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TWO WATER MOLDS CAUSING TOMATO ROOTLET INJURY

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TWO WATER MOLDS CAUSING TOMATO ROOTLET INJURY[1]

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INTRODUCTION

During May, 1926, the writer isolated some possibly parasitic fungi from affected rootlets of tomato (*Lycopersicum esculentum* Mill.) seedlings in greenhouses of the United States Department of Agriculture in the District of Columbia and at the Arlington Experiment Farm, Rosslyn, Va. The underground portions of these seedlings were, on the whole, in very good condition, injury being confined usually to a relatively small portion of the more minute rootlets, which exhibited yellowish or brownish discoloration at the tip and for several millimeters to a centimeter upward.

This material while scarcely in a condition to cause practical concern, yielded nevertheless a numerous array of fungous cultures referable to more than a dozen species, most of them to a certain degree parasitic. The genus Pythium was very liberally represented in this collection, as, indeed, might be expected from the very usual association of many of its members with rootlet-tip injury in numerous representatives of the higher plants. The mycelial form identified as *Rhizoctonia solani* Kühn (*Corticium vagum* B. & C.) made its appearance in scores of cases. A less predictable feature of these isolations was a frequent recurrence of two water molds, which, because of the paucity of information as to the association of members of the Saprolegniaceae with root injury in general, deserve further discussion.

OCCURRENCE OF APHANOMYCES EUTEICHES

One of the water molds which appeared more than a dozen times was, in the absence of cross inoculation experiments, provisionally identified as *Aphanomyces euteiches* Drech., a fungus known as the cause of a very destructive type of root rot of peas prevailing especially during seasons of excessive rainfall (*4*).[2] A comparison of the strains isolated from tomato roots with others derived from pea roots showed general agreement in morphological detail, including the very distinctive peculiarity presented in the sinuous inner contour of the thick oogonial wall. Mature oospores of the fungus were recognized in the tissues of a number of affected rootlet tips. With a few exceptions the rootlets that yielded *A. euteiches* yielded no other fungus. Appearances indicated actual though not severe pathogenicity—a degree of pathogenicity important not so much on account of any potential damage to tomatoes as on account of the increased opportunity afforded the parasite for

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[2] Reference is made by number (italic) to "Literature cited," p. 292.

maintaining itself until such time as an ensuing crop of peas becomes available. In regions where tomatoes and peas are cultivated on a example, in some sections of Maryland, the fungus would thus be sufficiently extensive scale to play important parts in a rotation, as for better able to survive from season to season in dangerous quantity.

Interchange

PLECTOSPIRA MYRIANDRA N. G.; N. SP.

OCCURRENCE

The second water mold, *Plectospira myriandra*, was encountered about as frequently as the first, but only on potted tomato seedlings from the greenhouses at Arlington farm. Its relationship to the host appeared to be quite similar, injury being limited to brownish discoloration and eventual death of occasional rootlet tips. The damage to the material examined was rather slight. Growths originally obtained on corn-meal agar plates were freed of contaminating bacteria by the method described by Brown (*1*), which consists in making transfers from the under side of thick, plain-agar plates. Thus purified, the fungus was subsequently cultivated on artificial media like corn-meal agar, Lima-bean agar, Lima-bean decoction, and pea decoction. Advantage was also taken of the well-known method of inducing zoospore formation by the transfer of vigorous mycelia to sterile water.

MORPHOLOGY

On corn-meal agar the fungus produces a colorless growth devoid of aerial mycelium, having much the general appearance of *Aphanomyces euteiches* or *A. helicoides* Minden (*7, p. 555–562*), and exhibiting, like these, a well-defined radial effect. Under the microscope the mycelium is seen to be composed of hyphae of low refringency, branching at moderate intervals. As in the species of Aphanomyces mentioned, each hyphal element maintains approximately the same diameter from its origin to its termination. The more delicate branches, such as are produced, for example, when portions of mycelium are transferred to water (fig. 1, A) and includding also the filaments bearing the antheridia, not infrequently measure less than 2μ in diameter—probably as slender mycelial elements as are known in the Saprolegniaceae.

Zoosporangia are obtained when fresh growth from liquid or agar media is transferred to water, corn-meal agar seemingly providing the most satisfactory substratum that has been used for their production. Under suitable conditions massive complexes are formed, composed of folded, inflated elements usually twice and not rarely three times the diameter of the largest vegetative hyphae and bearing short digitate branches—the whole a compact, intricate apparatus. The basal protions of Figure 1, B and D, represent two such structures of only moderate proportions. The larger examples, which measure more than 200μ in diameter, have a bulk five or ten times as great and are very easily seen with the naked eye. In Figure 1, C and F, are shown still simpler types, while the very simplest, consisting, indeed, of only a single inflated element, is represented in Figure 1, E. These complexes are apparently always borne terminally on a hypha of ordinary diameter, from which, on attaining definitive size,

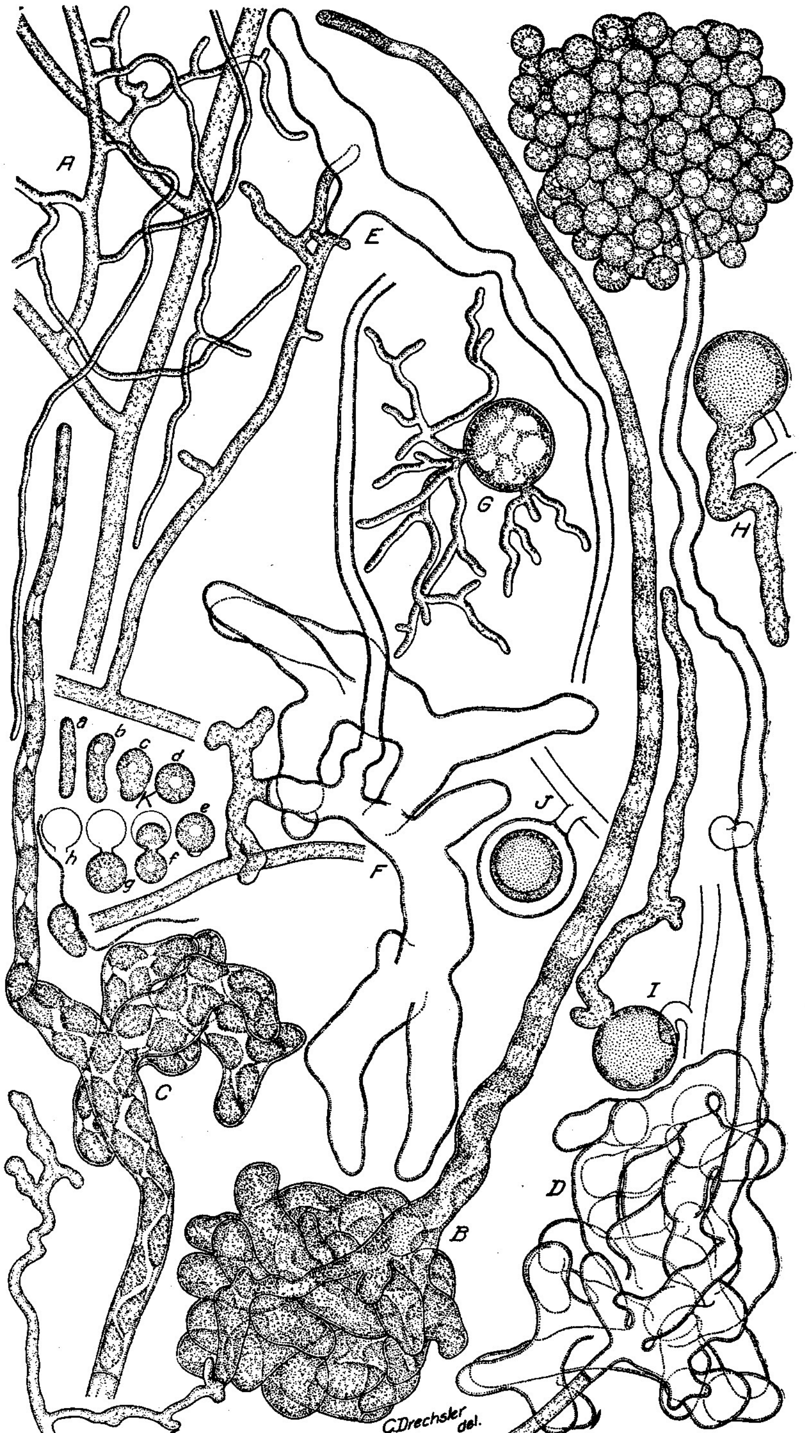

FIG. 1.—Vegetative condition, asexual reproduction, and germination of oospore of *Plectospira myriandra* as occurring in material grown in pure culture on corn-meal agar and transferred to sterile water. All figures drawn with aid of camera lucida, × 500. A, mycelium, the larger hyphae submerged in substratum, the very delicate filaments growing free into surrounding water; B, sporangium showing contents becoming separated into zoospore initials; C, sporangium of small size with zoospores ready for discharge; D, sporangium after evacuation with irregular mass of encysted zoospores at mouth of efferent element; E and F, two small sporangia after discharge; G, oospore in water germinating by delicate hyphae; H and I, oospore embedded in substratum germinating by single stout germ tubes; J, disappearance of oospore wall initiating process of germination; K, *a* to *h*, successive stages in rounding up and an encystment of zoospore followed by production of papilla, evacuation of contents, and escape in motile form

they are regularly set off by a fairly thick septum. As their enlargement nears completion, there is produced distally from one of the distended parts—often from one that appears as somewhat of a direct prolongation of the supporting hypha—a filamentous element of diminishing diameter that attains finally a length varying from 100μ to 600μ or 700μ. This filamentous element, which is sometimes marked by abrupt bends at irregular intervals or again may be of regular outline throughout its course, serves in the evacuation of the sporangium as an efferent tube.

Zoospore formation is associated, as in related forms, with protoplasmic changes (fig. 1, B), which in the efferent hypha differs in no particular from that characteristic of the genus Aphanomyces and results here also in a single series of protoplasts connected by delicate strands (fig. 1, C). Within the inflated elements the process results usually in two parallel series of such protoplasts. When the tip of the efferent filament gives way evacuation takes place, often with violent rapidity, at an approximate rate of perhaps 10 zoospores a second, so that scores of these bodies may be observed at the mouth of the tube in their original cylindrical shape, or in scarcely altered early stages of contraction. The total number of zoospores delivered from a single efferent element varies from a minimum of about 20 to 25 to a usual maximum of from 400 to 500, depending on the size of the sporangium involved. The sporangial complexes that exceed at all considerably the proportions of those shown in Figure 1, B and D, usually are provided with plural efferent tubes, each of which functions independently in evacuating portions of the aggregate apparatus, which may thus best be regarded as constituting a compound structure made up of the basal parts of a number of sporangia.

The zoospores remain massed at the mouth of the sporangium in an encysted condition for a period of approximately two hours (fig. 1, D), after which they escape from the cyst wall (fig. 1, K), swim about actively for a time, round up a second time, and under suitable conditions, germinate. The details associated with the discharge of the cyst contents are identical with those described for *Aphanomyces euteiches*, even with respect to the dimensions of the papilla and of the short evacuation tube that persists on the empty cyst wall.

The sexual stage of the fungus develops readily on various media, being produced in quantity, for example, on corn-meal agar. The oogonia appears as subspherical bodies, occasionally intercalary or laterally intercalary in position (fig. 2, D and E) but much more frequently formed terminally on relatively short branches from the stouter hyphae (fig. 2, A–C, F–I). These branches do not generally exceed in length the diameter of the oogonium and frequently measure considerably less. The septum delimiting the oogonium may be inserted nearly tangent to the subspherical part, but more typically is inserted somewhat lower, including, as it were, a portion of the stalk. In the mature parthenogenetic apparatus the oogonial wall often seems lightly sculptured internally, and at times it presents the appearance of numbers of minute, scattered pits. Whether these inconspicuous features are to be homologized with the pronounced pits distinctive of the oogonia of various genera of Saprolegniaceae is not altogether clear, but would seem very doubtful.

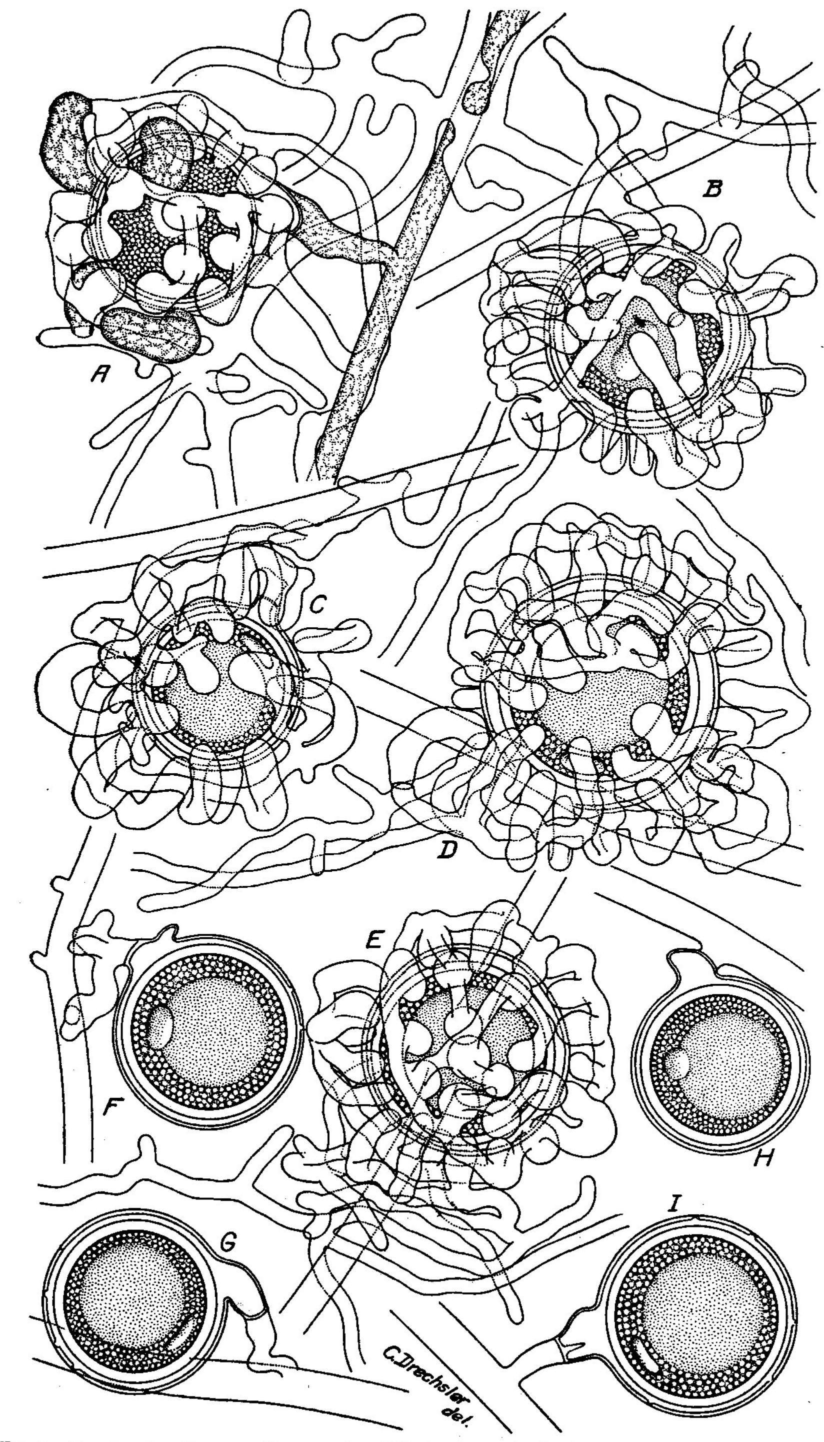

FIG. 2.—Sexual and parthenogenetic apparatus of *Plectospira myriandra* drawn from corn-meal agar cultures with aid of camera lucida, × 1,000. A to E, oogonia invested with numerous antheridial elements, terminal on short branches in A, B, and C; laterally intercalary in D and E (the four antheridia in A with contents shown by stippling are of potentially functional type); F to I, mature parthenogenetic oogonia with oospores

In most cultures from one-half to two-thirds of the eggs are usually produced parthenogenetically. (Fig. 2, F–I.) The remainder are formed in the presence of antheridial elements in extraordinary abundance. (Fig. 2, A–E.) The elements are uniformly of declinous origin, arising from delicate, sinuous hyphae. In the proximity of the oogonium one or several of these hyphae put out branches, the resultant filaments enveloping different regions of the female structure and giving off at short intervals, either directly or on short, spurlike branches, elements having the shape and the positional and contact relations usual for antheridia. Most of these elements never are delimited by septa and soon appear as empty structures in consequence either of the withdrawal or the degeneration of their contents. In most instances, however, several of the structures, usually terminal in position and often distinguished from the others by greater size, a thicker wall, and more extensive contact with the oogonium, will be found delimited by a septum and filled with dense protoplasmic material. (Fig. 2, A.) These structures have all the appearance of true antheridia. In more advanced material they are, like the rudimentary elements, devoid of contents.

Owing to the difficulty of accurate observation due to the intricacy of the apparatus, clear evidence as to the performance of these organs was not obtained, yet indications that their contents are discharged into the oogonium were not lacking. In any case, however, the presence of the antheridial elements could scarcely be interpreted as necessary for the development of the egg or as in any way promoting its formation. Indeed, the egg apparatus provided with them appeared in general to be considerably slower in maturing and slightly more subject to degeneration than the parthenogenetic apparatus, and it was also more likely to be of extreme dimensions. The antheridial elements are remarkable chiefly because they are exceptionally numerous; rarely fewer than 15 and sometimes as many as 35 were visible on the upper side and about the equatorial plane of the oogonium, and perhaps 10 or 20 more may be concealed beneath the oogonium. The virtual investment of the female organ with such a bristling array of curved structures is one of the striking characteristics of this species.

The oospore is regularly found singly in an oogonium, which it usually occupies almost completely, although there is nearly always a narrow space between the oospore wall and the oogonial wall which occasionally becomes more pronounced in width. The ripe oospore generally has a wall of moderate thickness, and contains a large spherical homogeneous structure somewhat eccentrically placed in a peripheral matrix. Within this matrix granulelike bodies of uniform size are arranged geometrically. They are often 3 or 4 layers deep on one side and 1 or 2 or more rarely 3 layers deep on the other. A strongly refringent homogeneous body oblate ellipsoidal in shape and 5μ or 6μ in diameter by 1.5μ to 2.0μ in thickness, can always be distinguished in the matrix, usually embedded between the outer and inner layer of granular structures, where it appears in profile as an elliptical occlusion. This body, entirely similar to homologous structures in the oospores of Aphanomyces, Pythium, and related genera, may possibly be the nucleus.

Germination of the oospores readily takes place on transfer to water. The oospore wall loses its visibility evidently as a result of some process of gelatinization (fig. 1, J), after which the contents become distributed throughout the interior of the oogonium. A single stout germ tube corresponding in diameter to the larger vegetative hyphae (fig. 1, H and I), or one or several more richly branching systems of hyphae corresponding to the more delicate mycelial elements (fig. 1, G), may be produced, apparently depending largely on whether the oospore is embedded in a matrix (corn-meal agar, in the trials under discussion) or is immediately in contact with water. In a few instances the single germ tube appeared to terminate abruptly in a small lobulate sporangium, making it seem probable that the production of germ sporangia, frequent in *Aphanomyces euteiches*, occurs here occasionally.

TAXONOMY

In the taxonomy of the Saprolegniaceae major importance has been assigned to the type of development exhibited by the zoospores as well as to the morphology of the zoosporangium. With respect to the former feature, the tomato parasite reveals complete similarity to Achlya and Aphanomyces; with respect to the latter, a partial combination of the characteristics of both these genera. In Achlya the zoospores are fashioned within a massive sporangium, usually of tapering-cylindrical shape, regular in outline, such apical modification for evacuation as may be present being generally very slight. Zoospores may be produced also by the gemmae that develop in many species. These bodies frequently occur in irregular forms, as is evidenced, for example, by Coker's figures of *Achlya flagellata* Coker (*2, pl. 37, fig. 7*) and *Achlya megasperma* Humphrey (*2, pl. 44, fig. 9*). Gemmae of this type and other structures, like some of the more irregular "Conidienstände" depicted by Maurizio (*6, Taf. I, figs. 13, 27*), show a certain degree of resemblance in shape to the simpler, more openly disposed sporangia produced by the fungus under consideration. However, even the most copiously branched gemmae of Achlya or, indeed, of any recognized genus of the Saprolegniaceae, fail to exhibit the measure of complexity and involvement found in the better developed sporangia of the fungus discussed in this paper. These latter, moreover, can scarcely be regarded as "Hemmungsbildungen," to which Klebs' (*5*) researches have reduced many of the structures designated as gemmae. Their production is most luxuriant from fresh mycelium that previous to its transfer to water has been well nourished and is not thereafter exposed to serious bacterial contamination—conditions under which development would not ordinarily be arrested. Nor do they require a resting period for zoospore production like the generality of gemmae. They are essentially short-lived bodies that in the absence of conditions suitable for the fashioning and discharging of zoospores soon degenerate, or, at best, proliferate vegetatively after a promiscuous manner.

With respect to the vegetative thallus a pronounced difference between the tomato parasite and Achlya is evident, the mycelium of the former being, of course, decidedly smaller in diameter. On the other hand, the similarity to the thallus of Aphanomyces is striking, not only as to the thickness of the hyphae, but also as to the general appearance under the microscope. This obvious resemblance, together with the uniseriate production of zoospores in the efferent hyphae, would seem indicative of a close affinity to that genus—an affinity that finds a peculiarly apt parallel in the genus Pythium, assuming for the latter the wider limits more generally adopted. The genus Pythium includes species entirely analogous to Aphanomyces, in which externally undifferentiated filaments serve as sporangia, as well as a series of forms like the widely distributed parasite, *P. aphanidermatum* (Eds.) Fitz., in which sporangia are represented by communicating systems of distended digitate or lobulate elements, often intricately involved—closely similar in composition to those described in this paper. Schröter (*8*) regarded the Pythium types possessing purely filamentous sporangia as sufficiently distinctive to establish a separate genus, Nematosporangium. As has been pointed out, such a disposition might prove profitable if accompanied by the addition of another genus including the forms with lobulate zoosporangia (*3*).

Assignment of the present fungus to Aphanomyces, to which it seems most closely related, could apparently be accomplished only in contravention of the feature most distinctive of that genus. The adoption of a new genus would seem less objectionable, especially as it is not improbable that as more terrestrial types of Saprolegniaceae come to light other forms of similar morphology may require taxonomic treatment. Such a genus is therefore proposed here under a name meaning "plaited coil"; and it is hoped that a further characterization of the fungus may be conveyed in the specific name suggestive of the abundance of antheridial elements.

DIAGNOSIS

Plectospira, n. g.

Mycelium slender, sparingly or moderately branched. Zoosporangia composed of inflated elements, often compacted into an irregular complex, within which zoospores are differentiated in two or more series, together with a prolonged filamentous element within which zoospores are formed in one series and by which the entire organ is evacuated. Zoospores encysting at the mouth of the efferent hypha, later escaping from their cysts and swarming. Oogonia intercalary or terminal. Antheridia absent or present. Oospores single and somewhat eccentric (subcentric) in internal structure.

Plectospira myriandra, n. sp.

Mycelium 1.8μ to 6μ in diameter. Inflated elements of sporangia 6μ to 18μ in diameter; efferent hyphae usually 5μ to 10μ at base, generally tapering more or less to a diameter 3.5μ to 4.5μ at tip. Sporangia sometimes very extensive and compound; then provided with plural efferent hyphae, each delivering up to an approximate maximum of 500 zoospores. Zoospores, after encystment, 6μ to 12μ in diameter, usually 9μ to 10μ, developing a papilla 2.5μ to 3μ in diameter and 1μ long, the cylindrical wall of which after evacuation persists on the empty cyst wall. Oogonium mostly terminal on short branches, more rarely laterally intercalary or intercalary, subspherical, smooth, 15μ to 33μ in diameter, usually 23 to 29μ, provided with a wall generally approximately 0.5μ, more rarely up to 1μ in thickness. Antheridia absent, or frequently 25 to 55 in number, mostly rudimentary, the smallest approximately 3μ in diameter and 5μ in length, often without delimiting septum; the largest, up to 6.5μ in diameter and 25μ in length, delimited by septum and often potentially functional in appearance; mostly straight, distended cylindrical or curved cylindrical; diclinous in origin, borne in close arrangement on a number of branching systems arising from delicate hyphae. Oospore, single, 13μ to 30μ, usually 20μ to 27μ in diameter, provided with a wall 1.1μ to 1.9μ, usually 1.5μ in thickness, slightly eccentric in internal structure. Mildly parasitic, causing discoloration and death of rootlet tips of *Lycopersicum esculentum* Mill. in greenhouse at Arlington Experiment Farm, Rosslyn, Va., in May, 1926.

In the foregoing diagnosis of the genus Plectospira, as generally in the definition of taxonomic groups within the Saprolegniaceae, more stress is laid upon the morphology and development of the structures concerned in asexual reproduction than upon the morphology of the sexual apparatus. Except for the internal structure of the oospores, the degree of importance to be attributed to the latter will remain problematical until congeneric forms have been discovered. The presence of antheridial elements in such abundance as to envelop the oogonium to a considerable extent, while not common, is far from being unknown among other genera of the Saprolegniaceae, rather extreme conditions of such envelopment being found, according to Von Minden (7), in *Achlya prolifera* (Nees) deBary and *Achlya oblongata* deBary. Among the forms with single oospores, some species of Aphanomyces show a certain degree of such envelopment, more, however, because of the relatively large size of the several antheridia present than because of their number.

A condition much more similar to that prevailing in *Plectospira myriandra* was described by de Wildeman (9) in an account of a fungus he found parasitic on the oogonia of Characeae and designated as *Achlyopsis entospora*, the type of a new genus. The oogonia were characterized as globose or elliptical, formed terminally on branches, and separated from the thallus by a thick septum often inserted at some distance from the swollen portion. Numerous antheridial filaments derived apparently from several mycelial hyphae sometimes completely invested the oogonium "comme d'un réseau ou d'une couche continue." The abundance of antheridial filaments was held to suggest Achlya, but because of the oospores occurring singly in the oogonia and the development of the latter within the host—two characters which the author regarded as exceptional in the Saprolegniaceae—the fungus was referred to the Peronosporaceae. No sporangial stage was recognized as associated with the parasite. In another portion of the text, however, the author described certain structures also found occurring within the affected oogonia of Characeae:

"Comme le montrent nos figures, ces mycéliums internes etaient constitués par un gros filament ramifié, contourné en tous sens et formant une masse pelotonnée plus ou moins volumineuse. De certaines portions de ce thalle coralloïde naissaient des filaments étroits s'enfonçant dans le milieu; d'autres portions naissaient des ramifications, primitivement assez larges, s'amincissant progressivement, traversant la paroi de l'oogone et se prolongeant dans l'eau."

Although de Wildeman recognized these coralloid structures as closely similar to certain mycelia produced by *Plasmopora viticola* (B. and C.) Berl. and De T., a comparison of his figures (9: Pl. XI, figs. 1–4) with Figure 1, B–E, reveals a much more complete similarity. There can, indeed, be little doubt that the "thalles coralloïdes" represent evacuated zoosporangia of the same external morphology as that characteristic of the tomato parasite. Some consideration, therefore, was given to the plausibility of a taxonomic disposition whereby this parasite was to be assigned to de Wildeman's genus appropriately emended by referring the coralloid structures to it and assuming for them the rôle of zoosporangia with Achlya-like development. It appeared best, however, to reject this alternative, as de Wildeman reported among certain other Phycomycetous forms present in the same material as his *Achlyopsis entospora*, all apparently in rather intimate confusion, two new species of Pythium, *P. characearum* and *P. gibbosum*. These species also were based solely upon their sexual stages. In the absence of information to the contrary, the probability is presented of the empty "thalles coralloïdes" representing evacuated lobulate zoosporangia of one or the other of these species of Pythium—a probability quite as strong as that they were associated with *Achlyopsis entospora* and represented zoosporangia that had become evacuated after the manner described in this paper for the tomato parasite. That the species to which the binomial *P. characearum* was applied may be a form with lobulate zoosporangia is made more probable by the description of its mycelium as "très abondant, il forme dans le milieu, des pelotes de filaments très enchevêtrés; il semble porter des grappes d'oosporanges."

The structure of the oospore of *Achlyopsis entospora*, with its very thick wall, and its more or less homogeneous contents containing 1 to 4 refringent globules, suggesting in these details little similarity to the structure of the oospore of the fungus affecting tomato rootlets, provides an additional reason for not referring the latter to de Wildeman's genus. As, however, the figures given by that author indicate that his material may have suffered considerable degeneration, it would be inadvisable to attribute too much significance to a comparison of such features.

SUMMARY

Two water molds have been found causing discoloration and death of occasional rootlet tips of tomato seedlings in greenhouses. One was identified as *Aphanomyces euteiches*, known previously as a cause of root rot of peas. The other is described as the type of a new genus resembling Aphanomyces in general habit and in zoospore development but differing from it in having a differentiated zoosporangium composed typically of an involved complex of inflated elements, and a filamentous efferent hypha.

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