a result of invasion by the fungus. The outlines of the cortical cells are only vaguely indicated, and the hyphal connections of both oogonia and antheridia are entirely lost. The thick oogonial envelope is shown rather clearly in most instances, as is also the oospore with its uniformly normal structure of wall and contents. × 440

duction of the sexual apparatus in nature is not without some relation to the processes of decomposition brought about by secondary organisms.

With respect to the orientation of the sex organs on the mycelium, *Aphanomyces cochlioides* shows general similarity to *A. euteiches* and *A. raphani*. The oogonium is borne terminally on a branch that usually is relatively short, its length often scarcely exceeding one-half the diameter of the supported female organ. (Fig. 3, B–G; fig. 4, B, C, E, G, H.) Near the origin of the oogonial stalk the parent hypha may bear a number of diverticulate branches or short protuberances, which generally engage the filament bearing the antheridial branches, the latter filament being similarly provided with diverticula by means of which reciprocal engagement is effected. (Figs. 3 and 4.) Aside from the enveloping disposition of the antheridial branches about the oogonium, firm contact is thus established without such spiral involvement of supporting elements as occurs often in the forms described in this paper as *A. camptostylus* and *A. cladogamus*. The sex organs are evidently regularly borne on separate parent hyphae, no androgynous condition ever having been observed. Occasionally the antheridia are supplied from two parent hyphae (fig. 3, E), but as a rule the male elements are all contributed by a single filament.

The further development of the oogonium is closely similar to that of the corresponding structure in *Aphanomyces euteiches*. The basal septum delimiting the female organ from its stalk, however, does not often protrude much into the oogonial cavity. Indeed, the greater completeness with which this cavity is occupied by the oospore leaves insufficient room for any prominent columellalike modification, although less pronounced instances occur fairly frequently. (Fig. 4, C, H.) The oogonial wall, as in the pea parasite, is represented by a thick envelope varying in thickness by virtue of irregularities in the inner contour. But whereas in *A. euteiches* its thickness sometimes considerably exceeds that of the oospore wall, in the beet fungus it maintains a more consistent approach to equality, the irregularities in the inner contour being considerably less pronounced than in the congeneric form.

The oospores of *Aphanomyces cochlioides* resulting from normal development become surrounded with walls of uniform, moderate thickness, the latter dimension being approximately equal to one-sixth or one-fifth of the radius of the spore itself. The contents, which in earlier stages consist of large granules occupying the entire oospore cavity without noticeable orientation, later become differentiated with the appearance of a spherical reserve globule that increases in size until in mature specimens its diameter is approximately three-fifths that of the oospore. At this stage the globule in question is somewhat eccentrically placed, being separated from the oospore wall on one side by two layers or even a single layer of granules, and on the other by two to four layers. The peripheral granules exhibit orientation suggestive of geometrical regularity. Embedded in the thicker portion of the granular layer is to be distinguished a strongly refringent body, subspherical or oblate ellipsoidal in shape, and evidently homologous to that described for *Plectospira myriandra*. In coloration the oospores show considerable variability, depending apparently on the character of the substratum. On maize-meal agar, for example, a practically colorless condition is common, while in disintegrating beet seedlings a bright-yellow or even golden colora-

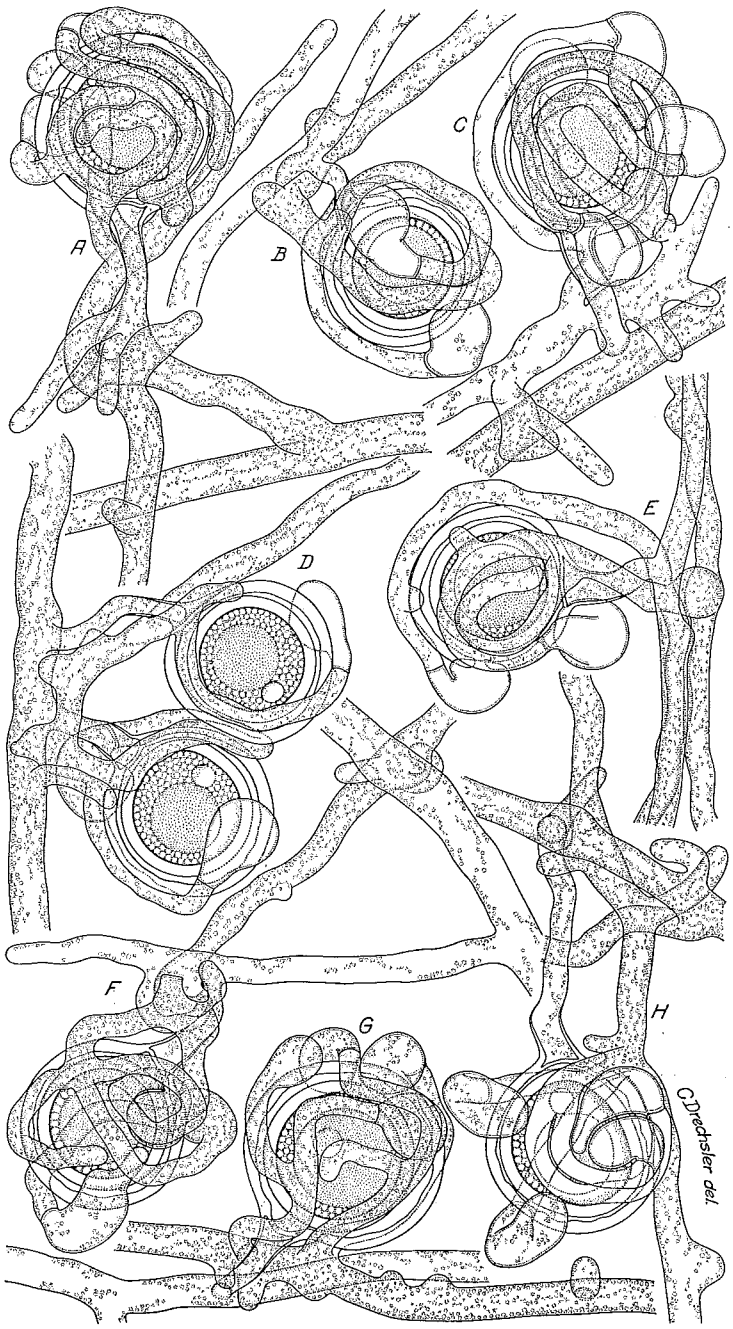

FIGURE 3.—Sexual apparatus of *Aphanomyces cochlioides*. A to H, Drawn from maize-mea agar cultures with the aid of a camera lucida. × 920

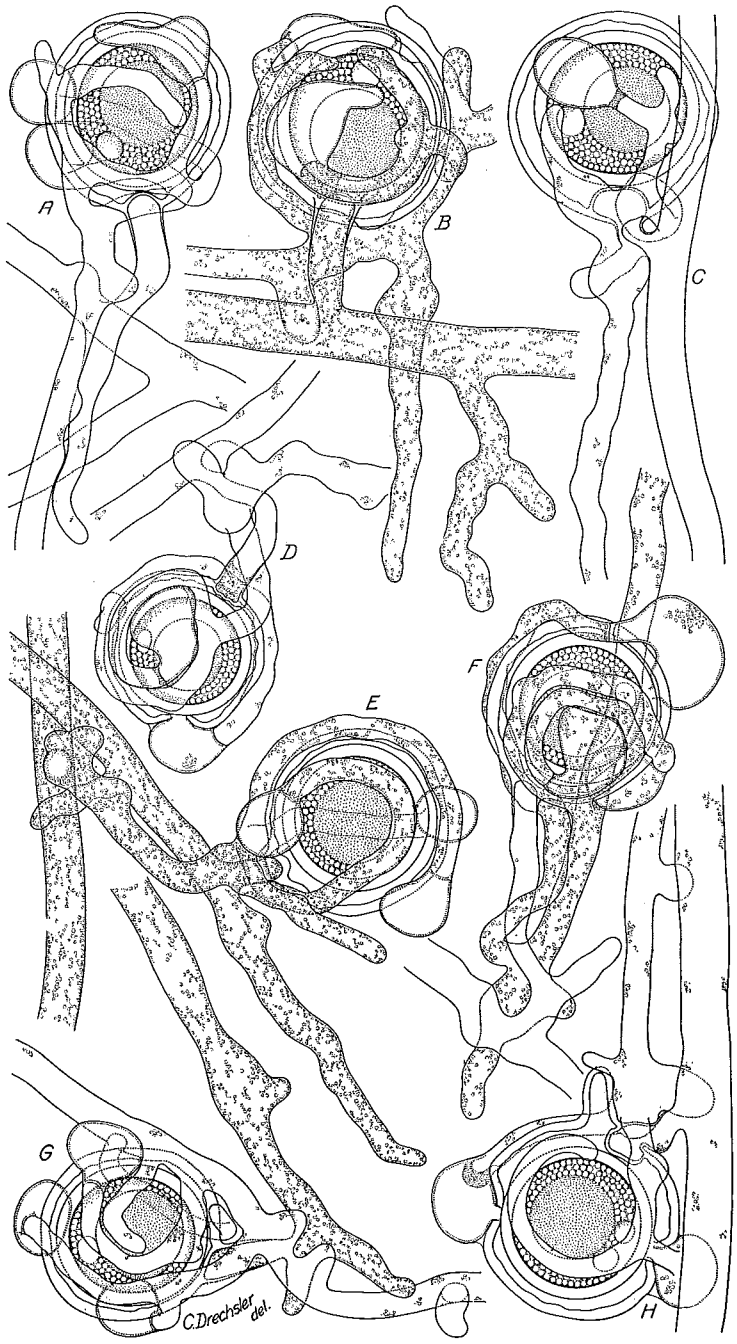

FIGURE 4.—Sexual apparatus of *Aphanomyces cochlioides*. A to H, Drawn from maize-meal agar cultures with the aid of a camera lucida. × 920

tion has been observed, having been brought about evidently by the concentration of pigment, especially in the oospore wall.

A rather intricate aspect is given to the sexual apparatus of *Aphanomyces cochlioides* because of the envelopment of the oogonium by the antheridial branches, to which reference has already been made. On the limited surface of the female organ are accommodated the somewhat rangy filamentous elements bearing terminally the three or four antheridia often present, and frequently in addition several sterile branches of variable lengths arising from antheridial branches or directly from the parent hypha of the latter. In many cases an involved arrangement results, the distal portion of the antheridial stalk being coiled on itself, with the male organ occupying a central position in the flat spiral thus formed. The antheridia themselves are inflated structures of ample size which in profile are seen to be, as it were, partly countersunk into depressions in the indurated oogonial envelope, as indeed, though less conspicuously are sometimes also the supporting branches. As the fertilization tube arises at the center of the depression where the oogonial wall is almost if not actually in contact with the oospore, it remains usually a relatively short structure, its length hardly exceeding the thickness of the oogonial wall. (Fig. 4, D, H.) Occasionally the antheridium is drawn out at the apical end into a short distal prolongation. (Fig. 3, F, H; fig. 4, A, B.) However, this modification is also present in other species of the genus, being especially well developed and frequent in *A. cladogamus*.

TAXONOMY

In spite of certain disturbing considerations, the available evidence is not too seriously at variance with the presumption that the water mold found parasitic on sugar beets in Michigan is specifically identical with the German form discussed by Peters. That author's statement that when diseased seedlings are put into water, mycelium develops only from the cotyledons, may be plausibly interpreted to mean that he regards the more extensive branching hyphae that usually grow out of the cotyledons as being essentially vegetative and the mostly unbranched elements growing out of stem and roots as constituting zoosporangia. In view of the almost complete transformation of actively growing mycelium into sporangial units, in this as in congeneric species, on the intervention of suitable conditions, such a distinction would appear somewhat difficult to maintain. If a distinction is to be recognized it would seem more appropriate to regard the unbranched extramatrical filaments from the stem mainly as evacuation hyphae, and the more profuse growth from the cotyledons as including also the extensive ramifying elements that constitute the bulkier portions of individual zoosporangia in the species generally. The diameters of mycelial hyphae given by Peters as 8 to 14 μ are more difficult to reconcile with the morphology of the American beet parasite, as they would seem to pertain to an appreciably coarser fungus. Measurements of filaments that have collapsed as a result of the withdrawal of the protoplasmic contents might yield such high values, but the suspicion that collapsed hyphal membranes may not have been distinguished from living hyphae can only be suggested. Satisfactory agreement, however, prevails between the two forms with reference to the habit of branching, giving as it does an impression of

rigidness. As this feature, as well as various others pertaining to mycelium, zoosporangia, and zoospores, is common to a number of congeners, it can hardly be regarded as of much taxonomic importance.

Beyond the information that several thin-walled antheridia, which appear devoid of contents during later stages, are applied to the oogonium and cover a large part of it, Peters gives little detail concerning the make-up of the sex apparatus. These details could hardly have been made out in the natural substratum, owing to the obscuring effect of the host tissue. As has been shown, investment of the oogonium in considerable measure by the antheridia in conjunction with antheridial stalks and associated sterile branches prevails regularly in the Michigan beet parasite. Among the groups of congeneric forms having smooth oogonia it would appear, however, that investment by antheridia and their hyphal connections constitutes a minor feature only in *Aphanomyces raphani* and *A. gordejevi* Skvort.; in the latter case, judging from Skvortzow's figures (*29: figs. 11 and 12*), as a result not alone of the paucity of male organs but also of their narrow apical contact with the female organ suggestive more of contact relationships encountered in the genus Pythium. Since rather extensive investment prevails in *A. euteiches*, *A. camptostylus*, and *A. cladogamus*, as well as presumably in *A. helicoides* and *A. laevis*, it can not be considered a feature especially distinctive of a particular species.

Peters's account (*25*) probably devotes more attention to the sizes of oogonia and oospores than to any other morphological character. In Table 1 the measurements derived from the German fungus on the natural substratum are summarized in connection with values obtained from the Michigan parasite both on the natural substratum and on artificial media.

TABLE 1.—*Comparison of certain measurements of Aphanomyces cochlioides with those reported by Peters for the Aphanomyces species parasitic on sugar beets in Germany*

Fungus measured	Oogonium				Oospore			
	Diameter (μ)		Thickness of wall (μ)		Diameter (μ)		Thickness of wall (μ)	
	Range	Average	Range	Average	Range	Average	Range	Average
German fungus	[a] 18.7–25.5	21.8	Thin to 1.5	------	[b] 14–22.1	18	5–6	------
	Not measurable.	------	Thin, collapsed.	------	15.2–22.8	18.5	----------	------
	20–27	23.5	Somewhat thickened.	------	15–24	20.2	----------	------
Michigan fungus:								
Corn-meal agar	20.2–28.6	24.1	1.1–2.4	1.6	25.8–23.6	19.1	1.3–2.0	1.7
Beet seedling in water	20.1–28.9	23.8	1.1–1.8	1.4	15.6–21.8	18.2	1.3–1.7	1.5

[a] Rarely 30 μ. [b] Rarely 23 μ.

A satisfactory agreement with respect to the diameters of the oospores is evident, and with respect to the diameters of the oogonia the difference is not greater than differences often appearing in measurements of different lots of material. On the other hand,

comparisons of the thickness of the oogonial and the oospore envelopes reveal marked disparity. In the German account the wall of the female organ was described as occasionally being so thin as to occur in a collapsed condition, in which event the oospore was provided with a wall only 3 μ thick, while oogonia with walls 1.5 μ thick bore oospores with walls 5 to 6 μ thick. As even the minimum given for the thickness of the oospore envelope is well above the maximum revealed in American material, a serious morphological difference might be supposed to exist here, provided both sets of measurements could be regarded as having been made on structures in comparable condition.

It would appear very questionable, however, whether the structures on which the measurements were based were quite comparable. In a previous publication (*20*) the explanation was suggested that Peters's highly variable and rather extreme findings relative to the thickness of the oospore wall may have been based on measurements of spores that had undergone the degenerative changes incident to death of the protoplasmic contents. Subsequent observations have supported that explanation. Examination of beet seedlings that had succumbed to the attack of the Michigan fungus often showed degeneration so prevalent that not a single normally developed sexual apparatus could be found among the several hundred present. In many of the oogonia the contents had disappeared completely, and in others they had become collected in amorphous lumpy masses, or were present in coarsely granular form, darker and less transparent than the normal contents. When oospores had been formed, analogous degenerative conditions were abundantly represented in them, often being associated with conspicuous swellings of the oospore walls.

Although in the absence of figures illustrating the make-up of its sex apparatus the water mold dealt with in Peters's publication can hardly on morphological grounds alone be definitely pronounced identical with the American parasite, it would be equally or even more difficult to assert its independence as a separate species. The knowledge that is available concerning the prevalence of water molds as parasites of the higher plants is greatly in favor of the same fungus being concerned in the two countries. The number of saprolegniaceous forms associated with diseases in flowering plants are so few that the several established instances may well be looked upon as somewhat anomalous. With more adequate attention being given to root troubles it can be expected that the number may be considerably augmented, the report by Sideris (*28*) of the association of pineapple wilt in Hawaii with several strains of Aphanomyces providing a recent increment; yet it is hardly probable that cases of a particular crop plant harboring two parasites of such exceptional affinity will soon become frequent. Although, to be sure, the parasitism of *Plectospira myriandra* and *A. cladogamus* on the tomato (*Lycopersicum esculentum* Mill.) constitutes precisely such a case, the presumption against the occurrence of two distinct saprolegniaceous forms on beets as causal agents of the same type of disease would, nevertheless, appear to be strong.

In any case the parasite attacking beets in Michigan is evidently not to be identified as *Aphanomyces laevis*. The oogonia of both forms, it is true, are spherical and smooth, and the measurements

given by De Bary for the diameters of those structures in *A. laevis* ($\frac{1}{86}$ to $\frac{1}{72}$ line) are not incomparably larger than the measurements obtained by the writer. However, judging from De Bary's illustrations, the oogonial wall of *A. laevis* is a relatively thin membrane, the oospores are considerably inferior to the female organs in size and the antheridia are androgynous (*2: fig. 17*) as well as diclinous (*2: fig. 18, a and b*) in origin and of an elongated vermiform shape with ventral lobulations. In descriptions under the same binomial, but based, in part at least, on independent studies by later writers, essentially the same morphological characters are cited. Thus in Humphrey's figures (*19: pl. 20, figs. 105 and 106*) the oogonial wall is represented as a single-contoured membrane separated from the oospore by a wide space, while the "clavate cylindric" antheridia "on short branches of androgynous or diclinous origin, sometimes even from the same branch" appear as longish wormlike structures. In Coker's figures (*5: pl. 55, figs. 3 to 7*) the oospore is revealed as scarcely any larger in proportion to the oogonium, and the latter is again drawn with a single-contoured wall; while the antheridia, "large, abundant on all oogonia and extensively wrapping them about" would seem in some instances so little inflated as hardly to exceed in diameter the "androgynous or diclinous" branches supporting them. The androgynous or diclinous origin of the antheridial branches is mentioned also in Minden's account (*23*). Quite at variance with the foregoing characterizations, the beet parasite exhibits an oogonial wall of considerable thickness, an oospore that leaves rather little unoccupied space within the oogonium, and short, strongly inflated antheridia borne on branches that are regularly of diclinous origin.

In the main the same distinctions would apply also in considering the relation of the sugar-beet parasite to *Aphanomyces helicoides* Minden, since the latter, according to the original description differs little from *A. laevis*, except that its antheridia and the hyphal elements supporting them display a strong helicoid tendency. This tendency is expressed in Minden's species in part by the antheridial branches together with the antheridia being wrapped spirally about the oogonium or applied to the latter in a helical coil the resulting arrangement being evidently not greatly unlike that obtaining in the case of the beet parasite. However, in addition, the antheridial branches of *A. helicoides* are described as being coiled about one another for longer stretches, or about hyphae, even when the latter do not bear oogonia, thus becoming massed in dense tangles in proximity to the female organs and their stalks. Such helicoid involvement of hyphal elements is apparently not characteristic of the beet parasite, the engagement of filaments bearing the male and the female components of a sexual apparatus being effected here, as in *A. euteiches* and *A. raphani*, through firm contact facilitated often by the presence of diverticulate proturberances. It may be added that with respect to diameter of the oogonium, *A. helicoides* would seem to represent a somewhat larger species, Minden's measurements for this dimension being 23 to 38 μ.

Morphological differences between the beet parasite and *Aphanomyces euteiches* are readily distinguished when the two organisms are grown on a suitable medium like maize-meal agar. As is indicated in Table 2, the oogonia and oospores of *A. euteiches* are considerably

larger than the corresponding structures of the other. The disparity is apparently most pronounced with respect to the maximum diameter of the oogonium, which in the pea parasite is approximately half again as large as that in the congeneric form. While the minimum values for this dimension as well as the minimum diameters of oospores are approximately equal for the two species, the averages for the linear dimensions of the structures in question nevertheless reveal a superiority of the pea parasite equivalent to about one-third of the values pertaining to the smaller form. Among other peculiarities separating the beet parasite from *A. euteiches* may be mentioned the crowded, frequently cochleate, arrangement of antheridial branches and antheridia on the oogonium prevailing in the former, as compared with the freer disposition usual in the latter. Moreover, the male organs of the beet parasite are less inclined toward irregularity, rarely being subject to extravagant development resulting, for example, in two inflated lobes occurring in series on the same supporting branch, or in structures arched after the manner of a measuring worm.

TABLE 2.—*Summary of measurements of oogonia and oospores of various root-inhabiting water molds grown in maize-meal agar plate cultures*

Name of fungus	Oogonia				Oospores					
	Diameter (μ)		Thickness of wall (μ)		Diameter (μ)		Thickness of wall (μ)		Diameter of reserve globule (μ)	
	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average
Aphanomyces camptostylus	19.4–26.1	22.9	0.6–1.2	0.9	16.3–21.2	18.8	1.1–2.0	1.5	9.0–13.2	11.0
Aphanomyces cochlioides	20.2–28.6	24.1	1.1–2.4	1.6	15.8–23.6	19.1	1.3–2.0	1.7	9.4–14.0	11.8
Aphanomyces cladogamus	18.8–33.0	26.8	.8–1.9	1.3	15.3–25.6	21.8	1.2–2.0	1.5	9.0–16.6	14.0
Aphanomyces euteiches	19.2–41.7	31.8	1.2–4.1	2.0	14.6–30.8	25.4	1.2–2.7	1.7	8.5–20.8	15.2
Aphanomyces raphani	26.5–51.5	38.3	1.1–2.6	1.6	18.9–39.1	28.1	1.2–2.6	1.8	12.8–28.6	18.5
Plectospira myriandra:										
Parthenogenic	21.3–29.8	25.2	.5–1.0	.7	18.7–26.6	22.1	1.1–1.6	1.5	11.8–17.5	14.5
With antheridia	23.0–35.6	28.6	.5–1.0	.7	20.7–31.9	25.3	1.2–1.9	1.5	13.2–19.2	16.1
Plectospira gemmifera	21.7–28.5	25.2	.5–1.0	.7	19.0–24.9	21.9	1.1–1.8	1.5	9.6–13.2	11.6

The inferiority of the beet parasite with respect to the linear dimensions of oogonia and oospores is even greater when the fungus is compared with *Aphanomyces raphani*. Similarly, since in the radish parasite an appreciably smaller proportion of the oogonial surface is occupied by male organs and their attachments, those structures are less crowded on the oogonium. The mycelium of the beet fungus is finer than that of *A. raphani*, of considerably faster growth, and not given to the production of relatively intricate, closely branching systems of hyphae.

As has been suggested previously, the positional relation of the antheridium to the oogonium illustrated in Skvortzow's (*29*) figures of *Aphanomyces gordejevi* would seem different from any species of

Aphanomyces that the writer has had occasion to examine. The description of the sporangium as "fadenförmig, von den vegetativen Faden nicht zu unterscheiden, mit seitlicher, kurzer Entleerungspapille," in the absence of any details concerning the mode of discharge or the process of zoospore formation, contributes little toward establishing the taxonomic status of the fungus. In any case the small diameters of its hyphae (1.8 to 4 μ) as well as the inferior diameters of its oogonia (13 to 17 μ) indicate a fungus of generally smaller dimensions than the beet parasite or any of the related forms discussed in this paper.

From the foregoing considerations it is apparent that the beet parasite presents morphological departures from the descriptions of the several congeneric species, the oogonia of which are regularly without protuberances. It was accordingly designated as a new species (*9*), the specific term "cochlioides" being intended to characterize the flat involute arrangement of the antheridium and the antheridial branch on the oogonium unaccompanied by helicoid involvement of filamentous parts with one another. A more formal description of the species may perhaps not be inappropriate here.

DESCRIPTION

Mycelium 3 to 9 μ in diameter, not given to abrupt fluctuations in thickness, sparingly or moderately branched, capable of extramatrical and even moderate aerial development. Sporangia formed by the direct transformation of vegetative mycelium, the delimiting septa occurring as plain or irregular cross walls or as more massive plugs; sometimes very extensive, including segments of axial hypha more than 3 mm. in length together with numerous ramifying elements, then provided with plural evacuation tubes; the latter varying usually from 0.1 to 3 mm. in length, often considerably contorted or with more regular Polythrinciumlike modification over longer stretches, frequently narrowing toward tip but not markedly attenuated, discharging from a few to over 300 zoospores. Zoospores on encystment after delivery 6 to 15 μ in diameter, usually 7 to 10 μ; diplanetic, developing a papilla approximately 2 μ in diameter, the cylindrical wall of which persists on the empty cyst membrane. Oogonium terminal on usually short branch, subspherical, smooth, usually 20 to 29 μ (average 24.1 μ) in diameter, provided with a wall of somewhat irregular inner contour 1.1 to 2.4 μ (average 1.6 μ) in thickness; the oogonial stalk and parent hypha making intimate contact with the antheridial branches, as well as with the one, or more rarely, two hyphae bearing them. Antheridia usually up to four, rarely up to five, in number, 6.5 to 10 μ in diameter, 9 to 18 μ in length, often curved, delimited from the stalk by a septum or plug inserted close to juncture of expansion and cylindrical filament, occasionally drawn out at apex into a narrow prolongation; frequently partly countersunk into an abrupt depression in the oogonial membrane, which is regularly perforated at the apex of the depression by the short fertilization tube; borne terminally on branches 2.2 to 3.8 μ in diameter, one or several in number, regularly of diclinous origin, which may bear one or more secondary branches terminating blindly or in another male organ; the branches together with the antheridia extensively wrapped about the oogonium, often being applied to the latter in involute cochleate disposition. Oospore single, subspherical, nearly colorless to deep yellow, 16 to 24 μ (average 19.1 μ) in diameter, surrounded by wall 1.3 to 2 μ (average 1.7 μ) in thickness, containing at early maturity a somewhat eccentrically placed reserve globule 9 to 14 μ (average 11.8 μ) in diameter. Destructive to seedlings of sugar beets (*Beta vulgaris* L.) near East Lansing and Saginaw, Mich.

PATHOGENICITY

The repeated isolation of *Aphanomyces cochlioides* from numerous specimens, noted in another connection, supplied insistent evidence of the pathological rôle played by the fungus in the causation of damping off of sugar-beet seedlings. To be sure, in many instances

the individual specimens yielded also other fungi known to be capable of producing substantially the same symptoms, and it may not unreasonably be assumed that these concomitant types were in a considerable measure primarily responsible for the damage observed. For, as in other examples of destruction from damping off or root rot in which an assortment of forms participate, it would seem that any one of the forms, whether favored by environmental conditions or aided by chance, might inaugurate the attack on the individual plant and perhaps retain the rôle of primary pathogene to the end. That rôle, however, apparently need not exclude the other forms, one or more of which may thus become well established in the affected tissues as secondary invaders, though capable in greater or smaller measure of performing, and, indeed, in other specimens actually performing, the part of primary parasite. It appears probable that the various species of Pythium and the different strains of Rhizoctonia are much more apt to be found occurring in such ambiguous biological relation than species of Phytophthora or Aphanomyces. However, with respect to the relation of *A. cochlioides* to damping off of beet seedlings under field conditions, it was fortunately not necessary to rely upon conjectures concerning the relative probability of this or that fungus representing the chief destructive agent, since an adequate proportion of the specimens yielded the fungus in question without any accompanying organisms to which pathological significance could be attributed.

The pathogenicity of *Aphanomyces cochlioides* was moreover demonstrated experimentally by several tests carried out in the greenhouse in series parallel with similar tests of six related forms (*A. euteiches*, *A. raphani*, *A. cladogamus*, *A. camptostylus*, *Plectospira myriandra*, and *P. gemmifera*). Seven-inch pots sealed at the bottom to prevent the entrance of foreign organisms were filled with sand, sterilized in an autoclave under pressure, and seeded to sugar beets, 35 seed balls being used to each pot. At the time of sowing and again on the fourth day thereafter, approximately 2 gm. of a maize-meal agar culture of each of the different fungi were incorporated in the upper inch of sand, in about a dozen small portions distributed as evenly as possible, the purpose of the later addition being to provide against the contingency of the fungus growth first added being acted upon unfavorably by contaminating forms and thus being rendered relatively inactive before the seedlings could become available for infection. The pots thus treated as well as a set of control pots to which no fungus was supplied were kept liberally though not excessively watered with sterilized water. As the individual seedlings damped off or revealed obvious injury they were carefully removed from the pot and taken to the laboratory for determination of the pathogene concerned. The routine for the determination consisted in placing the washed specimen in a shallow layer of water for 24 to 48 hours, examining it for the presence of the zoosporangial stage of the water mold involved, then removing it to filter paper where the free liquid was thoroughly blotted off, and planting it on maize-meal agar plates. During the ensuing week any resulting growth was examined in order to determine the presence or absence of other pathogenic types, as well as to establish the identity of the water mold present through the morphology of the sex apparatus produced.

The positive results from one of the parallel series of tests are given in Table 3. None of the pots to which had been added either *Aphanomyces raphani, Plectospira myriandra,* or *P. gemmifera* developed any cases of seedling injury except those attributable to *Phoma betae.* As it had not been deemed advisable to subject the seed to treatment with disinfectants, the last-named fungus made its appearance in all the series of pots, including the controls, causing a loss of two to five seedlings in each pot. In the particular series to which Table 3 refers, *A. euteiches* gave no indication of pathogenicity, although in another series its presence was discovered in two affected seedlings, in both instances, however, in association with *Phoma betae.* The strong presumption in favor of the latter fungus having been the primary causal agent leaves the performance of the pea parasite in the origin of beet-seedling troubles a negligible one.

TABLE 3.—*Results of inoculating sugar beets with parasitic species of Aphanomyces*

[Thirty-five seed balls sown Jan. 11, 1928, in each of the four pots in each set; soil autoclaved Jan. 11; 2 gm. of inoculum added Jan. 11 and again Jan. 15]

Inoculating fungus	Number of seedlings succumbing on dates shown															Seedlings surviving
	Jan. 21	Jan. 22	Jan. 23	Jan. 25	Jan. 27	Jan. 28	Jan. 30	Jan. 31	Feb. 2	Feb. 5	Feb. 7	Feb. 9	Feb. 11	Feb. 13	Total	
Aphanomyces cochlioides	5	11	12	14	13	7	4	13	11	18	9	14	21	4	156	1
Aphanomyces cladogamus	3	5	15	13	7	5	0	2	2	0	0	0	0	0	52	108
Aphanomyces camptostylus	4	10	6	13	10	0	0	1	0	3	0	0	0	0	47	111

In the pots to which *Aphanomyces cladogamus* and *A. camptostylus* had been added, damping off began to be manifested simultaneously (on the tenth day after sowing) with the appearance of the disease in the pots containing the beet parasite. For approximately a week the other two fungi displayed an aggressiveness scarcely less than that of *A. cochlioides.* During approximately another week their activity, though greatly reduced, was still noticeable, but later it ceased entirely. On the other hand, *A. cochlioides* continued to operate seemingly with undiminished efficacy, until 43 days after sowing only one healthy seedling survived its attack, and the experiment was necessarily terminated from lack of a proper supply of host plants. A sustained aggressiveness as a parasite of sugar beets was revealed by *A. cochlioides* in every series of tests undertaken. The inferiority in pathogenicity to beet seedlings evidenced by *A. cladogamus* and *A. camptostylus,* obtained, as these fungi were, from hosts with no close affinity to the beet, is, of course, far less at variance with expectations usual under the circumstances than their manifestation of any pronounced pathogenicity whatever.

The necessity of appropriate restraint in the interpretation of positive results ensuing from inoculation tests carried out with root fungi under usual experimental conditions is not too obvious to merit special emphasis. That the more tender growth encouraged by cultivation in a greenhouse is generally more amenable to infection is an opinion rather widely held. In addition, certain operations demanded by the exigencies of experimental study, as, for example,

soil sterilization with resultant obliteration not only of the extraneous competing forms, but also of the entire microflora and microfauna, may readily operate to bring into unusual relief biological propensities that under natural conditions remain without manifestation. Specious demonstations of parasitism are only too easily possible and that in passable obedience to Koch's canons. In the absence of any evidence that *Aphanomyces cladogamus* and *A. camptostylus* occur on the sugar beet in nature, it is not intended, as far as the beet is concerned, to assert for these fungi any status as parasites comparable with that of *A. cochlioides*.

APHANOMYCES CLADOGAMUS, N. SP.

INTRODUCTION AND HISTORY

In a previous paper (*8*) the writer reported the isolation from diseased tomato rootlets of a species of Aphanomyces that was provisionally identified as *A. euteiches*. The identification was based on similarities in the aspects and branching habits of the mycelia, on fair correspondence in the dimensions of oogonia and oospores, and more particularly on the thickening of the oogonial membrane, a characteristic then not known to occur in members of the genus other than the pea parasite. With the discovery of a thickened oogonial covering in the obviously distinct *A. raphani* and *A. cochlioides*, the species from the tomato was compared more closely with *A. euteiches*, with the result that rather definite morphological dissimilarities were revealed.

MORPHOLOGY OF SEXUAL APPARATUS

Probably the most distinctive of these differences is found in the origin of the antheridia, which in the fungus under consideration may be monoclinous or androgynous as well as diclinous. The one or several branches bearing the male organs may arise from the hyphal element supporting the oogonium, a condition frequent when that hyphal element constitutes in itself a filament of some length. (Fig. 5, A; fig. 6, C.) When the oogonium is borne on a short stalklike branch of the type usual in at least those members of the genus provided with smooth oogonia, the antheridial branches are more apt to have their origin from the same axial hypha as the oogonial stalk, and at variable distances from the latter. (Fig. 5, B, C, E; fig. 6, D, E.) The antheridial branches whether arising close to (fig. 6, D) or at some distance (fig. 5, C) from the oogonium frequently follow a somewhat circuitous course before making contact with the female organ. Even in such instances (fig. 5, C) the usual intimate contact relationship between oogonial stalk and antheridial branch may prevail. This relationship is frequently associated with helicoid involvement, which, however, is usually unlike that described for *A. helicoides*, being accomplished regularly by the winding of the oogonial support about the antheridial branch (fig. 5, D; fig. 6, A, E, F), although in instances so rare as to constitute exceptions the converse arrangement obtains. Often, especially when the origin of the antheridial branch is at some distance from that of the oogonial stalk, or when by virtue of a roundabout course the antheridial branch engages with the oogonium while directed toward the base of the latter, contact with the oogonial stalk may be brought about

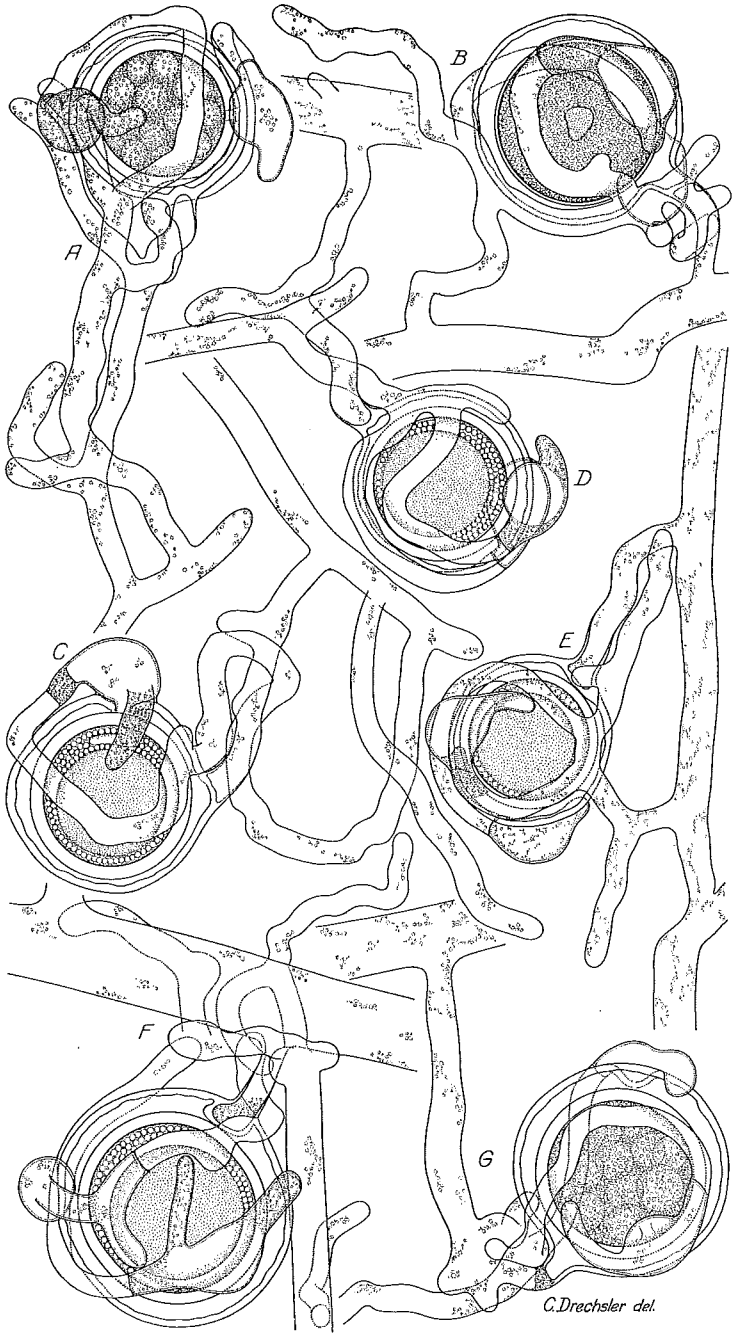

FIGURE 5.—Sexual apparatus of *Aphanomyces cladogamus*. A to G, Drawn from maize-meal agar cultures with the aid of a camera lucida. × 920. Antheridia of monoclinous origin in A, B, C, E; of diclinous origin in D, F, G. Oospores in a young condition with little evident internal organization in B; approaching maturity with reserve material in a number of globules in A, G; and in a mature condition in C, D, E, F

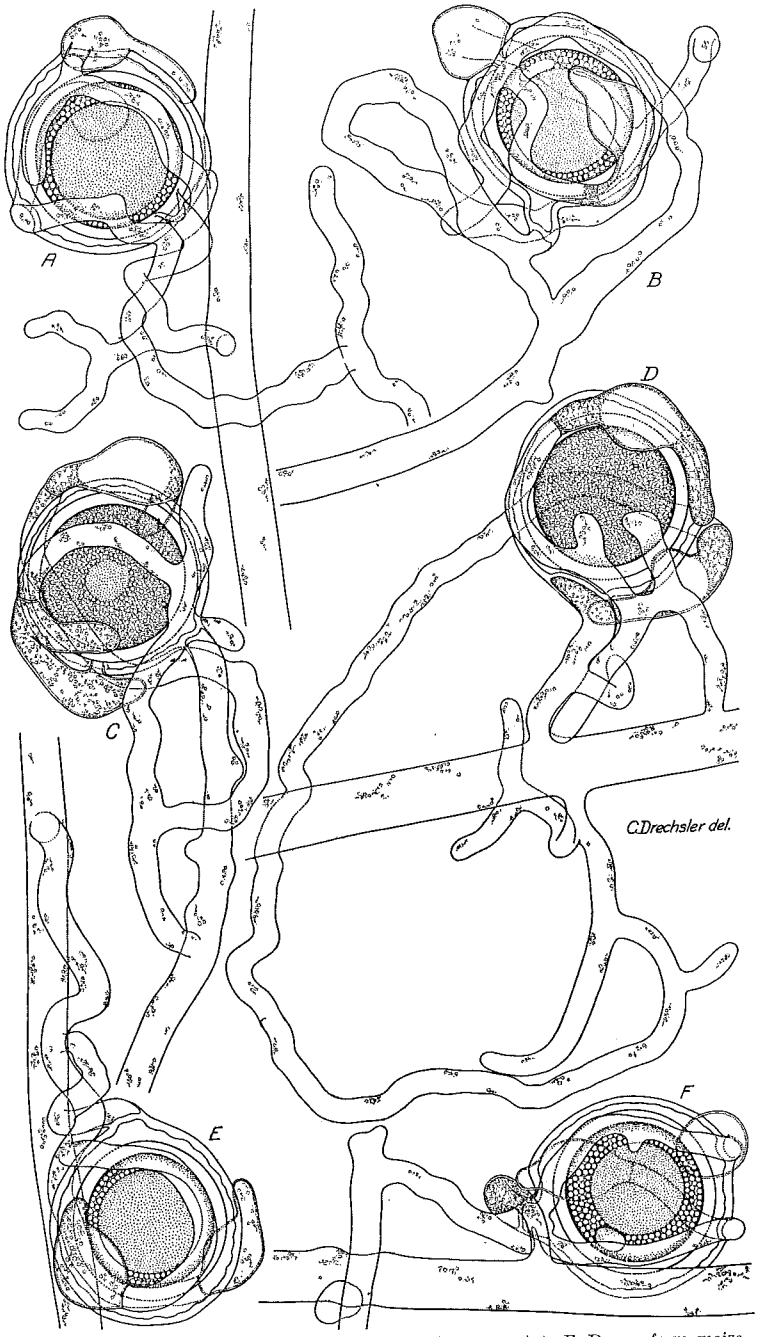

FIGURE 6.—Sexual apparatus of *Aphanomyces cladogamus*. A to F, Drawn from maize-meal agar culture with the aid of a camera lucida. × 920. In A, F, antheridia of diclinous origin; in B, D, E, of monoclinous origin. Oospores somewhat immature in C and D; mature in others

by the antheridial stalks becoming branched, one of the elements, usually the one that would appear to be the prolongation, passing downward along the female stalk, while the other bears a male organ. (Fig. 5, B, E; fig. 6, D.)

Although in the original description of *Aphanomyces euteiches* the diclinous origin of the antheridia was merely set forth as being typical, it may be mentioned that neither at the time the description was written nor subsequently has the writer ever observed in the sexual apparatus of the pea parasite antheridia of unquestionably monoclinous origin. To be sure, dubious cases equally capable of being interpreted as representing a monoclinous or a diclinous condition have been encountered among the hundreds that have been examined, yet in dealing with a complicated apparatus such examples can be assumed to occur with considerable frequency even when no male organs of monoclinous origin are actually present. At any rate, whenever the hyphal connections of the sex organs in *A. euteiches* could be clearly made out, the mycelial relation between oogonial and antheridial stalks was invariably too remote to be uncovered. In this respect the tomato-root fungus is widely different from the pea parasite, since in the former a monoclinous origin of the antheridia is very clearly exhibited in a large proportion of cases. The fungus reported from soil in North Carolina by Coker and Braxton (*6*) as *A. euteiches*, and illustrated in part as being provided with antheridia of androgynous origin, would seem, therefore, to have rather more similarity to the tomato parasite. The frequent occurrence of helicoid involvement of antheridial and oogonial branches in the tomato fungus and its absence in *A. euteiches* provide another obvious specific distinction that can be extended to apply also to *A. cochlioides* and *A. raphani*.

Although in the tomato-rootlet fungus under consideration the antheridial branches are wrapped about the oogonium for goodly distances, they are not usually quite so numerous, and they do not give off sterile secondary branches quite so freely as in *Aphanomyces cochlioides*. The crowding of male organs and their supporting filaments on the oogonium is consequently less pronounced, and an approach to a cochleate arrangement of these structures is only occasionally observed. A feature more noticeable in this than in congeneric species is the frequent insertion of the septum delimiting the antheridium at some little distance below the inflated part. As the male organ thus often comes to include at the proximal end a more than negligible portion of the filament which, besides, sometimes bears a spurlike branch, and at the distal end an apical prolongation of variable length, it represents a generally rangier structure than that of other species. (Fig. 5, F; fig. 6, B, C, D.) The occurrence of two antheridia in series as in *A. euteiches*, or of several successive ventral lobulations in the inflated part of a single antheridium, represented, for example, in De Bary's figures of *A. laevis*, has not been noted. In the mature sex apparatus the antheridia and also, though less conspicuously, the hyphae supporting them, are often found partly countersunk in abrupt depressions in the oogonium, much as in certain other forms with thick oogonial envelopes. (Fig. 5, C; fig. 6, A, B, F.)

As indicated in Table 2, the tomato-root fungus gives somewhat larger measurements for diameters of oogonium and of oospore than

Aphanomyces cochlioides, the difference, however, being too small to deserve emphasis in a discussion of specific distinctions. On the other hand, comparison with *A. euteiches* and *A. raphani* reveals differences relative to these dimensions of a more decisive magnitude. The oogonial envelope appears perceptibly inferior to the oospore wall in thickness.

ASEXUAL REPRODUCTION

The zoosporangial stage of the tomato parasite presents few peculiarities. The evacuation tubes that grow out of infected beet seedlings into water, or that are produced when mycelia grown in maize-seedling decoction are transferred to water, are usually stout filaments of considerable length. One of the smallest of these structures, comprising, however, the entire sporangium is shown in Figure 7, D, *a* and *b*. They show little or no tendency to taper toward the apex or to develop the Polythrinciumlike irregularities recorded for *A. raphani* and *A. cochlioides*. A feature of some interest that has been observed also in *A. camptostylus* is the frequent appearance on the more distal portion of the elements in question of several very short lateral diverticula. Although at times these remain closed (fig. 7, C, *b*), and hence play no part in the process of evacuation, at other times one or all of them are found to open at the tip exactly in the same way as the tip of the hypha itself and to permit the passage of escaping zoospores. (Fig. 7, B, *b*.) As the apices of the lateral stubs usually do not open until after a large number of zoospores have accumulated about the main orifice, it is possible that the supernumerary modifications serve the special function of providing alternative channels of egress when the terminal opening has become more or less obstructed.

The development of the motile stage takes place in the same manner as in other members of the genus. (Fig. 7, E, *a–g*.) It must be mentioned, however, that the writer's procedure which in other species yielded swimming zoospores in countless numbers, while not entirely unsuccessful with the fungus under consideration, yielded rather few motile spores in comparison with the vast quantities of encysted individuals present. Apparently the swimming stage in this form is not encouraged by quite the same conditions as in related forms.

TAXONOMY

Because of the morphological differences pointed out, it is evident that the tomato-root parasite is not identical with *A. euteiches*. Consequently, as far as the members of the genus Aphanomyces known to be parasitic are concerned, no grounds remain for suspecting that the cultivation of tomatoes in rotation with peas may be detrimental to the latter crop. Whether the pathogenicity on sugar-beet seedlings observed under experimental greenhouse conditions might be manifested under field conditions and in the presence of a natural soil flora, is problematical. The fungus appears worthy of recognition as an independent species, for which the term "cladogamus," descriptive of the frequent origin of the sex organs on coordinate branches of the same hypha, may not be inappropriate.

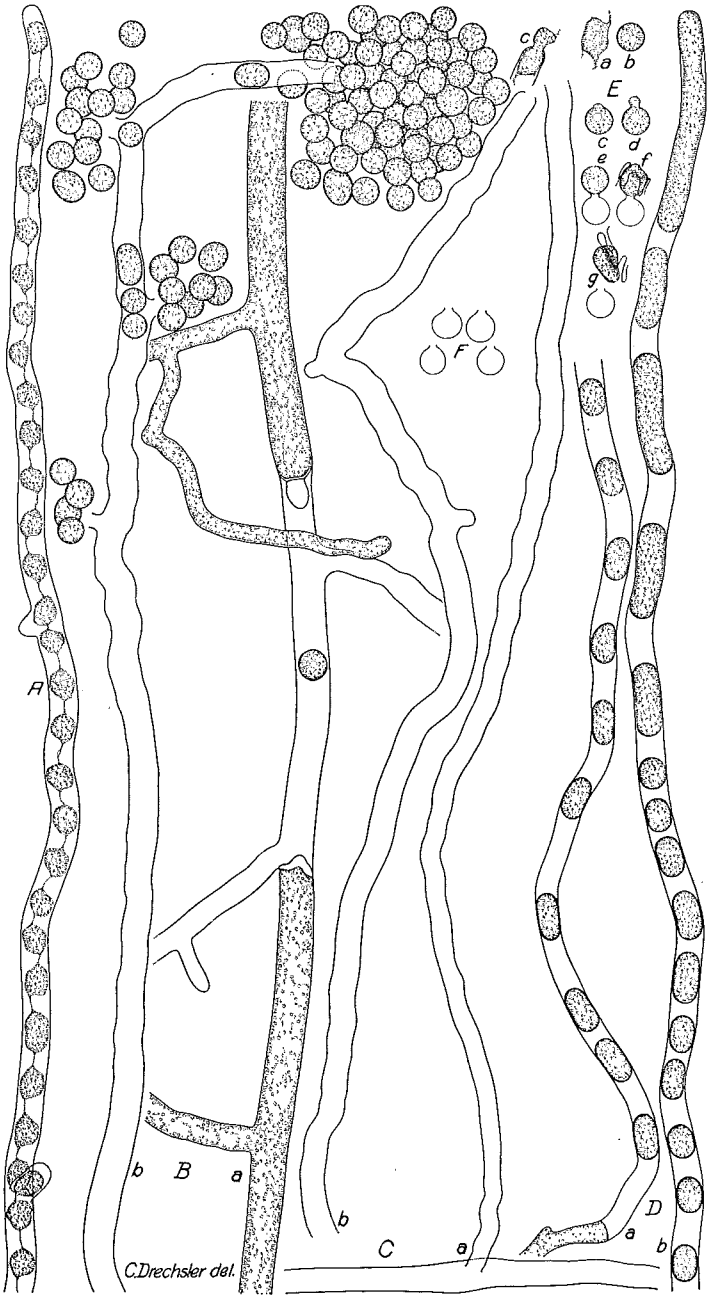

FIGURE 7.—Asexual reproduction of *Aphanomyces cladogamus*. All figures drawn with the aid of a camera lucida from material grown in maize-seedling decoction. × 460.

(For explanatory legend see opposite page)

DESCRIPTION

Aphanomyces cladogamus, n. sp.

Mycelium 3.5 to 10 μ in diameter, not given to abrupt fluctuations in thickness, sparingly or moderately branched, with moderate extramatrical and scant aerial development. Sporangia formed by the direct transformation of the vegetative mycelium, the delimiting septa occurring as narrow cross walls or more massive plugs; sometimes very extensive, including segments of axial hyphae more than 2 mm. in length with numerous ramifying elements, then often provided with plural evacuation tubes; the latter up to 2 mm. or more in length, usually stout, often maintaining diameters of 7 to 8.5 μ, not markedly narrowing toward the apex, often bearing on the distal portion up to four short, lateral protuberances which like the tip of the axial structure may open up to permit the egress of zoospores. Zoospores varying in number from a few to over 300 from a single orifice, on encystment usually 7 to 10 μ in diameter, diplanetic, developing a papilla 2 to 2.5 μ in diameter, the cylindrical wall of which persists on the empty cyst membrane. Oogonia terminal on short or longer branches, subspherical, smooth, mostly 19 to 33 μ (average 26.8 μ) in diameter, provided with a wall of somewhat irregular inner contour, 0.8 to 1.9 μ (average 1.3 μ) in thickness; the oogonial stalk regularly making contact with an antheridial branch and sometimes coiling about the latter. Antheridia two, or more rarely three, in number, consisting of an inflated part usually approximately 13 μ long and 10 μ in diameter, often together with an apical prolongation 3.5 to 4 μ in diameter and up to 13 μ or more long, and a similar proximal extension up to 18 μ long, and occasionally bearing a branch; the inflated part often sunk into an abrupt depression in the oogonial wall, which latter is perforated at the apex of the depression by the short fertilization tube; borne terminally on branches mostly 2.5 to 3.6 μ in diameter, one or two in number, of diclinous or monoclinous origin, often bearing one or more secondary branches that terminate blindly or in another male organ, usually somewhat depressed into the oogonium which they encircle somewhat extensively. Oospore single, subspherical, mostly nearly colorless, 15.3 to 25.6 μ (average 21.8 μ) in diameter, surrounded by a wall 1.2 to 2 μ (average 1.5 μ) in thickness, containing at early maturity a somewhat eccentrically placed reserve globule 9 to 17 μ (average 14 μ) in diameter. Mildly parasitic on *Lycopersicum esculentum* Mill., causing discoloration and death of rootlet tips in greenhouse in Washington, D. C., and under experimental conditions destructive to seedlings of *Beta vulgaris* L.

APHANOMYCES CAMPTOSTYLUS, N. SP.

ORIGIN AND PATHOGENICITY

The fungus to be discussed under the binomial *Aphanomyces camptostylus* was found included in an assortment of several dozen cultures representing mostly pythiaceous types received from F. R. Jones, who had isolated them from the underground parts of various wild and cultivated plants. According to the notes accompanying the cultures, the organism in question was derived from the root of an oat plant collected by M. B. Linford at Sauk City, Wis., June 5, 1924. Evidently because the isolations were made in the course of an investigation of disease in a crop not closely related to the grasses, no information was made available concerning the condition of the

EXPLANATORY LEGEND FOR FIGURE 7

A.—Distal portion of an evacuation tube immediately before discharge, showing two lateral protuberances.

B.—Portions of a sporangium, *a* representing a short segment of an axial filament on which the two branches constituting the main bulk of the sporangium have their origin, *b* representing the distal portion of an evacuation tube with spores massed before three lateral orifices as well as before the terminal orifice.

C.—Evacuation tube drawn in two sections, the proximal one, *a* showing origin from converted mycelial hypha, and the distal one, *b*, revealing two lateral protuberances which failed to open, all the zoospores consequently being discharged through the terminal orifice, which happened to be somewhat constricted, necessitating constriction of the zoospores in passing out, as is indicated in *c*.

D.—Evacuation tube constituting entire small sporangium, which failed to become evacuated, entailing the rounding up of the zoospores and some larger masses within.

E.—Successive stages, *a* to *g*, in the rounding up of a discharged protoplast, and the development of the motile stage from the encysted condition.

F.—Evacuated cyst membranes.

particular specimen utilized. It may be mentioned, however, that in certain seasons a yellowish, sickly appearance generally indicative of root trouble is not infrequently observed in oats growing on poorly drained land. The direct unfavorable effect of an excessive water supply may well be complicated here by the activity of soil organisms. In Denmark, for example, *Pythium debaryanum* was incriminated by Gram and Rostrup (*16*) as the cause of root blight of oats, while more recently Beaumont (*3*) found the same parasite implicated in the causation of damping off in that crop in England, plants that recovered from the earlier attack remaining backward throughout the season.

It is not intended in the present account to deal with the question of the probability of a biological relationship that may have obtained

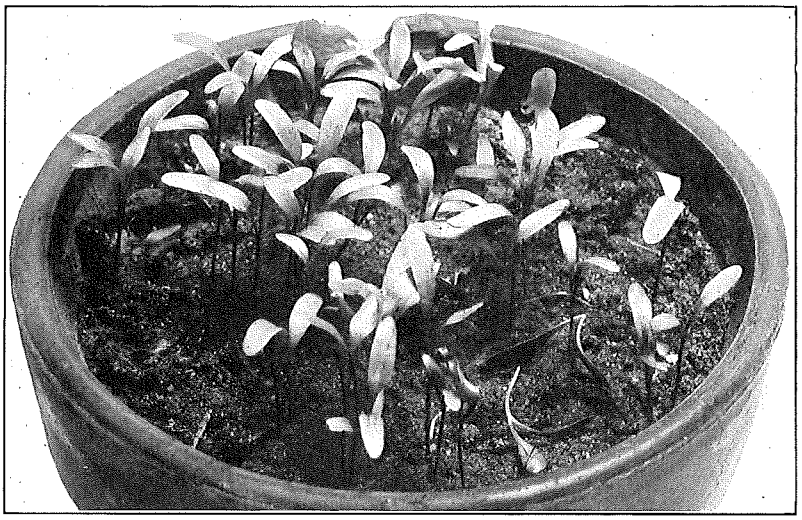

FIGURE 8.—Sugar-beet seedlings in a pot of sterilized soil to which was added at the time of seeding, and again four days afterwards, 2 gm. of a maize-meal agar culture of *Aphanomyces camptostylus*. Photographed 17 days after seeding to show 5 plants that had succumbed to attack by the fungus, which was subsequently reisolated from the affected tissue of each diseased specimen. × ½

between the fungus and the host material from which the isolation was made. Less uncertainty attaches to the pathogenicity of the organism to sugar-beet seedlings, since under the conditions of the inoculation experiments described in another connection its capacity to serve as an efficient cause of damping off was readily apparent. (Fig. 8.) As has been mentioned, this capacity diminished greatly somewhat more than two weeks after sowing and disappeared completely at the end of another week. Whether similar results could be effected under the more rigorous conditions prevailing in the field and in the presence of a natural soil flora is not known.

MORPHOLOGY OF SEXUAL APPARATUS

Although the fungus under consideration approaches more closely to *Aphanomyces cochlioides* in the dimensions of its oogonia and oospores, peculiarities in the arrangement of the sex organs reveal a greater similarity to *A. cladogamus*. The helicoid disposition of the oogonial stalk about the antheridial hypha often discernible in

the latter species is considerably more frequent here. In addition, the involvement is more extensive, since not only the stalk directly supporting the female organ (fig. 9, C; fig. 10, A–C) but more often also one or more branches arising from that stalk (fig. 9, B; fig. 10, D) or from the parent filament (fig. 9, H; fig. 10, E) may participate in a greater or smaller measure. Twining of hyphal parts related to the antherdium about the oogonial support is exceptional as in *A. cladogamus*. The direction of rotation is indiscriminately sinistrorse or dextrorse, depending apparently altogether on the positional relation of the parts when contact is established. In spite of the prevalence of the spiral arrangement, the type of engagement common to congeneric forms by mere contact of filaments bearing the sex organs with one another and with such diverticulate protuberances as may be present is not infrequent. (Fig. 9, D–G, I; fig. 10, F, G.)

Owing to the number of antheridia often present and the length of the branches supporting them, a crowded arrangement of these structures on the oogonium frequently results. The similarity to *Aphanomyces cochlioides* thus occasioned may extend to the disposition of the parts in cochleate involution. (Fig. 9, F, H.) The antheridium, like that especially of *A. cladogamus*, is frequently provided with an apical prolongation, a proximal extension, and occasionally even with a lateral process. (Fig. 10, C.) Outgrowths from the dorsal surface of the male organ, such as are found in *A. euteiches*, have never been observed in the species in question, nor, indeed, in any of the other forms discussed in this paper except occasionally in *A. raphani*. (Fig. 14, C.)

On casual microscopic examination, the antheridial branches of *Aphanomyces camptostylus* would appear to arise regularly from hyphae of which the mycelial connection with the oogonium is too remote to be discovered. Closer inspection of a large number of the sexual apparatus and tracing the filaments concerned through several ramifications will, nevertheless, though in relatively few instances, reveal such connection. In Figure 10, G, is illustrated the closest approximation in origin of oogonia and antheridia that the writer has observed. This would still be too remote for ready detection when more or less obscured by overlying mycelium. The infrequency of demonstrable mycelial connection between male and female components of the sex apparatus in the oat-root fungus contrasts strongly with the abundance of the monoclinous relationship in *A. cladogamus*.

The oogonia of *Aphanomyces camptostylus* are formed promptly and in great numbers when the organism is grown on a suitable medium like maize-meal agar. On this substratum they are appreciably smaller than the corresponding structures of the congeneric forms mentioned in Table 2, and besides differ from the latter in the inferior thickness of the membrane surrounding them. When produced in the tissue of diseased beet seedlings, the female organs become somewhat larger and their wall noticeably thicker, so that the specific differences separating the fungus from *A. cochlioides* and *A. cladogamus* with respect to the two features, is less obvious. In general, however, the rather frail appearance characteristic of the oogonia and the antheridia of the form in question enables it to be distinguished from the sturdier beet parasite as well as from the tomato-root fungus at a glance and without the necessity of measurements.

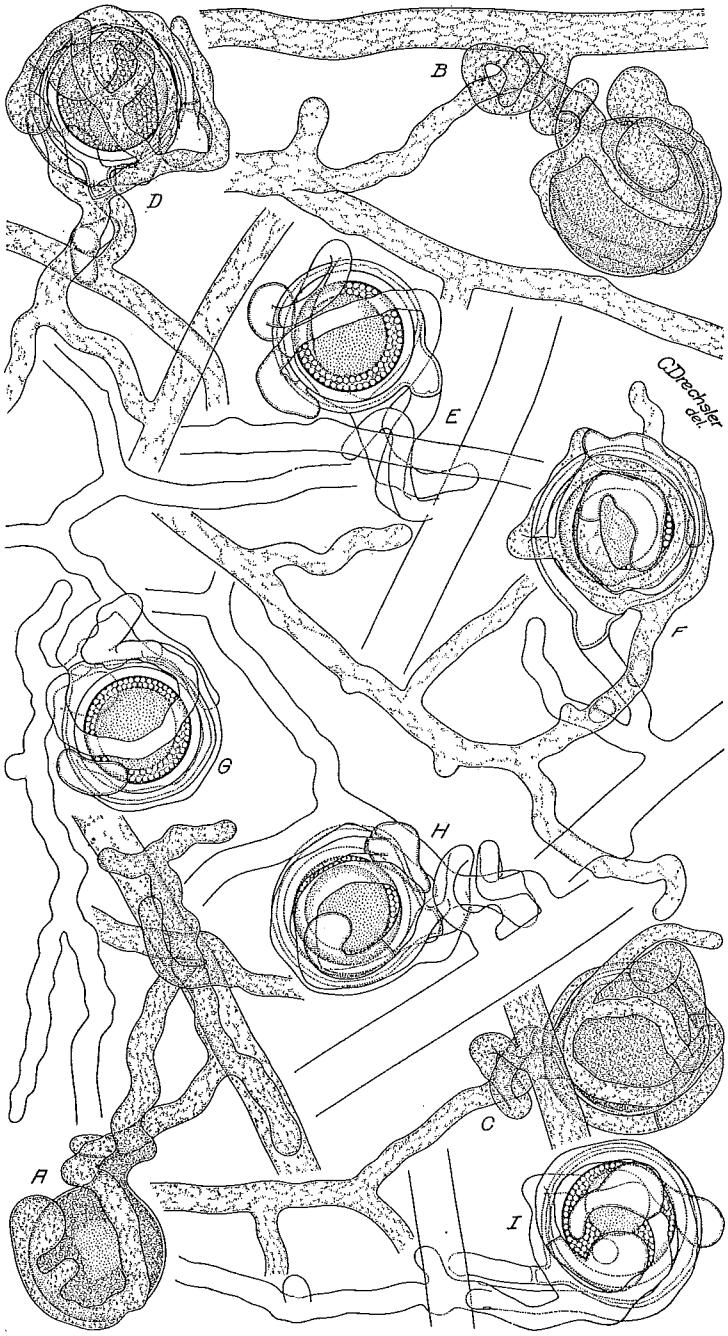

FIGURE 9.—Sexual apparatus of *Aphanomyces camptostylus:* A to I, Drawn from a maize-meal agar culture with the aid of a camera lucida. × 920

A.—Early stage before the insertion of the septum cutting off the oogonium.
B, C.—Oogonia delimited by septa.
D.—Oospore some time previous to maturity, the reserve globule being relatively small.
E to I.—Mature oospores.

FIGURE 10.—Sexual apparatus of *Aphanomyces camptostylus*: A to G, Drawn from a maize-meal agar culture with the aid of a camera lucida. × 920

A, B.—Earlier stages preceding fertilization.
C to G.—Mature conditions; G representing also a somewhat exceptional instance in which the mycelial connection between oospore and antheridum is apparent.

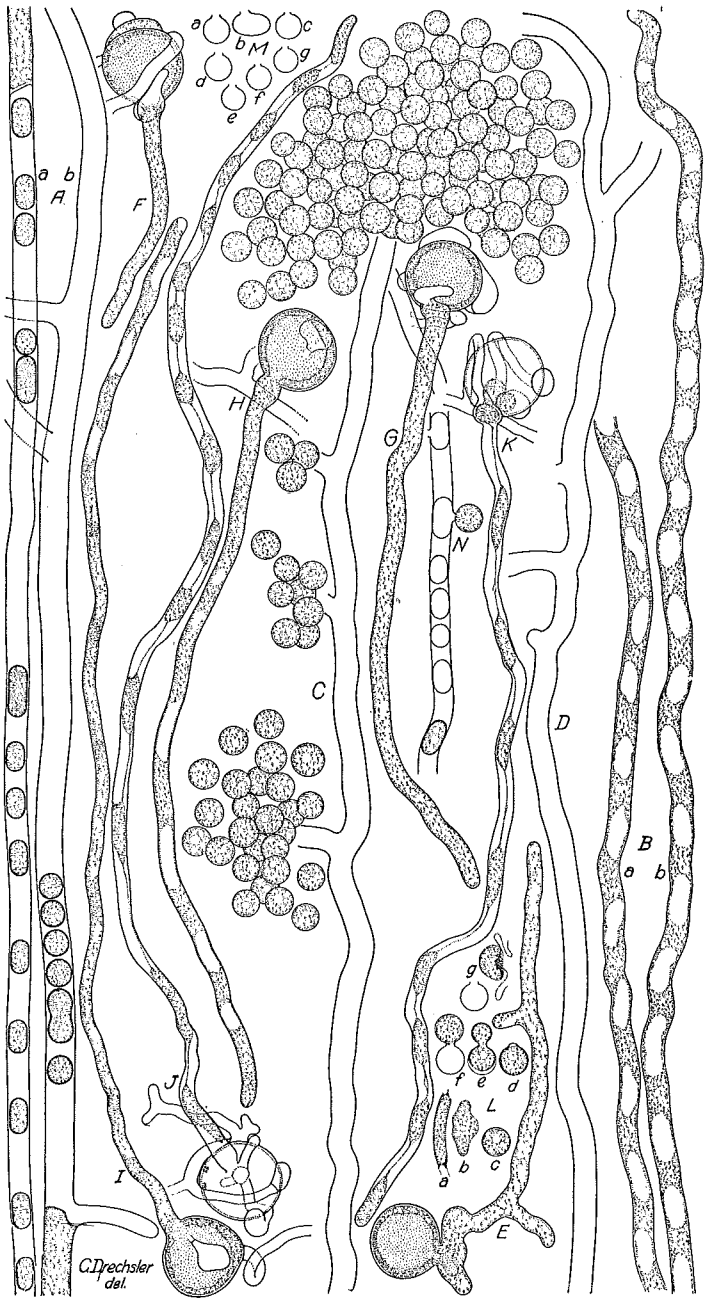

FIGURE. 11.—Asexual reproduction of *Aphanomyces camptostylus*. All figures drawn with the aid of a camera lucida from material grown on maize-meal agar, or in maize-seedling decoction. × 445.

(For explanatory legend see opposite page)

The oospores of *Aphanomyces camptostylus*, as is shown in Table 2, present approximately the same degree of inferiority in size to those of the other forms as do the oogonia. During their development they are given to degeneration in an unusually small measure, although on exposure to summer temperatures after the first month they show considerably more breakdown internally than the sexual spores of *A. raphani*, *A. euteiches*, or *A. cochlioides*. As in the last-named species the oospore, practically colorless on maize-meal agar, may in the tissues of beet seedlings exhibit a bright-yellow coloration, again apparently as a result of heavy absorption of pigment by the oospore wall.

GERMINATION OF OOSPORES

Probably the most interesting characteristic of the oospores of the oat fungus is found in their unusual readiness to germinate. When these bodies are transferred to water in minute bits of maize-meal agar, germination ensues in numerous instances within 18 hours, while through repeated changes of water the process can subsequently be induced in practically all. (Fig. 11, F, G.) As in the case of *Aphanomyces euteiches*, the process either yields a mycelium directly (fig. 11, E) or results in the production of a germ hypha of limited length within which, as well as within the oogonial envelope, zoospores are differentiated to be discharged from the open tip in the manner characteristic of the genus (fig. 11, H–K). The sequence of events from the solution of the oospore wall to the encystment of the discharged protoplasts offers no significant departure from that described for the pea parasite. Generally the number of zoospores liberated is somewhat less than in *A. euteiches*, a fact presumably not without relation to the smaller size of the germinating structures.

ASEXUAL REPRODUCTION

The conversion of the vegetative thallus into zoosporangia likewise can be brought about whenever desired. When mycelium grown in maize-seedling decoction is transferred to water, stout evacuation hyphae without marked apical attenuations, rather similar to those of *A. cladogamus*, are produced. (Fig. 11, B.) These hyphae also often exhibit in the distal portion a number of branches generally very short but at times tending toward greater length, which frequently though not always serve in the evacuation of the delimited zoospores by providing additional means of egress. (Fig. 11, C, D.) The discharged spores after encystment give rise to motile zoospores readily and in quantity, development following the

EXPLANATORY LEGEND FOR FIGURE 11

A.—End portions, *a*, the distal, *b*, the proximal of an axial filament over 3 mm. long of an extensive sporangium, resulting from the transfer of a maize-meal agar culture to water; showing character of delimiting septa and a number of zoospores that rounded up within the sporangium.

B.—Contiguous portions, *a* and *b*, of an evacuation tube some time previous to discharge.

C.—Evacuation tube provided with three lateral orifices in addition to the apical one, all of which had permitted egress of zoospores.

D.—Evacuation tube with three lateral orifices in addition to the apical one, and with a lateral protuberance that failed to open.

E.—Oospore germinating by the production of a branching vegetative hypha.

F, G.—Earlier stages in the germination of oospores, not yet certain whether sporangial or vegetative

H–K.—Oospores germinating by production of a germ sporangium in different stages of development

L.—Successive stages, *a* to *g*, in the rounding up of discharged protoplast, and the development of a motile form from encysted condition.

M.—Several evacuated cyst envelopes *a*, *c*, *d*, *f*, and *g* representing usual types, *b* and *e* types somewhat more irregular in shape.

N.—Evacuation of spores that had encysted within a sporangium after the manner of Dictyuchus.

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course described in the species previously discussed. Occasionally when zoospores are retained within the filamentous sporangium, the production of the motile stage may nevertheless ensue, being then accomplished after the manner prevailing in the genus Dictyuchus. (Fig. 11, N.) Such departure from normal development, an example of which was recorded earlier by Sorokine (*30*) in his account of *Aphanomyces stellatus* De Bary, is naturally to be interpreted as a promiscuous irregularity of no special taxonomic significance. As in related forms, failure of zoospores to be discharged is especially frequent in the extensive sporangial units consisting of portions of axial hyphae often over 3 mm. in length, together with numerous branching systems, which result from the transfer of largish pieces of agar culture to water. (Figure 11, A, *a* and *b*.)

In applying the term "camptostylus" it is hoped that the frequent helicoid disposition of the oogonial stalk about the antheridial filament, which constitutes one of the more conspicuous characteristics of the species, may be brought into relief.

Description

Aphanomyces camptostylus, n. sp.

Mycelium 2.5 to 9.5 μ in diameter, not given to abrupt fluctuations in thickness, sparingly or moderately branched, with moderate extramatrical and very scant aerial development. Sporangia formed by the direct transformation of vegetative mycelium, the delimiting septa occurring as narrow cross walls or as curved or irregular partitions; sometimes very extensive, including segments of axial hyphae more than 3 mm. in length, together with numerous ramifying elements, then often provided with plural evacuation hyphae; the latter up to 2 mm. or more in length, usually rather stout, often maintaining a diameter of 6 to 7.5 μ, not markedly narrowing toward the apex, bearing often on the distal portion one to four lateral protuberances or branches up to 30 μ in length, which, like the tip of the axial filament, may open up to permit the egress of zoospores. Zoospores varying in number from a few to over 300 from a single orifice, on encystment usually 7 to 10 μ in diameter, diplanetic, developing a papilla approximately 2 to 2.5 μ in diameter, the cylindrical wall of which persists on the empty cyst membrane. Oogonia terminal on short branches or on longer hyphal elements, subspherical, smooth, mostly 19 to 26 μ (average 22.9 μ) in diameter, provided with a wall of somewhat irregular inner contour, 0.6 to 1.2 μ (average 0.9 μ) in thickness; the oogonial stalk regularly making contact with an antheridial branch, the stalk itself and often one or several branches arising from it or from its parent filament frequently coiling about the antheridial hypha. Antheridia usually up to four in number, consisting of an inflated part 4.5 to 9 μ in diameter, 8 to 11 μ in length, frequently together with narrower apical proximal or lateral extensions; regularly functional, communicating with interior of oogonium by very short fertilization tube; borne terminally on supporting branches 2 to 3 μ in diameter. The latter branches mostly of diclinous origin, the mycelial connection with the oogonium rarely demonstrable and then somewhat remote; encircling the oogonium extensively and when crowded often disposed with the male organ in cochleate arrangement. Oospore single, subspherical, colorless or yellowish, mostly 16 to 21 μ (average 18.8 μ) in diameter, surrounded by a wall 1.1 to 2 μ (average 1.5 μ) thick, and containing at early maturity a slightly eccentric reserve globule 9 to 13 μ (average 11.0 μ) in diameter; germinating readily either by the direct production of a branching vegetative mycelium, or by the production of an unbranched germ sporangium often 200 to 400 μ in length and discharging commonly 10 to 17 zoospores. Isolated from root of *Avena sativa* L. collected near Sauk City, Wis.; under experimental conditions destructive to young seedlings of *Beta vulgaris* L.

APHANOMYCES RAPHANI KENDRICK

INTRODUCTION AND HISTORY

In an abstract (*1*) published in 1912, Barrett recorded the widespread occurrence throughout the country of a serious disease of radish, distinguished by a peculiar browning or blackening of a portion or all of the root, accompanied often by deformity due to local retardation of growth. The tissues were described as remaining of a normal texture without unpleasant taste or odor during the early stages of infection, but as becoming affected later by rots due to saprophytic forms. Under certain conditions seedlings were reported as also becoming infected, the infection resulting in a shrinkage of the stem at the surface of the ground and their eventual death. The disease, which in many localities in Illinois rendered the crop entirely or in large part unmarketable, was attributed to *Aphanomyces laevis*. Beyond a characterization of the mycelium in the host tissue as intercellular, very delicate, evanescent, and easily overlooked, of the swarm spores as being produced in large numbers especially in wet situations, and of the oospores as being easily produced, little detail concerning the peculiarities of the parasite was given.

While during subsequent years, Barrett's statement concerning the distribution and prevalence of black root, as the disease came to be designated, was given support from various sources, his reference of the trouble to *Aphanomyces laevis* remained without confirmation. It is not surprising, therefore, that after the appearance of a paper by Edson (*12*) in which a similar disease was attributed to the fungus later described as *Rheosporangium aphanidermatum*, the latter parasite came to be cited customarily as the cause of black root, to the general neglect of any mention of *A. laevis*. With the publication in 1927 of an abstract by Kendrick (*21*), the effective pathogene was finally revealed as being indeed a member of the genus Aphanomyces, which, because of certain morphological characters distinguishing it from De Bary's fungus as well as from several other congeneric forms having smooth oogonia, was described as a new species, *A. raphani*. In a more complete account that appeared later (*22*), the same author gave a comprehensive discussion of the symptoms, history, distribution, economic importance, and etiology of the disease. The morphological aspects and taxonomic relations of the fungus were set forth at some length and its pathogenic nature was brought more formally into relief by means of controlled inoculation experiments carried out with the use of pure cultures on artificial media.

ORIGIN AND ISOLATION OF CULTURES STUDIED

As the radish disease described by Kendrick had regularly been observed, according to information supplied by G. H. Coons, in the vicinity of Lansing, Mich., the locality in which beet seedlings were found subject to attack by *Aphanomyces cochlioides*, comparison of the two obviously related parasites became highly appropriate. To further such comparison, specimens of radishes affected with black root were collected in a market garden at Dimondale, Mich., on July 1 and again on July 8, 1927. It may be mentioned that the damage attributable to the disease was not excessive, being confined

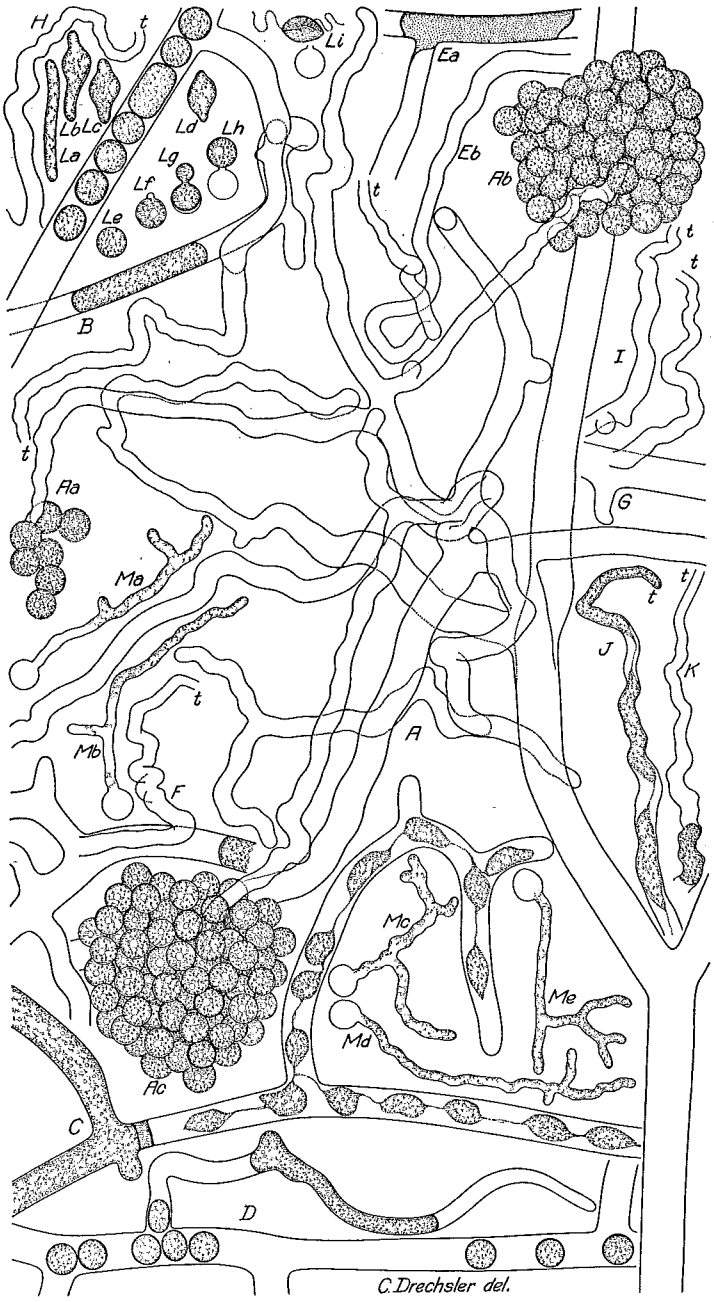

FIGURE 12.—Asexual reproduction of *Aphanomyces raphani*. All figures drawn with the aid of a camera lucida from material grown on maize-meal agar. × 450

(For explanatory legend see opposite page)

apparently to a number of separate small areas each showing from a few to several dozen affected plants, a distribution similar to that evident in the case of pea-root rot in fields not yet generally infested with *A. euteiches*. In the specimens examined infection was found of varying extent, being limited sometimes to small incipient lesions and at other times manifested in discoloration of large portions of the surface, together with extensive blackening of underlying tissues as well as with pronounced malformations.

On treating the material according to the procedure already outlined, the pieces of diseased tissue placed in water regularly yielded hyphae having the low refringency characteristic of the mycelium of the more delicate Saprolegniaceae. With repeated renewal of water these hyphae served as evacuation tubes in the discharge of an abundance of zoospores entirely after the manner prevailing in the genus Aphanomyces. When the pieces of tissue were subsequently transferred to corn-meal agar plates, there were produced mycelium and oospores, which left no doubt as to the identity of the fungus with *A. raphani*. Of more than a dozen cultures, all freed of bacteria and each derived from a separate specimen, none showed any noticeable departure in morphology from the others. As the readiness with which the parasite was identified in itself testifies to the general adequacy of Kendrick's description, it will be sufficient here to indicate certain comparative aspects and to remark briefly on the few details concerning which an interpretation at variance with that in the original account might be entertained.

VEGETATIVE HABIT

The mycelium of *Aphanomyces raphani*, which in its larger elements reveals a diameter of 8 to 14 μ, is obviously coarser than that of any of the related root parasites discussed in this paper. On maize-meal agar its rate of growth is considerably slower and its habit strikingly different. In the related forms the thallus presents a general uniformity in the distribution of its elements, the long hyphae branching at rather generous intervals, neither axial filaments nor branches being addicted to frequent, abrupt, or marked changes in diameter or in direction. In the radish parasite, on the other hand, growth tends toward concentration in ramifying systems arising here and there from the wider and longer axial filaments. Within these systems, closer branching prevails. The generally rapid tapering of the relatively short branching elements, sometimes combined with promiscuous unevenness in diameter, and their frequent abrupt turns add to the appearance of irregular intricacy. (Fig. 12, A.)

EXPLANATORY LEGEND FOR FIGURE 12

A.—Portion of an extensive sporangium evacuated through plural efferent hyphae, three of which, *Aa*, *Ab*, and *Ac*, are shown with their respective groups of zoospores.

B.—Portion of a sporangium showing a number of zoospores encysted within, and an evacuation hypha with the open tip *t*.

C.—Portion of a sporangium showing a thick delimiting septum, and zoospores in a condition immediately preceding discharge.

D.—Portion of a sporangium, showing some encysted zoospores within, and a mass of undivided protoplasm contracted in a branch.

E.—Portions of a sporangium; *Ea* showing a massive delimiting plug, *Eb* representing the contorted evacuation tube with open tip *t*.

F-K.—Evacuation tubes showing degrees of contortion, variations in length, and diameters of tip *t*, functional in providing channel of egress.

L.—Successive stages, *La* to *Li*, in contraction of discharged protoplast, and development of motile zoospore from encysted condition.

M.—Zoospores, *Ma* to *Me*, germinating by production of germ tubes.

ASEXUAL REPRODUCTION

When pieces of a maize-meal agar culture, for example, are transferred to water, the mycelium is promptly converted into zoosporangial units delimited by narrower partitions (fig. 12, C, F) or by more massive plugs. (Fig. 12, *Ea.*) Liberation of zoospores follows usually after about four hours. Owing to the more compact branching, the plural evacuation tubes arise in closer proximity to one another than in other forms, so that the same microscopic field frequently affords a view of several of these structures functioning simultaneously. Thus, whereas the origin of the two evacuation tubes close to each other that is shown in an illustration of *Aphanomyces euteiches* in a previous paper (*20: pl. 6, C*) represents for the pea parasite a rather unusual approximation of these parts, the origin of the three tubes shown in Figure 12, A, is far from exceptional for *A. raphani.* Indeed, in the material from which the latter figure was drawn several additional evacuation tubes arose from the same sporangial unit and so near to those shown as barely to avoid inclusion within the limits of the drawing.

The evacuation hyphae of *Aphanomyces raphani* are mostly of moderate length, rarely exhibiting the more extreme development observable in *A. cochlioides, A. cladogamus,* and *A. camptostylus.* In many instances these structures taper markedly toward their tips (fig. 12, B, E–K), while in others the decrease in diameter is not pronounced (fig. 12, *Aa* and *Ab*). As in *A. cochlioides,* a Polythrinciumlike modification is often apparent. (Fig. 12, *Aa, Bb,* B, *Eb,* H.) Stronger distortions are not infrequent (fig. 12, *Eb,* F), and while these sometimes occur in the form of spiral twists, it is doubtful whether a spiral tendency is to be considered among the regular characteristics of the species.

The encysted protoplasts round up at the orifices of the evacuation tubes and give rise to the swimming spores quite as in other members of the genus. (Fig. 12, *La–Li.*) The isthmus through which the contents of the cyst pass measures approximately 2 μ in diameter. On germination a single germ tube measuring from 2 to 2.7 μ is usually produced. (Fig. 12, *Ma–Me.*) Irregularities resulting from frustrated processes, like the incomplete division of sporangial contents, or the retention of zoospores in the sporangial hyphae (fig. 12, B, D) or in the narrowing evacuation tubes (fig. 12, K) occur as in related forms.

MORPHOLOGY OF SEXUAL APPARATUS

The arrangement of the sexual apparatus of *Aphanomyces raphani* would seem less intricate than that of the other root parasites discussed in the present account, although the stalks supporting the male and female organs, together with their parent hyphae, regularly show intimate contact of the kind exhibited by *A. euteiches* and *A. cochlioides.* According to Kendrick, "the antheridial stalk arises from the same hypha as the oogonial stalk, or from a hypha in close proximity to the oogonium." The first portion of that statement, asserting a monoclinous origin of the antheridial stalk, the present writer has been unable to confirm. In all apparatus examined the male and the female organs were found derived from separate hyphae of which the mycelial connection was apparently too remote to be traced.

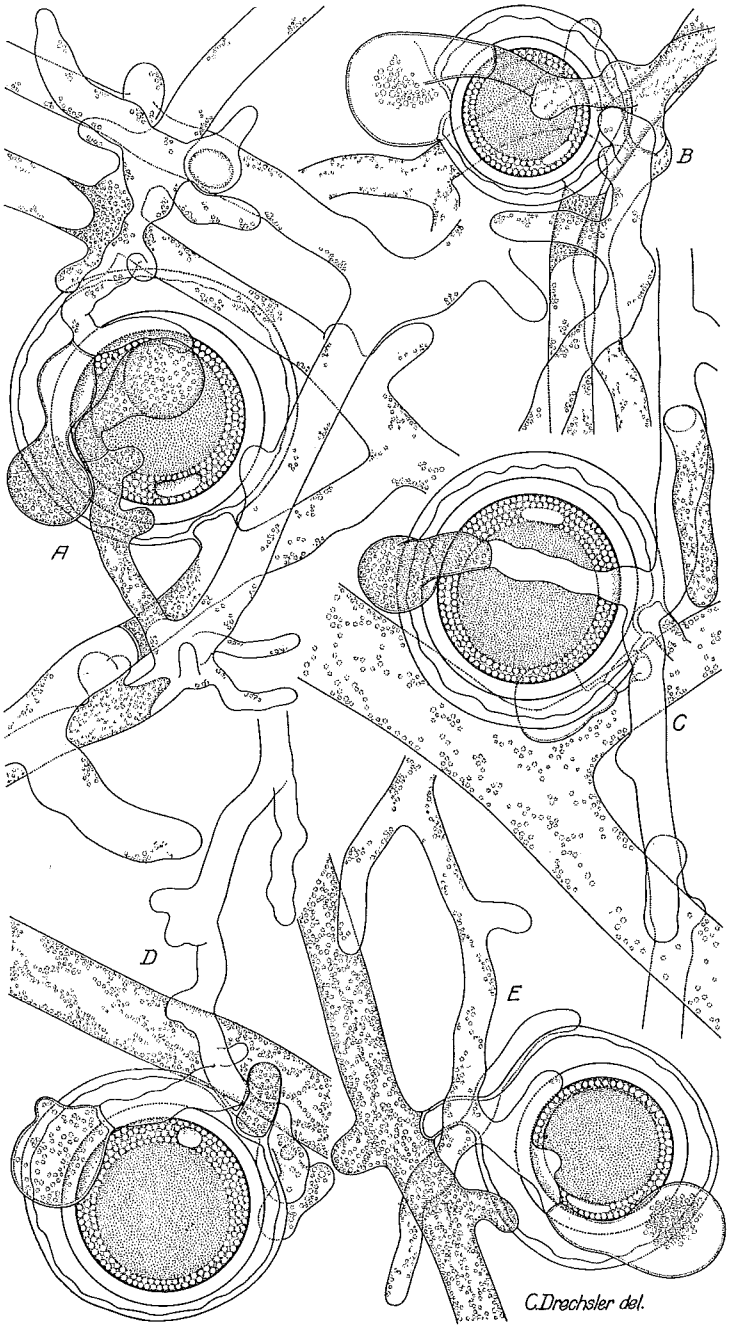

FIGURE 13.—Sexual apparatus of *Aphanomyces raphani*. A–E, Drawn from maize-meal agar cultures with the aid of a camera lucida. ×920

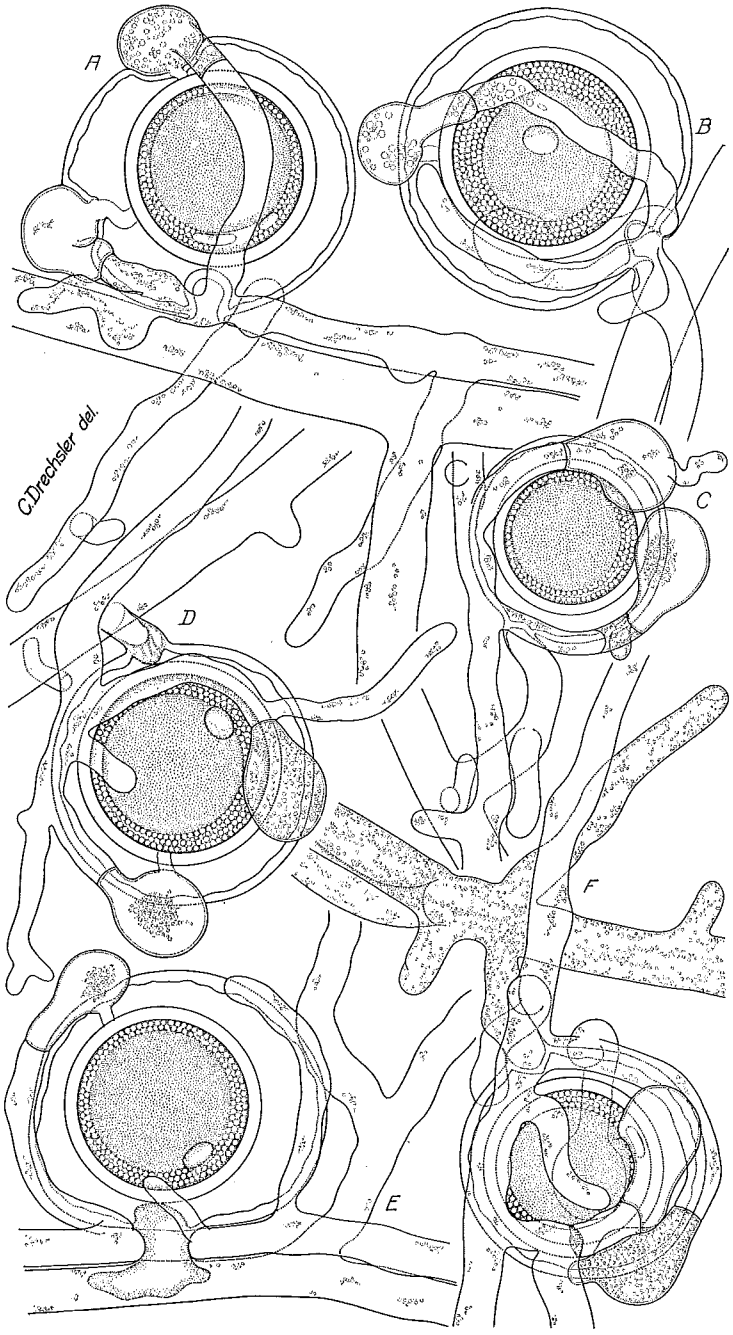

FIGURE 14.—Sexual apparatus of *Aphanomyces raphani*. A–F, Drawn from maize-meal agar cultures with the aid of a camera lucida. × 920

The number of antheridia to an oogonium is usually two (fig. 13, A, C; fig. 14, A–D), although the presence of a single male organ is not rare (fig. 13, B, D, E; fig. 14, E), and the occurrence of three far from exceptional (fig. 14, F). As the delimiting septum is frequently set at some distance from the terminal inflated part, a proximal extension of variable length and of a diameter approaching that of the supporting branch is often included. Apical prolongations are less common, though occasionally present (fig. 14, F), as are also dorsal diverticula (fig. 14, C) of the type encountered in *A. euteiches*. The inflated portion of the antheridium in profile view appears partly countersunk into a corresponding depression on the surface of the oogonium. At the apex of the depression the fertilization tube, which may be very short (fig. 13, B) or of appreciable length (fig. 14, A), perforates the envelope of the female organ.

As is evident from an inspection of Table 2, the oogonia and oospores of *A. raphani* are considerably larger than the corresponding bodies in the four other species listed. The average values for the diameter of these structures show fairly satisfactory agreement with those given by Kendrick. When, however, the values indicated for thickness of the oospore wall are compared with those given in the original account, namely, 2.5 to 4.5 μ (average 3.6 μ), a serious lack of agreement is revealed, which can hardly be attributed to the employment of different methods of measuring. It appears not improbable that the explanation that was suggested for the apparently excessive figures given by Peters for the same dimension in the case of the sugar-beet parasite may have some application in this instance. Of the four oospores represented in the photomicrographs included in Kendrick's paper, only one (*22: fig. 10, A*) would seem to exhibit a type of internal structure encountered in normal development, and in this oospore the wall is manifestly of only moderate thickness; whereas the other three oospores (*22: fig. 10, B*), of which the external envelope appears to be, indeed, of a thickness corresponding to the published values, exhibit a type of internal organization—a central granular lump embedded in a larger quantity of apparently more homogeneous material—not characteristic of normal development. While a certain increase in thickness of the confining membrane is a phenomenon usual in the aging of oospores, excessive thickening suggestive generally of gelatinous swelling is more plausibly to be interpreted as a concomitant of protoplasmic degeneration.

PLECTOSPIRA GEMMIFERA, N. SP.

INTRODUCTION

The genus Plectospira was recently erected by the writer (*8*) to accommodate a fungus, *P. myriandra*, having the general mycelial habit and type of zoospore development common to species of Aphanomyces, but dissimilar from the latter in exhibiting a differentiated zoosporangium composed typically of a complex of inflated elements, together with well-developed evacuation hyphae. Oospores were found produced in oogonia which either were entirely free of any suggestion of antheridia or were enveloped in an abundance of male elements surpassing any hitherto described for any fungus definitely referred to the Saprolegniaceae. In the absence of more than a single species it was uncertain whether the association of the lobulate

zoosporangium with the peculiarly intricate sex apparatus represented a somewhat casual conjunction or a combination of more taxonomic moment. Information pertinent to the question is provided by a consideration of a congeneric form isolated by R. D. Rands from a diseased root of sugar cane (*Saccharum officinarum* L.) collected near Thibodaux, La., April 21, 1927, the fungus presumably having been present in parasitic relationship.

MYCELIAL CHARACTERISTICS

The mycelium of the sugar-cane organism, like that of *Plectospira myriandra*, is in general somewhat more delicate than that of the several parasitic species of Aphanomyces dealt with in this paper, the longer axial hyphae not attaining so great a diameter, while the finer branches show noticeably more extensive development. On maize-meal agar and at summer temperatures it shows a considerably faster rate of extension than the mycelium of the congeneric form from the tomato, which in turn grows more rapidly in linear measure than the various species of Aphanomyces studied. Aerial growth is not usually evident in plate cultures when maize-meal agar is employed as a substratum, but in tube cultures a slight arachnoid development may appear generally over the sloping surface and a more profuse flocculent covering at the upper end.

ASEXUAL REPRODUCTION

Temporary Zoosporangia

When vegetative mycelium is transferred to water the zoosporangial stage is initiated by the development of strongly swollen structures that in consequence of a highly variable measure of branching may assume final form as relatively simple bodies (fig. 15, C) or as larger, intricate complexes (fig. 15, B). Their enlargement in either case is accomplished by active translocation of protoplasm from undifferentiated hyphae. When definitive dimensions are attained, and necessarily not until then, the specialized structure is delimited by the insertion in the single hyphal branch on which it is borne of a partition, which generally occurs in the form of a simple thin septum. (Fig. 15, C.) The swollen bodies thus set off have a characteristic dark appearance, due evidently to a certain degree of opacity in the densely granular contents. It may not be amiss to emphasize the fact that the structure under consideration is in outward form and in function essentially different from the vegetative mycelium, and that in this species as in the congeneric one from tomato rootlets mycelium produced in the course of vegetative growth is never converted directly into sporangia.

The ensuing sequence of events is much as in *Plectospira myriandra*. From the lobulate structure are produced one to sometimes more than a dozen longish filamentous elements, the number depending on the size of the specimen concerned. In Figure 16, A, is represented an instance of development moderate in this regard though at a later stage, the swollen complex being of medium proportions and having given rise to four filamentous outgrowths. (Fig. 16, *Ac–Af.*) At about the time such outgrowths attain definitive length a reorganization in protoplasmic contents becomes apparent throughout the structure. Vacuoles at first not conspicuous increase in number and

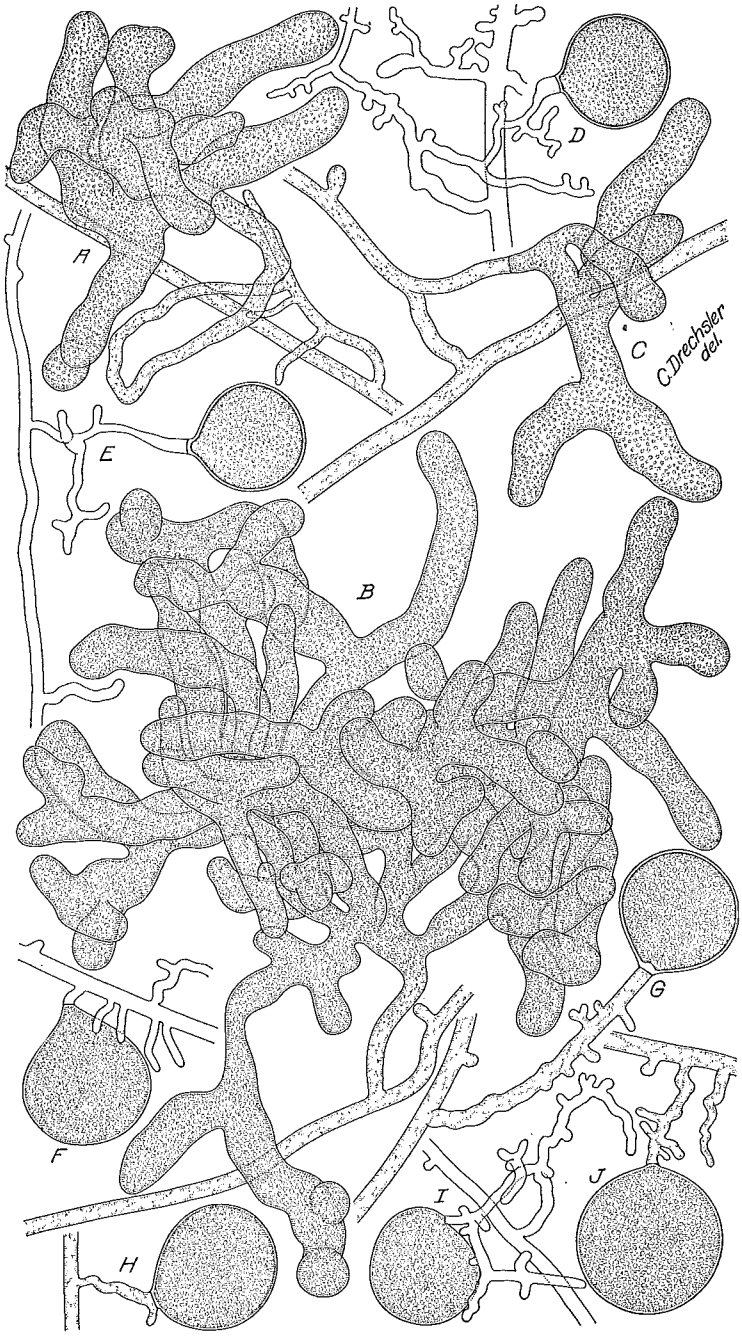

FIGURE 15.—Asexual reproductive structures of *Plectospira gemmifera* drawn from a maize-meal agar culture with the aid of a camera lucida. × 460

A, B, C.—Temporary sporangia of the type produced when mycelium is transferred to water, the delimiting septum not yet inserted in A and B, but present in C.
D–J.—Gemmae, showing differences in shape and mycelial attachment.

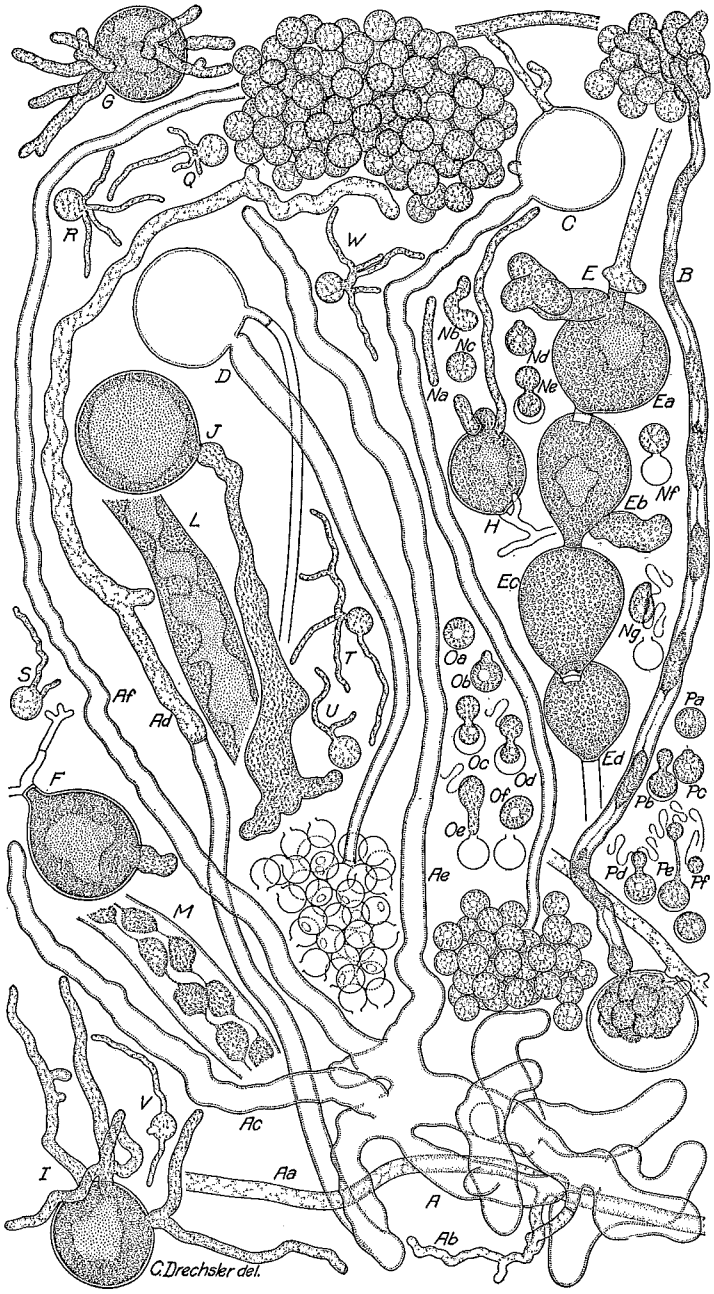

FIGURE 16.—Asexual reproduction of *Plectospira gemmifera*. All drawing made with the aid of a camera lucida from structures resulting from the transfer of growth on maize-meal agar to water. × 445

(For explanatory legend see opposite page)

size and ultimately coalesce into a practically continuous lacuna around which the more substantial, finely granular material is disposed in a peripheral layer of variable thickness. (Fig.16, L.) Through a rather abrupt orientation the granular material is converted into individualized protoplasts connected by delicate strands, two series being present in the swollen basal elements (fig. 16, M), while only a single series is contained within the more slender outgrowths. When very soon thereafter one or more of the filamentous outgrowths gives way at the apex, discharge of the protoplasts ensues, usually with remarkable rapidity. The outgrowths not serving as efferent hyphae (fig. 16, *Ac–Ae*) are evacuated downward in the same way as are the lobulations. Although comparable to the vegetative hyphae in diameter, and accommodating only one series of protoplasts, the outgrowths in general are hardly to be homologized with the outwardly undifferentiated hyphae that make up the body of the individual sporangial unit in members of the genus Aphanomyces. For, as has been stated, they originate from specialized structures and manifestly are designed for the specific function of effecting the evacuation of those structures, even when in the many instances of supernumerary development a considerable proportion do not actually participate in the discharge of that function. They are therefore undoubtedly to be homologized with the evacuation hyphae of Aphanomyces, which they resemble, moreover, in their capricious courses and their characteristically uneven diameters.

Gemmae

In the discussion of *Plectospira myriandra* the opinion was expressed that the lobulate structures described for that species were not analogous to the gemmae of water molds generally. Their luxuriant development under conditions optimum for immediate zoospore formation and their prompt degeneration when such conditions come to an end were held to remove them from the category of resting bodies. Effectual confirmation of that view is supplied by the sugarcane parasite in question, for this fungus, on a suitable substratum like maize-meal agar and in the absence of free water, develops an abundance of relatively large bodies, typically subspherical or somewhat pyriform, which even at summer temperatures are capable of surviving for several months. Usually they are borne terminally on the more delicate mycelial ramifications (fig. 15, D–F, I, J), but not rarely they may be supported by stouter branches (fig. 15, G);

EXPLANATORY LEGEND FOR FIGURE 16

A.—Medium-sized sporangium borne on a short secondary branch from primary branch *Ab*, which is borne on a longer filament *Aa*; evacuation effected through evacuation hypha *Af*, the similar elements *Ac*, *Ad*, and *Ae*, not functioning; the distal portion of element *Ad* is cut off and is continuing growth as a vegetative hypha.

B.—Gemma discharging zoospores after germination as a sporangium.

C.—Gemma after the discharge of zoospores and their encystment before the orifice of the evacuation hypha.

D.—Evacuated gemma with group of empty cyst envelopes.

E.—Series of four intercalary gemmae, *Ea* to *Ed*, of which *Ea* and *Eb* have begun to germinate.

F.—Gemma germinating presumably as sporangium by a single stout germ hypha.

G, H, I.—Gemmae germinating vegetatively by a number of slender germ hyphae.

J.—Gemma giving rise to a small temporary sporangium.

L.—Portion of a sporangium showing lacuna traversing the swollen element shortly before the separation of the protoplasm into zoospores.

M.—Same, after reorganization of contents in two series of zoospores.

N.—Successive stages, *Na* to *Ng*, in the contraction of discharged zoospores, and the development of the motile form from the encysted condition.

O.—Successive stages, *Oa* to *Of*, in the abnormal evacuation of the encysted structure.

P.—Successive stages, *Pa* to *Pf*, in abnormal development of encysted structure, with only partial evacuation.

Q–W.—Zoospores germinating by production of one or several delicate germ tubes.

sometimes, too, they occur in an intercalary position either separately or in series (fig. 16, E). Like the temporary sporangia, and evidently because of a similar concentration of granular material, they present a darkish, opaque appearance. The surrounding membrane is of appreciable though not pronounced thickness. If these subspherical bodies are transferred to water within a week or two after their formation they promptly produce one (fig. 16, *Eb*, F) or not infrequently two or three evacuation hyphae of the same type as those put out by the lobulate sporangia; as in the latter structures the contents throughout then become divided into zoospores, which are discharged through one (fig. 16, B–D) or less often two of the hyphae. The entire sequence of events, in many instances transpiring in little more than two hours, is essentially similar to the sequence followed in the germination of oospores of *Aphanomyces euteiches* and *A. camptostylus*, except that the preliminary solution of the oospore wall and the distribution of the contents within the oogonial cavity find no counterpart here. When old material is employed, as, for example, that provided by cultures three months after being planted, germination is considerably less prompt and proceeds regularly by the direct production of vegetative hyphae. (Fig. 16, G–I.) The latter are usually more slender than evacuation hyphae, but may under proper conditions, even at a relatively small distance from their origins, broaden out into swollen sporangial elements. (Fig. 16, J.)

Undoubtedly the typically subspherical structures produced under conditions not suitable for zoospore formation, and capable of surviving for a period of some duration, correspond to certain of the various bodies customarily recognized by students of the water molds as gemmae. A tendency toward lobulation evident in nearly all material lends color to the suspicion that in spite of their usual globose shape so obviously different from that of the temporary sporangia, they may nevertheless represent "Hemmungsbildungen" entirely homologous with the latter. In any case the occurrence of such structures in the more delicate water molds would seem somewhat unusual. In this connection mention may be made of the conidia discussed by Sorokine (*30*) in his account of a form designated as *Aphanomyces stellatus* De Bary, which, except for requiring an extended resting period, apparently germinated much after the manner of the asexual resting bodies produced by the sugar-cane fungus. Another irregularity reported by Sorokine, consisting in the occasional formation of zoospores in several rows within sporangia much broader than the prevailing type for the species, provides an additional feature that might suggest a closer affinity. However, the figures (*30: pl. 7, figs. 11 and 12*) given by the Russian botanist showing the relation of oogonium and antheridium reveal a simplicity rarely if ever realized in any of the species of Aphanomyces occuring on the roots of higher plants, and certainly altogether antithetical to the complexity characteristic of the sex apparatus in the sugar-cane fungus.

The association in one organism of the two types of asexual reproductive structures described invites comparison with the occurrence of outwardly similar bodies in the genus Pythium. As has been noted previously (*8*), the lobulate type of zoosporangium is well represented there, being found in *P. aphanidermatum* and more

than a dozen congeneric forms. The subspherical sporangium of the type represented in *P. debaryanum*, with which must be homologized the similar chlamydospore or conidium that germinates by a germ tube, also occurs widely, especially among terrestrial species. However, in spite of the rich assortment of forms that have been available for study, the writer has so far failed to discover any instance of the production of both types of sporangia by one and the same species of Pythium. Nor have such instances been convincingly recorded in the pertinent literature. To be sure, Serbinow (*27*) ascribed to *P. perniciosum* Serb. the production not only of intercalary subspherical chlamydospores but also of more irregular saclike structures participating often in zoospore formation. His figures leave little doubt that the two categories of asexual reproductive bodies in question were present in his material. But indications are not lacking, especially in his illustrations of the sexual stage, that he was dealing not with one but with two species of Pythium, such mixture of congeneric forms being far from unusual in various sorts of material and hardly amenable to separation by the method of isolation employed by Serbinow. In any case, whatever may be the correct explanation of the anomalous findings of that writer, the combination of a subspherical sporangium or a chlamydospore with a lobulate sporangium would seem at least infrequent in the genus Pythium. An adjustment in the latter genus to the limitation of asexual reproduction to a single type of structure is probably entailed in the longevity of the sporangial complexes of such species as *P. complens* Fischer and *P. arrhenomanes*, which, formed often in the absence of free water, may survive for weeks or even for months.

Development of Zoospores

The encysted protoplasts, whether discharged from lobulate sporangia or from subspherical gemmae, give rise freely to swimming zoospores. (Fig. 16, *Na–Ng.*) After a resting period of approximately two hours, gradual development of a papilla, evacuation of the cyst membrane in the course of about 10 seconds, acquisition of mobility by the adhering spore in 10 to 20 minutes, follow in much the same sequence as in the species of Aphanomyces previously discussed. The swimming spore later rounds up in the usual manner and germinates by the production of one or several delicate germ tubes. (Fig. 16, Q–W.) Two instances of abnormal emergence observed in a preparation inadequately supplied with water may be described for whatever interest may attach to them. In one, evacuation of contents started apparently in the normal way (fig. 16, *Oa–Oc*), but after approximately one-half of the material had escaped from the cyst envelope it came to a halt. A single cilium made its appearance near the apex of the external lobe of the hourglass-shaped protoplast (fig. 16, *Od*) and lashed about vigorously for a period of 25 minutes. Finally the protoplast, as if by much effort, drew out through the papilla. (Fig. 16, *Oe.*) Instead of its swimming away as was expected, all motion stopped very abruptly, with the exception that the protoplast contracted into an ellipsoidal shape near the orifice of the cyst membrane. (Fig. 16, *Of.*) In the second instance only about one-fifth of the protoplasm passed through the orifice. (Fig. 16, *Pb.*) On the small protruding lobe two cilia were developed (fig.

16, *Pd*), which maintained violent activity for 20 minutes, when the entire lobe drew away from the cyst, remaining connected for a minute by a very slender thread of protoplasmic material (fig. 16, *Pe*). The activity of the cilia continued undiminished until the protoplasmic connection gave way, whereupon it stopped instantly. After the rounding up of the small globule of protoplasm and the detachment of the cilia (fig. 16, *Pf*), no further changes were observed. In the same preparation other instances of frustrated evacuation were manifested in the abrupt cessation of all locomotor activity on the instant that contact between liberated body and cyst was interrupted. This cessation could hardly have been due to any deficiency of water serious enough to prevent locomotion, since normally developed specimens were actively swimming about in all portions of the microscopic field. That the abnormality was nevertheless associated with water deficiency became evident when on the addition of water further instances of emergence everywhere in the preparation followed normal courses.

SEXUAL REPRODUCTION

The sexual stage of the sugar-cane parasite is somewhat difficult to induce, having been secured in quantity only once, and then in a maize-meal agar culture. The subspherical oogonia are borne for the most part terminally on short branches (fig. 17, A–C) or longer filaments (fig. 17, D, E), in either case being delimited by a narrow septum inserted usually at the juncture of spherical and cylindrical parts. Antheridia are always present and, as in *Plectospira myriandra*, in extraordinary numbers. In the liberally supplied apparatus shown in Figure 17, D, for example, 45 male elements are revealed in the upper and equatorial aspects, while approximately half that number may well be assumed to be concealed on the under side. Even in the rather poorly provided apparatus shown in Figure 17, B, no less than 23 are visible, with perhaps a dozen underneath. The majority of these elements obviously do not participate in any sexual function, their evacuation at an early stage being accomplished rather by the movement of protoplasm into communicating parts unhindered in the absence of a delimiting cross wall. However, in every apparatus, several antheridia characterized by relatively large dimensions and a thicker wall, and delimited by a septum, usually stand out conspicuously from their fellows. These have all the appearance of functional organs. At times indications of a fertilization tube are perceptible, but the intricacy of the apparatus creates such a confused optical condition that the evidence of the existence of such a structure is never entirely convincing. It might, however, be inferred with considerable plausibility that those of the larger antheridia that become evacuated at an early stage contribute their contents to the oogonium, while others that retain their contents until an advanced stage in the maturation of the oospore can hardly be supposed to fulfill any important sexual function.

The antheridial elements of the sugar-cane fungus, like those of *Plectospira myriandra*, regularly arise from one (fig. 17, A, B, C, E) or several (fig. 17, D) hyphae other than those bearing the oogonia. In proximity to the female organ a number of branches which usually bear secondary branches of limited growth are produced. The ramifying system thus formed, bearing the pouchlike outgrowths that con-

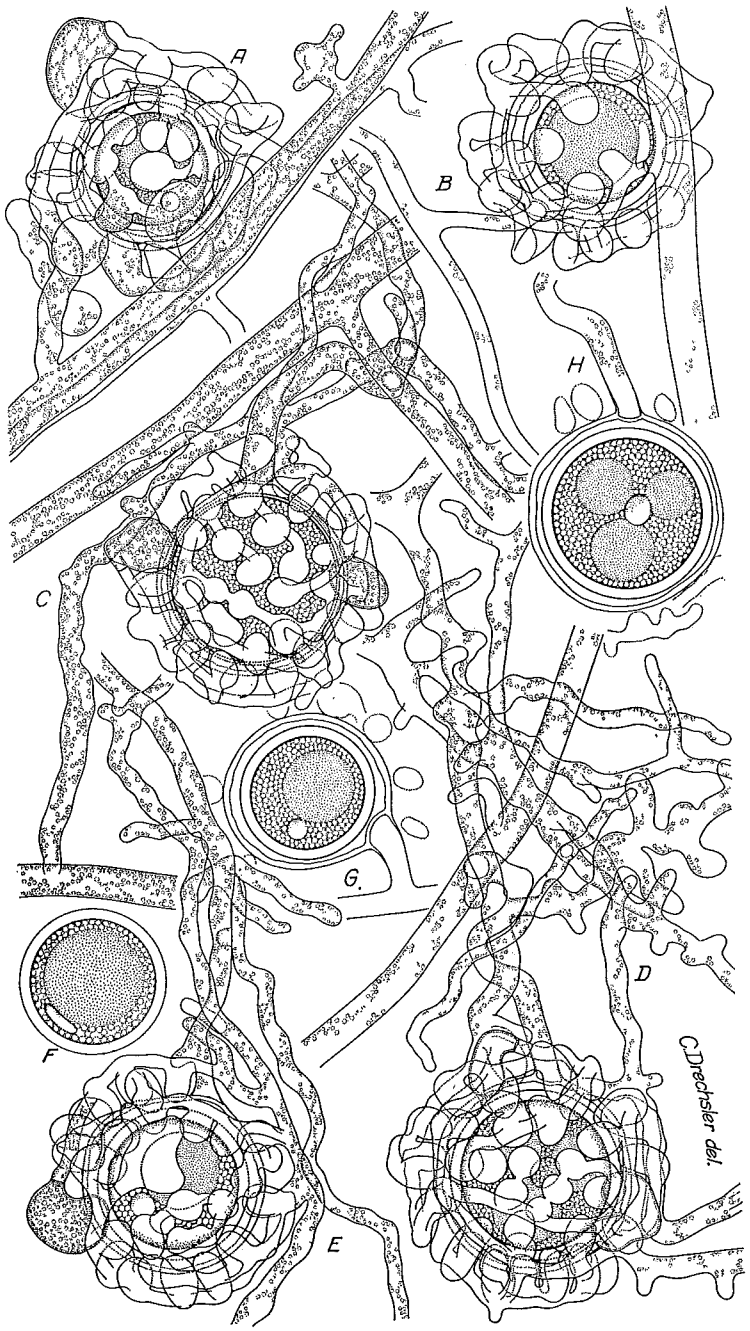

FIGURE 17.—Sexual reproduction of *Plectospira gemmifera*. All figures drawn with the aid of a camera lucida from structures developed on maize-meal agar. × 920

A–E.—Sexual apparatus showing mycelial relationship, male and female organs, and investment of oogonia by numerous male elements.

F.—Mature oospore.

G, H.—Oogonium with nearly mature oospore shown in A and D, respectively, but without overlying antheridia; several male elements in equatorial plane are indicated by stippled outline of optical view.

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stitute the numerous male elements, envelops the oogonium in a maze of structures that under low magnification present the appearance of a halo. In the cane parasite this halo on an average is of more moderate width than in *P. myriandra*, owing to a pronounced up-curving habit exhibited frequently by the antheridia of the latter species. With respect to the sizes of their oogonia and oospores the two congeneric forms, as indicated in Table 2, show no great differences, the agreement being especially close if only the parthenogenetic apparatus of the tomato fungus is considered. As it would seem that the function served in the species described earlier by an abundance of oospores of parthenogenetic origin is here provided for by a liberal production of gemmae, the term "gemmifera" is proposed as a specific name for the fungus under consideration.

DESCRIPTION

Plectospira gemmifera, n. sp.

Mycelium 2 to 7 μ in diameter. Inflated sporangial elements 6 to 20 μ in diameter; efferent hyphae usually 5 to 10 μ at base, tapering generally to a diameter of 3 to 5 μ at tip. Sporangia sometimes very extensive, then often provided with plural efferent hyphae of which several may function, each delivering up to 400 or more zoospores. Gemmae produced in quantity, typically subspherical or pyriform, 35 to 60 μ (average 44.7 μ) in diameter; germinating without resting period, often as a sporangium, then giving rise to 25 to 100 zoospores, sometimes with the production of several supernumerary evacuation hyphae in addition to the one or two functional ones, or, especially after aging several months, germinating directly by the production of one or several vegetative hyphae. Zoospores on encystment usually 7 to 10 μ, in oversized examples up to 17 μ, in diameter; diplanetic, the cyst membrane after evacuation revealing a persistent cylindrical papillar modification approximately 2.5 μ in diameter. Oogonia mostly terminal on short branches or on longer hyphae, subspherical, 22 to 29 μ (average 25.2 μ) in diameter, provided with a wall 0.5 to 1 μ in thickness. Antheridia always present, 20 to 45 visible in upper and equatorial aspects, the total number probably ranging from 30 to 65 or more; mostly rudimentary, the smaller ones approximately 3 μ in diameter and 5 μ in length and usually without delimiting septum, the larger ones up to 8 μ in diameter and 15 μ in length, delimited by septum and evidently capable of function; mostly straight distended cylindrical, or curved cylindrical; borne in close arrangement on branches enveloping the oogonium, which arise from one or less often two or more hyphae separate from the hypha bearing the female organ. Oospore single, colorless, usually 19 to 25 μ (average 21.9 μ) in diameter, provided with a wall 1.1 to 1.8 μ (average 1.5 μ) in thickness, slightly eccentric (subcentric) in internal structure. Isolated from flaccid water-soaked rootlet of *Saccharum officinarum* L. collected near Thibodaux, La., in April, 1927.

SUMMARY

A water mold presumably identical with the one reported by Peters as the cause of "Wurzelbrand" of beets in Germany under the binomial *Aphanomyces laevis* De Bary was found attacking sugar-beet seedlings in Michigan. The fungus to which the name *A. cochlioides* was applied earlier reveals departures from De Bary's description in the smaller diameters of the oogonium and the oospore, in the greater thickness of the oogonial wall, in the regular diclinous origin of the antheridial branches, in the crowded disposition of these branches and the male organs on the oogonium, resulting frequently in cochleate arrangement, and in the absence from the antheridium of ventral lobulations. The excessive values given by Peters for thickness of the oospore wall are attributed to the use of degenerate material.

In inoculation experiments carried out in the greenhouse, *Aphanomyces cochlioides* demonstrated pronounced effectiveness in the causation of damping off of sugar-beet seedlings. A lesser degree of pathogenicity was manifested by two congeneric forms, one originally isolated from an oat root and the other from a diseased tomato root. These are described as new species under the binomials *A. camptostylus* and *A. cladogamus*, respectively. *A. camptostylus* is characterized by somewhat smaller oogonia and oospores than the beet parasite, a relatively thin oogonial membrane, frequent extensive spiral involvement of hyphal parts supporting the antheridia by those bearing the oogonium, and ready germination of oospores by the production either of vegetative germ tubes or a germ sporangium. *A. cladogamus* exhibits larger oogonia and oospores than *A. cochlioides*, a perceptibly thinner oogonial membrane, frequent monoclinous origin of antheridial branches, and at times moderate involvement of hyphal parts supporting the antheridium by those bearing the female organ.

Aphanomyces euteiches and *A. raphani* showed no appreciable pathogenicity to sugar-beet seedlings. These species resemble *A. cochlioides* in the absence of spiral involvement of hyphal parts supporting the sex organs, but differ from it in not exhibiting cochleate arrangment of antheridia and antheridial branches on the oogonium, as well as in the considerably greater dimensions of oogonium and oospore. Kendrick's observations on the characteristics of the blackroot parasite were in general confirmed; however, no instances of antheridial branches arising from the same hypha as the oogonial stalk were encountered, and his values for thickness of oospore wall would appear to have been based on degenerating material.

Plectospira myriandra in greenhouse experiments proved innocuous to sugar-beet seedlings, as did also a congeneric form, originally isolated from a diseased sugar-cane root collected in Louisiana, and now described as a new species under the name *P. gemmifera*. Of special taxonomic interest in relation to the definition of the genus to which these forms are assigned is the production by the cane-root fungus, in association with the specialized lobulate zoosporangium, of the same sort of intricate sexual apparatus with its extraordinary number of antheridial elements, mostly nonfunctional, as was described for the type species. The parthenogenetic oospores formed so liberally by *P. myriandra* are absent here, being replaced by relatively large subspherical asexual bodies, or gemmae, capable of surviving for several months, though not requiring any resting period for germination. On the intervention of suitable conditions the gemmae may give rise to germ zoosporangia often provided with one or two supernumerary evacuation hyphae, frequently nonfunctional; or, especially after some aging, they may directly produce a vegetative mycelium.

A method of isolating fungi from decaying host tissues, which has been found especially useful in instances involving parasitism by members of genera like Aphanomyces, Pythium, and Phytophthora, is described in detail. Pieces of the material are first placed in water; on the resumption of vegetative growth by the fungus they are removed, the free water thoroughly blotted off between absorbent paper, and the transfer to agar plates carried out in the usual manner.

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