TWO SPECIES OF PYTHIUM OCCURRING IN SOUTHERN STATES

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In several recent papers (19, 20, 21, 22) supplementary discussion and illustrations have been supplied for 11 of the 15 species of *Pythium* that I presented as new in diagnoses published 12 years ago (14). Similar treatment is accorded herein to *P. myriotylum*, one of the remaining 4 species, which occurs in some southern States as a parasite on economic plants. In addition, another fungus, likewise of southern origin, though not intimately akin to *P. myriotylum*, is described as a new member of the same genus. For facility of comparison all figures relating to the formation of oospores in both fungi are given at the same magnification (*i.e.*, × 1000) used for such illustrations in previous accounts; and similar magnification is employed for illustrations showing early stages in the germination of oospores. Owing to limitations of space, the figures pertaining to asexual reproduction by sporangia of mycelial origin are again given at half this magnification (*i.e.*, × 500), as are also the equally rangy figures showing zoosporangia produced in the later stages of oospore germination. The smaller scale of magnification applies no less advantageously to the drawings of the rangy branching clusters of appressoria in *P. myriotylum*. The elaborate intrication of hyphae, often observable when either of the 2 fungi is encountered by any one of several other oomycetes associated with root rot, also pertains to the vegetative stage; but as it entails frequently a complicated arrangement of parts, the larger scale of magnification has appeared generally preferable for the drawings relating to antagonistic or parasitic action in dual cultures.

PYTHIUM MYRIOTYLUM

In May, 1924, the late Dr. W. A. Orton, then in charge of investigations on truck crop diseases in the Bureau of Plant Industry, submitted to me a tomato (*Lycopersicon esculentum* Mill.) plant from South Carolina, with instructions to isolate from its discolored rootlets whatever parasitic fungi might be present in them. Among the several oomycetes obtained in pure culture from the affected material was a species of *Pythium*, which, except for its less massive development of sporangial lobules and its much readier production of zoospores in irrigated agar preparations, strongly resembled an allied form that I had often isolated from discolored roots of maize (*Zea mays* L.). The same species of *Pythium* was recognized the next month in a culture derived from a decaying cucumber (*Cucumis sativus* L.) fruit selected in Philadelphia as being illustrative of damage observed in a carload lot of cucumbers shipped from South Carolina. When the fungus was introduced by wound inoculation into sound marketable cucumbers, a rapid watery decay resulted, much like the "cottony leak" attributable to *P.*
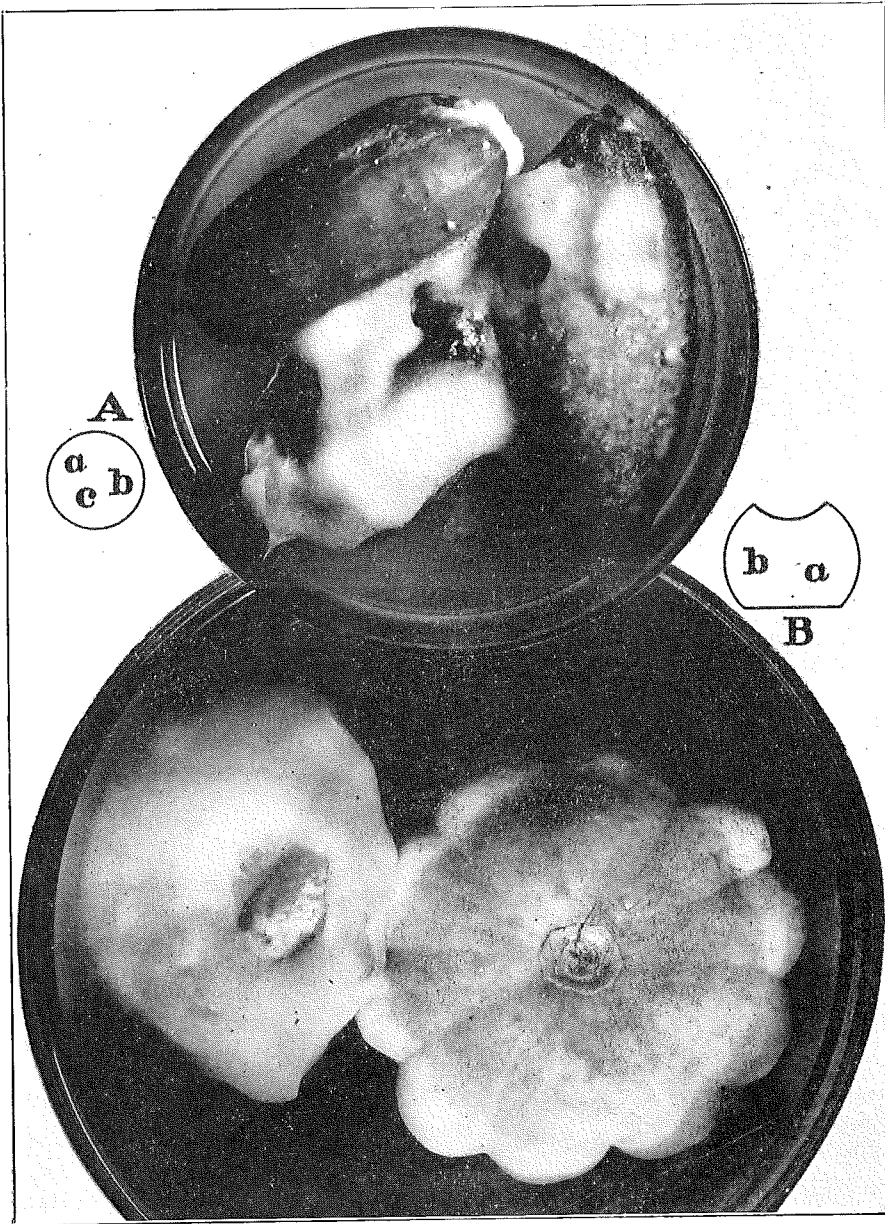
FIG. 1.   A. Three cucumbers (a, b, c) infected with *Pythium myriotylum;* photographed 5 days after inoculation of specimen c was accomplished by placing on its uninjured epidermis a sizable slab taken from a young maize-meal-agar plate culture of the fungus, and then incubating in a damp chamber at 28° C.   The parasite during the period of incubation spontaneously invaded the neighboring specimens a and b; about × ⅖. B. Two specimens of pattypan squash (a, b), each inoculated with *P. myriotylum* through an incision at the flower scar; extensive infection of the older and harder specimen (a) is shown only in a water-soaked area reaching nearly to the scalloped margin; the younger and tenderer specimen (b), invaded throughout, has become clothed in aerial mycelium; about × ½.

butleri Subr. For that matter, wounding was found unnecessary for successful infection. Under humid conditions and at fairly high temperatures (25 to 35° C.) mere application of sizable slabs of agar medium, newly permeated with mycelium, to uninjured epidermis consistently led to watery decay of the vegetable, and to its envelopment in abundant cottony mycelium (Fig. 1, A, a, b, c), which, on encountering adjacent cucumbers, caused their destruction in turn. Although this strong tendency toward aerial parasitism on cucumbers had been found alien to the maize-root fungus (9) its recognition as a physiological feature regularly associated with less pronounced sporangial lobulation and readier zoospore development required confirmation in additional cultures of separate origin.

Eleven such cultures, among a larger number referable to *Pythium butleri* and *P. acanthicum* Drechsl., were isolated early in June, 1925, from separate watermelon (*Citrullus vulgaris* Schrad.) fruits found partly destroyed by blossom-end rot in fields near Leesburg and Bradenton, Florida, and Thomasville, Georgia. A few weeks later 5 other cultures of the fungus under consideration were isolated from separate eggplant (*Solanum melongena* L.) fruits found damaged in fields near Bradenton by a ground rot closely resembling the "cottony leak" in the 18 similar specimens which yielded the cultures of *P. butleri* that, in a report (10) published at the time, were referred to *P. aphanidermatum* (Eds.) Fitzp. Under warm, humid conditions, introduction of the fungus into watermelon and eggplant fruits by wound inoculation always eventuated in watery decay of these vegetable products. The uninjured epidermis of both these fruits proved resistant to invasion, though in a few instances blossom-end infection, quite similar to spontaneous infection, resulted when a slab of agar medium newly permeated with mycelium was applied to the flower scar for 2 or 3 days. Eggplant fruits invaded by the fungus, whether from artificial inoculation or spontaneous infection, often afforded a copious growth of aerial mycelium (Fig. 2, A, B), but infected watermelons, with their hard rind of strongly indurated tissues, permitted no external development of the parasite. When slabs of agar medium, newly permeated with mycelium derived from any of the 16 cultures isolated from watermelon and eggplant fruits, were applied to uninjured cucumbers, watery decay promptly ensued, with abundant production of cottony aerial mycelium; and the aerial mycelium, on reaching other uninjured cucumbers, would closely invest them, penetrate into them, and thus destroy them in turn. In view of their capacity for aerial parasitism, as well as of their rather moderate sporangial lobulation and their ready production of zoospores in irrigated agar preparations, these 16 cultures, while generally resembling the maize parasite, differed from it in the same particulars as the 2 cultures previously isolated from material originating in South Carolina. The 18 cultures, therefore, were considered to belong to a separate species, which, in 1930, I described under the binomial *P. myriotylum*, 2 years after I had presented the maize parasite as *P. arrhenomanes* (12).

Thus at the time of its description *Pythium myriotylum* had become known to me as a root-rotting parasite only through a single encounter. Later, in 1931, I recognized the species in a number of cultures received from S. C. J. Jochems of the Deli Proefstation at Medan, Sumatra, where they had been isolated in connection with studies on a serious foot rot most
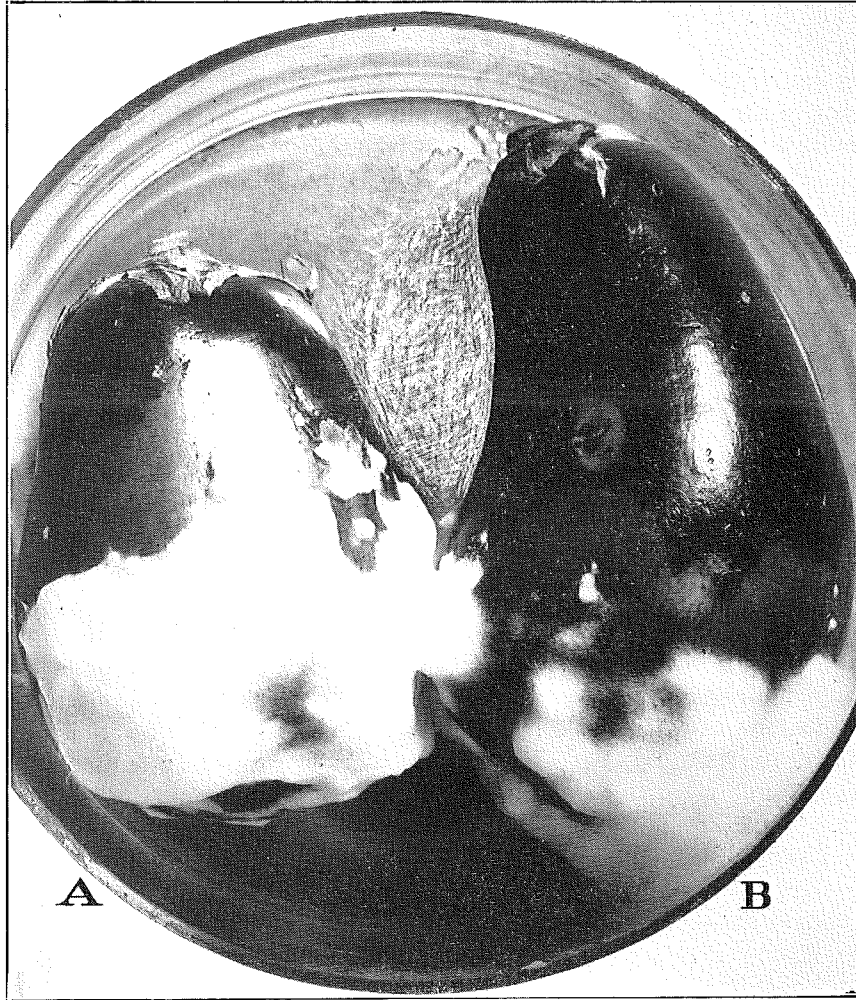

Fig. 2. Two eggplant fruits (A and B) infected with *Pythium myriotylum*; photographed 5 days after each had been inoculated by introducing the fungus into an incision at the stylar scar; incubated in a damp chamber at 28° C.; about × ½.

often attacking tobacco (*Nicotiana tabacum* L.) seedlings newly transplanted from seedbed to field. In an extended account, published in 1927, Jochems (25) had given a full description of the disease in question, denominating it as "parasitaire Stengelverbranding" or "parasitic stemburn," and had indicated as causal organisms 4 unidentified species of

Pythium, which he designated provisionally by the letters A, B, C, D, though holding that *P. debaryanum* Hesse and *P. aphanidermatum* were probably included among them. Somewhat earlier, to be sure, in a list of plant diseases and pests attacking cultivated plants in the Dutch East Indies (23), responsibility for causation of "Stengelverbranding" was attributed to species of *Pythium* cited under the 4 binomials *P. debaryanum, P. butleri, P. polyandrum,* and *P. nicotianae.* Descriptions applicable to the latter 2 binomials have never been supplied. However, in 1934, Meurs (27) made known that the fungus cited by van Hall (23) in 1925 as *P. polyandrum* was the same as *P. myriotylum,* which species, together with 2 congeneric forms, he had discovered in scores of cultures isolated by him from diseased tissues of tobacco plants found affected with stemburn in Sumatra during the period from 1929 to 1932. With respect to the nomenclature of the parasite, Meurs dismissed van Hall's binomial from consideration as a *nomen nudum.* In the meantime, incidentally, the specific component of that binomial—or rather an orthographic variant not considered different according to established rules of botanical nomenclature (3, p. 22, Art. 70, Note 4) —had been applied by Sideris (32) to a closely related and unquestionably congeneric fungus which he described as new under the binomial *Nematosporangium polyandron,* but which Rands and Dopp (30) have subsequently found referable to *P. arrhenomanes.*

Further indication that *Pythium myriotylum* occurs in affected vegetative parts of crop plants was provided when the fungus came to light in one of 3 pure cultures submitted to me for identification in March, 1940, by Dr. M. N. Walker of the Watermelon and Ornamental Field Laboratory at Leesburg, Fla., where they had been isolated from watermelon seedlings (35). Recently, too, the parasite has been reported unambiguously by additional observers in foreign lands. In 1936 Park (29) noted its occurrence as the cause of a soft rot affecting ginger (*Zingiber officinale* Rosc.) imported from India and planted in Ceylon. Subsequently Uppal (33) stated that the fungus causing soft rot of ginger in Surat had been determined to be *P. myriotylum.* According to Wager (34) *P. myriotylum,* together with 2 congeneric forms, was isolated once from papaw (*Carica papaya* L.) plants affected with "foot rot" in South Africa. Though a species of *Pythium,* isolated in Sierra Leone from garden bean (*Phaseolus vulgaris* L.) plants affected with wilt, was determined only rather indefinitely as being near *P. myriotylum,* Deighton's account (7) of the manner in which under moist conditions its woolly mycelium soon covered the stems of infected plants and then spread over the ground to attack other seedlings in its path, very aptly describes the growth habit of *P. myriotylum.*

That *Pythium myriotylum* may really have been the species encountered by Deighton appears the more likely from its aggressive parasitism on snap beans under experimental conditions (Fig. 3, A, B). Such parasitism on the uninjured immature edible pods of garden beans was reported in 1927 by Harter and Whitney (24), who found the fungus capable of bringing
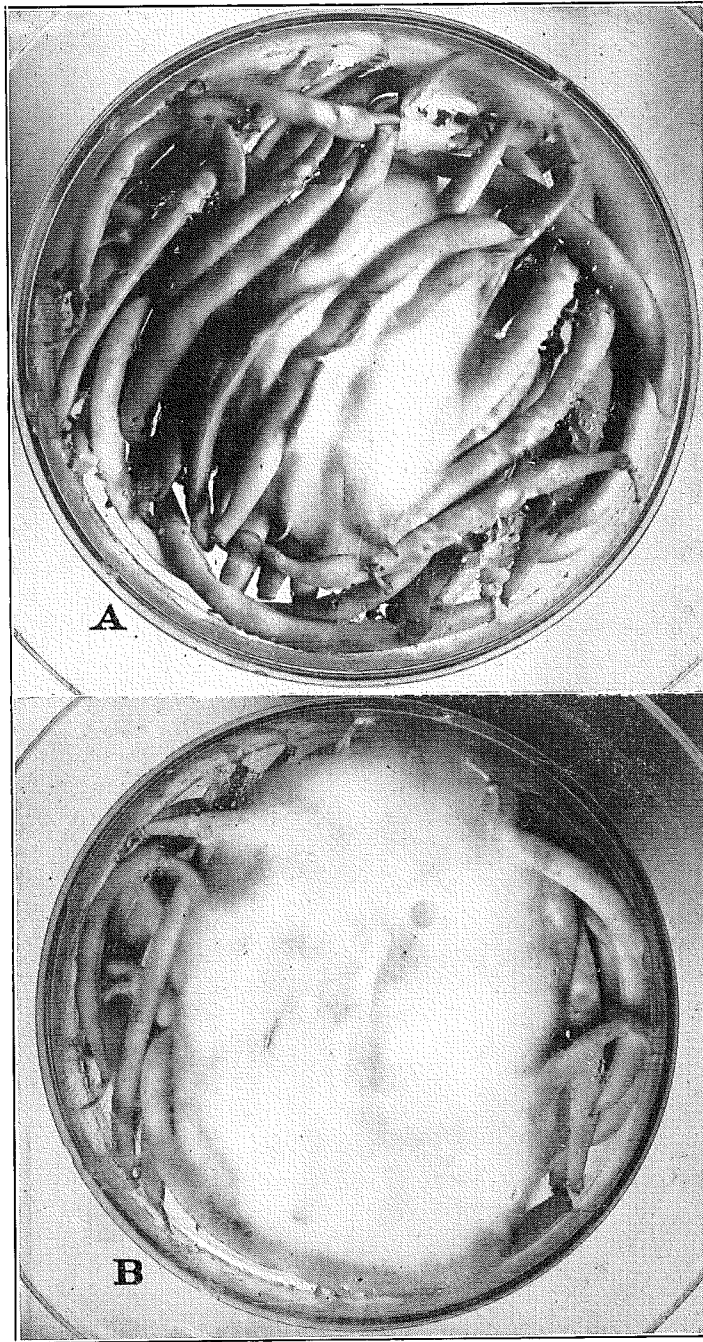
FIG. 3. String beans showing progressive attack by *Pythium myriotylum*, after a sizable slab of maize-meal-agar medium, permeated with mycelium, had been placed on the uninjured pods in the center of the dish. A. Photographed after incubation at 28° C. for 3 days. B. Same material photographed 2 days later, that is, after incubation at 28° for 5 days; about × ½.

about a decay of this vegetable product very similar to the "nesting" caused spontaneously in transit by the congeneric *P. butleri*, designated by them as *P. aphanidermatum*.   Like cottony leak of cucumbers, the decay of bulked snap beans caused by *P. myriotylum* is accompanied by production of profuse cottony mycelium.   Profuse aerial mycelium is usually produced also in the destruction of summer squashes (*Cucurbita pepo* L.) under moist conditions, following artificial inoculation with the fungus; the larger, older, and somewhat harder specimens of this vegetable (Fig. 1, B, a) permitting such production less promptly than younger and tenderer specimens (Fig. 1, B, b).   Wound inoculation of the parasite into muskmelons (*Cucumis melo* L.) and Cassaba melons (*C. melo* var. *inodorus* Naudin) ordinarily leads to no conspicuous cottony development externally, though the flesh within the hard rind often becomes extensively permeated with mycelium, and, though aerial mycelium often is produced within the central cavity.

In pure culture, especially at rather high temperatures, *Pythium myriotylum* extends its mycelium more rapidly than most allied forms.   Accordingly, it must be reckoned, together with such familiar pathogenic species as *P. ultimum* and *P. butleri*, among the fastest-growing of fungi. Further similarity to the 2 species mentioned is apparent not only in the somewhat coarse texture of its mycelium, but also in the disorderly arrangement and promiscuous ramification of its mycelial filaments.   On maize-meal-agar media, or on other media not rich in nutrients, aerial mycelium may be produced in only small quantity, particularly if the overlying or surrounding air is deficient in moisture.   However, if a humid atmosphere is maintained in a Petri dish containing a plate culture prepared with a medium rich in nutrients as, for example, Lima-bean agar, the dish often becomes completely filled with aerial mycelium of such density and firmness that the basal part of the container may be held up loosely suspended from the lid.

Microscopic examination of the under side of a Petri-dish culture prepared with maize-meal agar, usually shows everywhere a generous scattering of knob-like appressoria in contact with the glass floor.   When thus produced at the interface of agar medium and glass, these organs commonly measure about 7 or 8 µ in diameter, and consist of swollen terminations on rather short lateral branches, though their development as lateral protuberances on longer hyphal elements is not infrequent (Fig. 4, A, B).   With respect to the abundance of such intramatrical appressoria, *P. myriotylum* appears comparable to *P. butleri* and other strongly parasitic members of the genus.   However, with respect to the abundance of its production of appressoria on the surface of solid objects encountered by its aerial mycelium, the fungus is unrivalled by any congeneric form known to me.   In Petri-dish cultures with copious aerial development the ceiling of the container often is beset everywhere with dense clusters of appressoria borne on systems of closely ramifying branches (Fig. 4, C, D).   The adhesive organs formed here usually measure from 8 to 11 µ in diameter, and thus are notice-
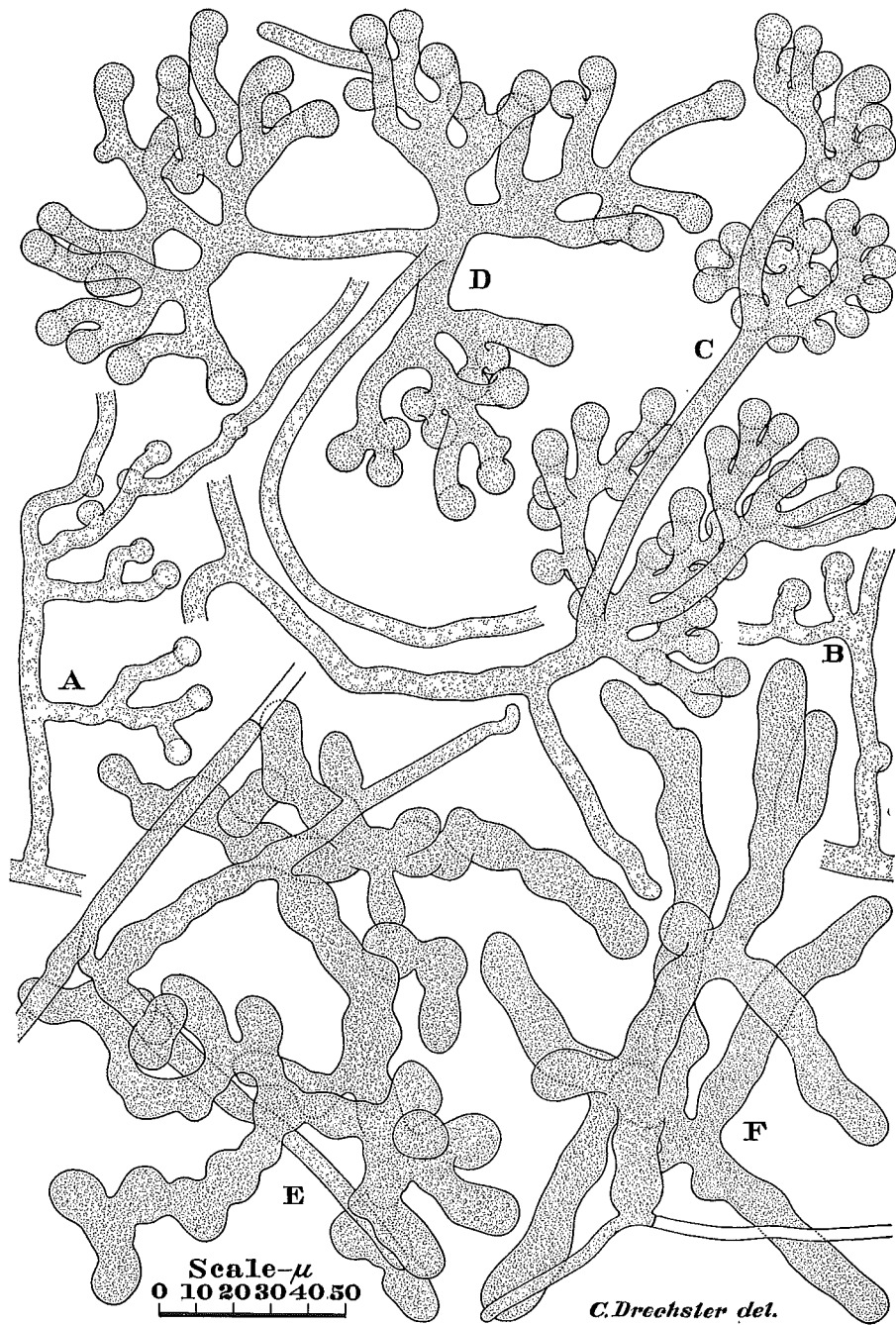
FIG. 4. Vegetative and asexual reproductive structures of *Pythium myriotylum* produced in Petri-dish cultures; drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. A, B. Portions of submerged mycelium bearing appressoria in contact with floor of container. C, D. Portions of aerial mycelium bearing appressoria in contact with ceiling of container. E, F. Systems of swollen sporangial branches formed on surface of maize-meal-agar medium.

ably larger than the homologous organs produced under the medium.   Unquestionably the abundant development of aerial appressoria by the fungus is related to its capacity to operate as an aerial parasite in the causation of such trouble in bulked vegetables as cottony leak or nesting.

When grown in plate cultures on maize-meal agar or Lima-bean agar, *Pythium myriotylum* often produces, especially in areas where the surface of the medium is moist with water of condensation, more or less branched systems of swollen digitate elements (Fig. 4, E, F; Fig. 5, A).   Like the homologous, though usually more extensive, ramifying complexes produced under similar conditions by *P. butleri* and *P. complens* Fischer (= *P. torulosum* Coker and Patterson), these branched systems, on being bathed in fresh water, give rise to evacuation tubes and undergo transformation into zoosporangia; the smaller systems usually being converted into single zoosporangia, the more extensive ones into plural zoosporangia.   Development of swollen branches takes place in some measure also when young undifferentiated mycelium is supplied with fresh water under conditions suitable for prompt production of zoospores; noticeably swollen elements being formed when, for example, small pieces of a newly invaded watermelon or cucumber, or small slabs excised from a young Lima-bean agar culture, are transferred to a shallow layer of water.   Though some of the sporangia partitioned off as separate reproductive units in such a preparation may include perhaps a dozen swollen branches (Fig. 5, B) together with hyphal parts of smaller volume and an evacuation tube (Fig. 5, B, t), many other sporangia may consist of a rather long portion of unmodified filament together with only a few swollen branches (Fig. 5, C, D).   Indeed, many of the smaller sporangia may include no perceptibly swollen elements whatever (Fig. 5, E).   Although an evacuation tube arising from a swollen part (Fig. 5, B, t; F, t), or one arising laterally from a rather wide undifferentiated hyphal part (Fig. 5, E, t), is usually recognizable as a special structure, similar distinctiveness is ordinarily absent when an evacuation tube is formed through prolongation of an outwardly unmodified branch or filament (Fig. 5, C, D).   Functional frustration of evacuation tubes seems rather more frequent here than in most congeneric forms; the frustrated tube soon being walled off in whole or in part by deposition of a septum (Fig. 5, F, a), and a new evacuation tube thereupon being put forth from some other position (Fig. 5, F, t).

Zoospore formation in *Pythium myriotylum* is associated with the sequence of internal changes usual for members of the genus.   As the evacuation tube attains definitive length, and forms a hyaline cap of dehiscence (Fig. 5, B, t), the protoplasmic contents of the sporangium show increasing vacuolization.   The hyaline cap suddenly yields to become inflated into a vesicular membrane as the migrating sporangial contents accumulate within it.   Once the migration is completed the undifferentiated granular mass (Fig. 5, C; F, t) undergoes division, and in the course of about 20 or 25 minutes is transformed into laterally biciliate, motile zoospores (Fig. 5, D,
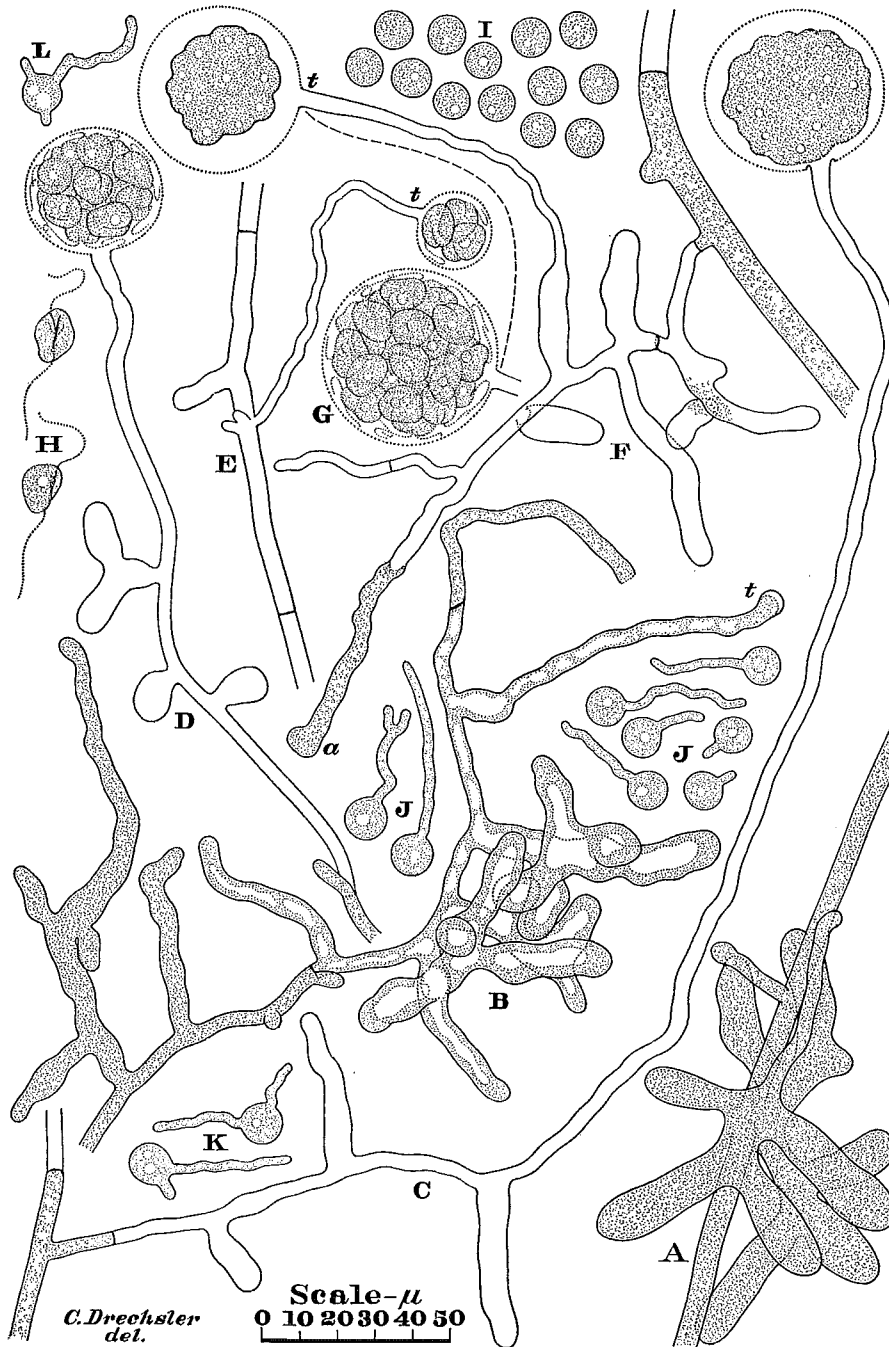
FIG. 5. Asexual reproductive apparatus of *Pythium myriotylum;* × 500 throughout. A. Hypha bearing distended sporangial branches. B. Vacuolate sporangium shortly before discharge. C. Sporangium shortly after discharge. D, E. Empty sporangia with their zoospores nearly ready to escape. F. Sporangium that, after frustration of its first evacuation tube (a), has formed a vesicle at the tip of a second tube (t). G. Same vesicle at a later stage. H. Motile zoospores. I. Encysted zoospores. J, K, L. Germinating zoospores. (t, evacuation tube.)
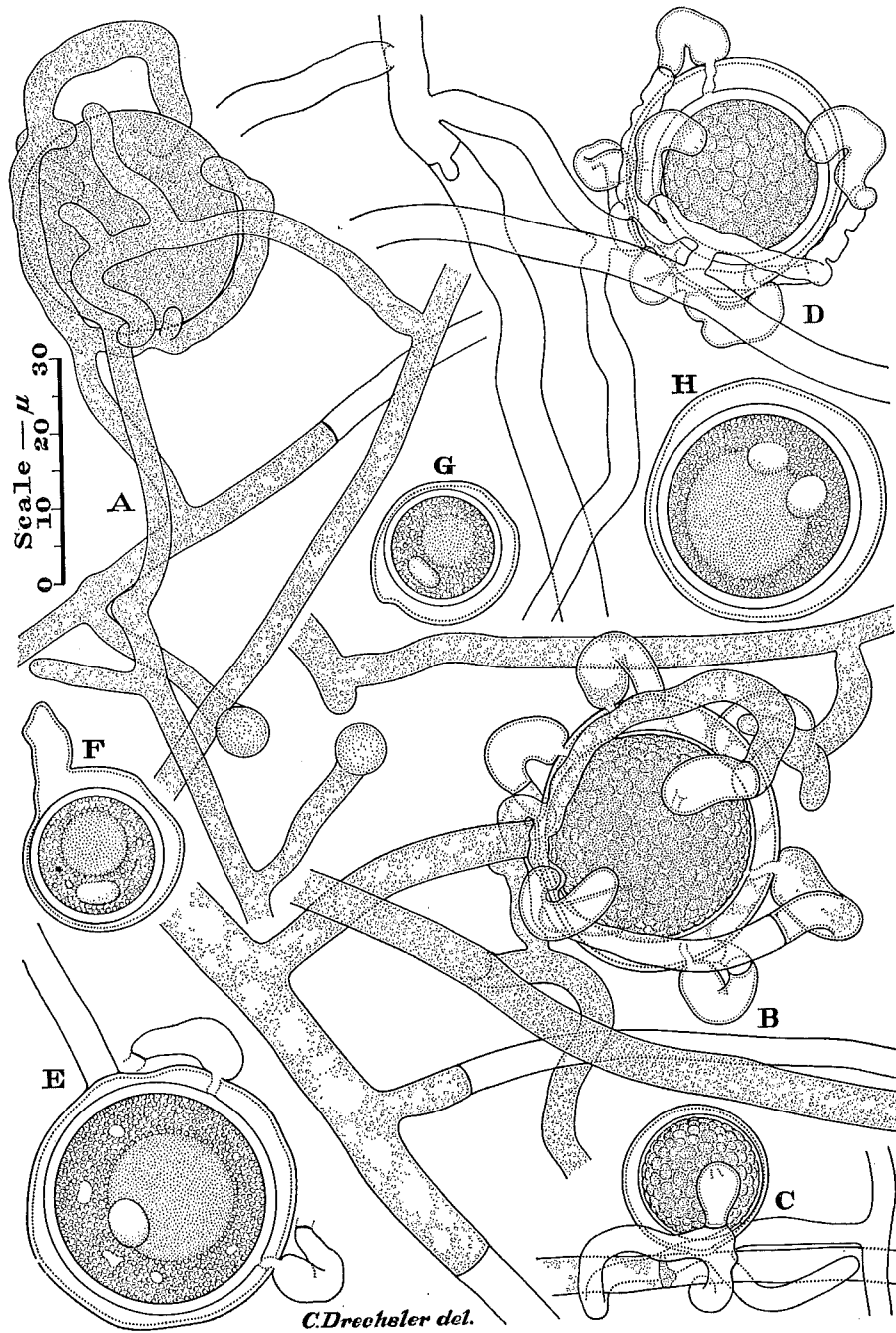
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FIG. 6.   Sexual reproductive apparatus of *Pythium myriotylum* formed in maize-meal-agar cultures; drawn to a uniform magnification with aid of a camera lucida; ×1000 throughout.   A–D. Immature units showing antheridial relationships.   E. Large oogonium with mature oospore; showing two antheridia in profile view.   F–H. Oogonia, each with a mature oospore.

E, G). After increasingly violent activity the zoospores escape from the disintegrating vesicle and swim about for some time (Fig. 5, H) to round up into subspherical cysts (Fig. 5, I), which sooner or later germinate by the production of a single germ tube (Fig. 5, J), or of 2 (Fig. 5, K) or even 3 (Fig. 5, L) germ tubes.

Pythium myriotylum, whether growing in the tissues of host plants or in various artificial agar media, usually shows fairly abundant sexual reproduction. The earlier stages in the enlargement of the subspherical oogonium ordinarily precede development of any accompanying antheridia, though, as a rule, antheridial branches are found wrapped extensively about the expanding female organ before it attains definitive size (Fig. 6, A). The enveloping branches soon give rise terminally or, less often, somewhat laterally, to crook-necked male organs, each delimited proximally by a cross-wall. At nearly the same time the oogonium, having completed its growth, likewise is delimited from its supporting hypha. Contraction of the oogonial contents into a spherical body of lumpy structure (Fig. 6, B, C, D; Fig. 7, A, B, C) ensues, with intrusion of fertilization tubes, and movement of antheridial materials through them. The spherical body, after laying down a thick wall, undergoes internal reorganization whereby it is converted into a yellowish oospore that at early maturity (Fig. 6, E, F, G; Fig. 7, D) reveals the unitary structure most usual in oospores of the genus,—its single reserve globule being surrounded by a parietal layer of granular protoplasm, in which a single subspherical or oblate ellipsoidal refringent body is imbedded. After several weeks of further ripening, some oospores will frequently be found to contain 2 refringent bodies (Fig. 6, H; Fig. 7, E); and additional increase in number of these small but conspicuous structural elements may come with more prolonged aging. With extended aging, too, the parietal layer often acquires a more coarsely granular texture almost like the texture of the homologous layer in species of *Aphanomyces*.

The sexual apparatus of *Pythium myriotylum* shows much variety with respect to the origin and positional relationships of its component parts. Most often, perhaps, the oogonia are borne terminally on branches of variable lengths (Fig. 6, A, B, D; Fig. 7, A, B, C), yet their occurrence in lateral (Fig. 6, C; Fig. 7, E) or intercalary (Fig. 7, D) positions is not infrequent. The male complement may be supplied wholly from a single parent filament (Fig. 6, D; Fig. 7, A, B), though often 2 (Fig. 6, A, B) or 3 (Fig. 7, C) such filaments, which generally have no close mycelial connection with the oogonium, may contribute antheridial branches. Almost invariably the antheridial branches are not only wrapped about the oogonium itself, but are to some extent intricated with the filament supporting that organ; the intrication, however, being of an irregular or haphazard sort little suggestive of the symmetrical helicoid involvement characteristic of *P. helicoides* Drechsl. and *P. palingenes* Drechsl. Undersized oogonia may be supplied with only 1 (Fig. 6, C) or 2 (Fig. 7, A) antheridia, whereas larger specimens very often have 6 (Fig. 6, D), 7 (Fig. 6, B) or 8 (Fig. 7, B, C) male organs
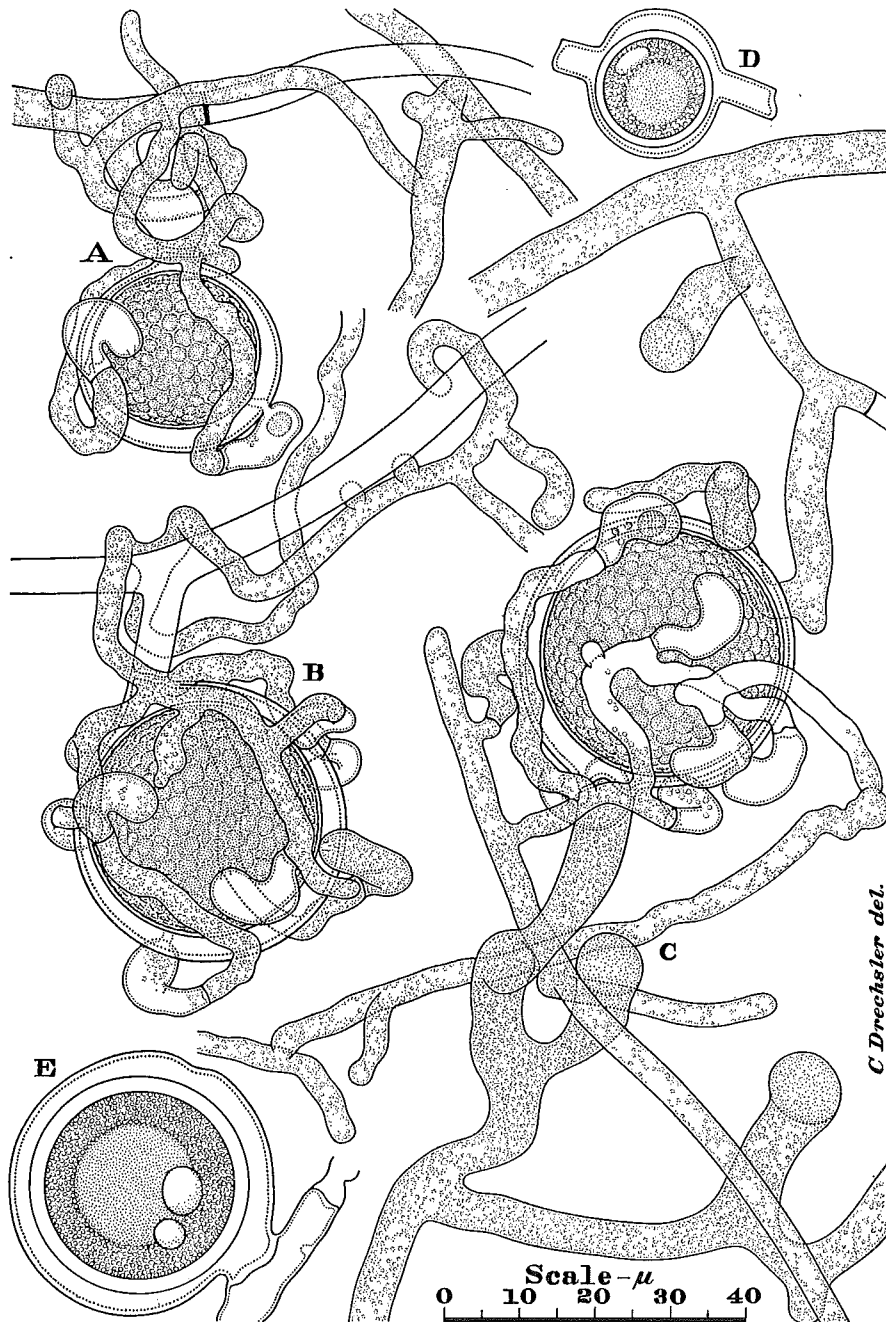
FIG. 7. Sexual reproductive apparatus of *Pythium myriotylum* formed in maize-meal-agar cultures; drawn to a uniform magnification with the aid of a camera lucida; ×1000 throughout. A–C. Immature units showing antheridial relationships. D, E. Oogonia, each with a mature oospore.

formed in contact with them. Somewhat comparable arrangement of sex organs and associated hyphal parts is recognizable in *P. arrhenomanes, P. graminicolum* Subr., *P. periilum* Drechsl., and *P. scleroteichum* Drechsl., despite certain readily noticeable differences pointed out earlier (17, p. 885); and it seems probable that *P. myriotylum* is more closely allied to these species than to *P. butleri*, even though the latter offers much greater similarities of parasitic habit. Analogous enwrapment of the oogonium by its male complement appears suggested also in Matthews' illustrations (26, Pl. 11, Figs, 1, 2, 3, 6) of her *P. catenulatum*.

Like *Pythium arrhenomanes* and *P. scleroteichum*, though perhaps in lesser degree, *P. myriotylum* is annoyingly given to degeneration of its sexual apparatus. Often its oogonia form oospores that degenerate before the correct unitary organization of early maturity has been achieved. Indeed, frequently degeneration sets in before even an oosphere becomes recognizable. When this happens the affected oogonium may utilize, without much evident advantage, some or all of its contents in putting forth a disorderly array of hyphal outgrowths; or, again, it may suffer spectacular invasion by a tangle of hyphae often originating from one or several of the attendant antheridia. Occasionally, the tangled mass of hyphae gives rise to a new oogonium and new antheridia, which then proceed with normal development; so that eventually an oospore of small size, yet of correct internal structure, comes into being within the old oogonial envelope.

When cultures of *Pythium myriotylum* show much degeneration they usually show also a pronounced tendency toward morphological extravagances that finds some expression even in those units of sexual apparatus that yield oospores of correct internal structure. Many of the antheridia may then be abnormally long and conspicuously upcurved or contorted, while an excessively large proportion of the oogonia and oospores may depart widely from average dimensions. Relatively large dimensions of both oogonia and oospores often accompany degeneration caused by low temperature and nutritional deficiency. Thus, when grown at 14° C. on filtered maize-meal-decoction agar the strain of *P. myriotylum* isolated from a decaying cucumber in 1924 gave 28.8 μ as the average diameter of good oospores, and 35.4 μ as the average diameter of mature oogonia. When the same strain was grown at 28° C. on maize-meal agar that contained in suspension a substantial quantity of finely divided maize-meal, and that had been slightly acidulated with hydrochloric acid, measurements on 200 sexual units selected at random gave values of 20.8 μ and 26.5 μ for average diameter of oospore and oogonium, respectively. The smaller values were mentioned in the diagnosis of the species, as they were derived from material evidently far more representative of normal development than the material that had given the larger values. For the cultures grown at the higher temperature on the more nutritious medium yielded sexual apparatus in extraordinary abundance and with virtually no degeneration; whereas, those grown at the lower temperature on the filtered medium yielded such appa-
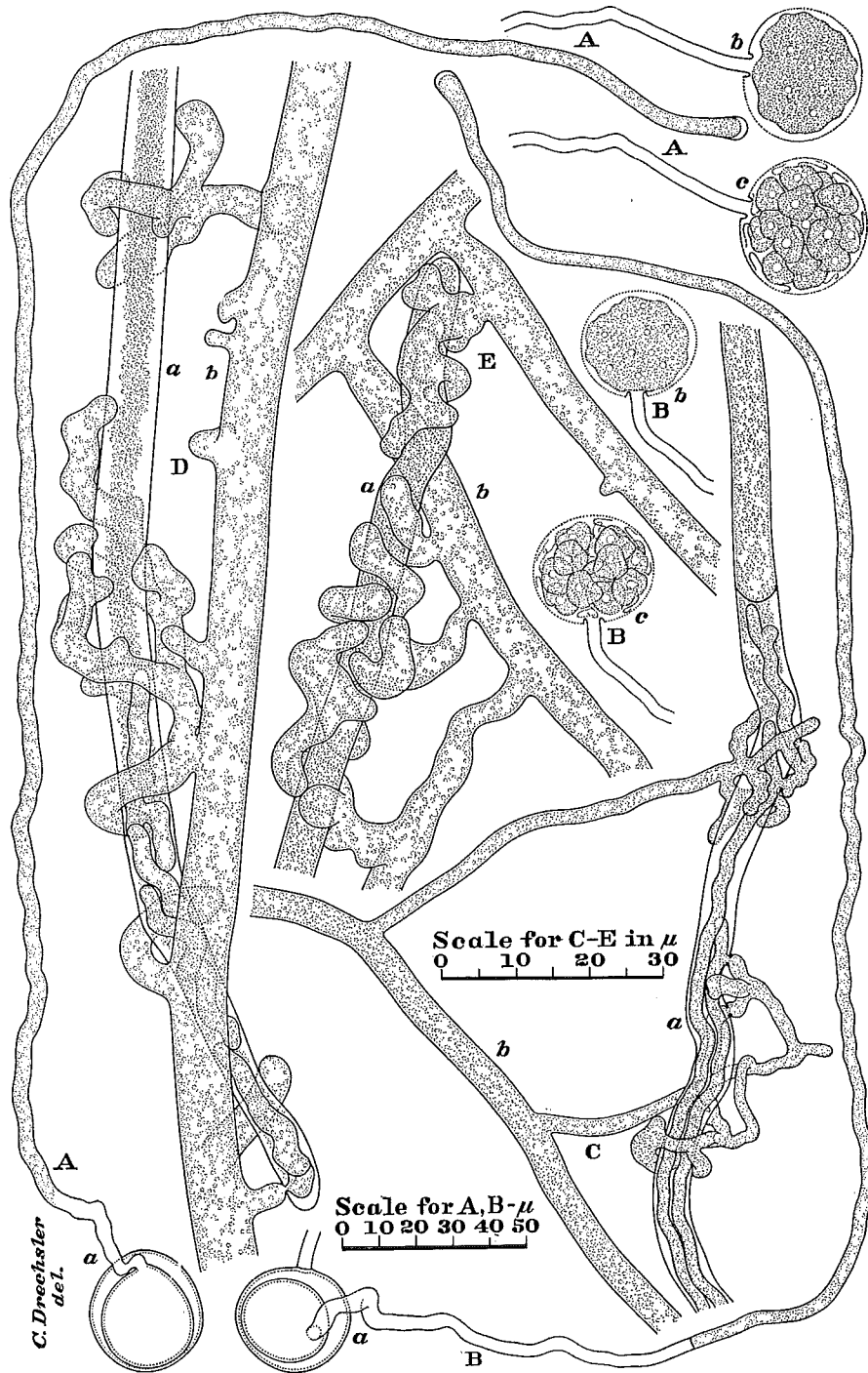
FIG. 8.   A, B. Oospores of *Pythium myriotylum*, each germinating by production of a zoosporangium: a, long germ zoosporangium shortly before discharge; b, vesicle with discharged granular contents; c, vesicle with zoospores ready to escape; × 500.   C. Hypha of *P. myriotylum* (a) parasitized by *P. periplocum* (b); × 1000.   D. Hypha of *P. myriotylum* (a) attacked by pansy strain of *Aphanomyces cladogamus* (b); × 1000.   E. Hypha of *P. myriotylum* (a) attacked by *Plectospira myriandra* (b); × 1000.

ratus only in moderate quantity, much of it vitiated by degeneration. Associated with the more abundant production of oogonia and oospores, and with virtual absence of degeneration, was a high degree of uniformity with respect to size. The 200 measurements of oogonial diameter from which the average of 26.5 μ was computed showed a distribution of values, expressed to the nearest integral number of microns, as follows: 16 μ, 2; 20 μ, 1; 22 μ, 1; 23 μ, 6; 24 μ, 7; 25 μ, 25; 26 μ, 54; 27 μ, 50; 28 μ, 29; 29 μ, 16; 30 μ, 5; 31 μ, 1; 32 μ, 1; 33 μ, 2; while the accompanying measurements for diameter of oospore showed the following distribution: 12 μ, 1; 15 μ, 2; 17 μ, 1; 18 μ, 6; 19 μ, 21; 20 μ, 41; 21 μ, 67; 22 μ, 37; 23 μ, 15; 24 μ, 6; 25 μ, 2; 26 μ, 1.

Oospores of correct internal structure have been found capable of germination after 2 or 3 years of storage at temperatures between 5 and 10° C. Like the reproductive bodies, sexual or asexual, of oomycetes generally, they produce vegetative germ hyphae when transferred to a fresh substratum devoid of free liquid water, or to a liquid medium containing a considerable quantity of nutrients in solution. When transferred to water containing little or no food substance they germinate more frequently by putting forth a simple filament, which in several observed instances was 500 to 600 μ long and 3 to 4 μ wide. After the protoplasmic contents have completely passed from the chamber of the oospore, and sometimes have further been evacuated from a proximal portion of the filament, the tip of the filament forms a hyaline cap of dehiscence (Fig. 8, A, a; B, a). Discharge of the germ sporangium thus brought into being proceeds as with zoosporangia of mycelial origin; the hyaline cap becoming inflated into a vesicle as it receives the migrating granular material (Fig. 8, A, b; B, b). The undifferentiated protoplasmic mass undergoes division into a swarm of laterally biciliate zoospores (Fig. 8, A, c; B, c), numbering usually 10 to 16 individuals, which escape when the vesicular membrane finally yields under the impact of their increasingly violent movements.

A NEW PROLIFEROUS PYTHIUM OF THE HELICOIDES SERIES

In June, 1939, A. A. Dunlap of Texas Agricultural Experiment Station submitted for identification a culture of a pythiaceous fungus that, according to an accompanying letter, had been isolated from roots of wheat plants sent from the Panhandle region of Texas. Since the numerous oospores visible in the substratum were unmistakably of multiplicate internal structure the fungus was at once presumed to belong with the series of proliferous forms typified in *Pythium helicoides*. This presumption was found amply justified when the asexual reproductive stage came to light. As a member of the *helicoides* series the fungus invites attention by unusual simplicity in the make-up of its sexual apparatus.

Growing at moderate temperatures in pure culture on agar media soft enough to offer no appreciable impediment, the fungus from Texas extends its mycelium more slowly than most species of *Pythium*. A relatively rich

medium, as for example, Lima-bean agar, induces development of rather copious aerial mycelium both in Petri dishes and in test tubes. Such aerial mycelium has been observed to persist more than 2 years under fairly dry conditions, without matting down on the substratum. Some aerial growth may often be observed also when the fungus is propagated in test tubes on maize-meal-decoction agar, a medium containing nutrients in moderate or in small concentration; though in Petri dishes this substratum usually affords development only of intramatrical mycelium that presents to the naked eye a somewhat lustrous radiating appearance modified occasionally by cloud-like variegation expressive of local differences in density of texture. Under the microscope, as might be expected, the intramatrical filaments are revealed, on the one hand, as being less haphazard in their courses than the filaments making up the diffuse mycelium of *P. myriotylum*, while, on the other hand, they show less parallelism of arrangement than is evident in the conspicuously lustrous mycelia of *P. vexans* de Bary (=*P. complectens* Braun) and *P. complens*. Intramatrical hyphal branches in plate cultures ordinarily fail to form appressoria on encountering the glass floor of the Petri dish,—a failure contrasting markedly with the behavior of the closely related *P. polytylum* Drechsl. (14), which, under similar conditions, gives rise to numerous adhesive organs.

Asexual reproduction may conveniently be induced by excising sizable slabs from a Lima-bean-agar plate culture permeated with young mycelium, and transferring them to a shallow layer of sterile water in a sterilized Petri dish. At temperatures between 20 and 25° C. each irrigated slab promptly extends into the surrounding liquid a fringe of extramatrical mycelium often 5 to 10 mm. in width. Within perhaps 15 or 18 hours after the transfer some of the filaments composing the fringe give rise individually to a terminal enlargement with a papillate protrusion at its apex (Fig. 9, A). Once it attains definitive size the enlargement is delimited from the supporting filament through deposition of a basal septum (Fig. 9, B, C, D). The terminal papilla may become prolonged into an evacuation tube of somewhat variable length. Should this tube suffer functional frustration, 1 (Fig. 9, E) or 2 (Fig. 9, F) similar tubes are put forth from other positions. Sooner or later the undifferentiated protoplasmic content of the sporangium passes into a vesicle formed through inflation of a hyaline cap at the tip of the successful evacuation tube (Fig. 9, G). In this vesicle the granular protoplasm is fashioned into laterally biciliate zoospores, wholly after the manner typical of the genus *Pythium* (Fig. 9, H). Often, especially when discharge is delayed while one or more longish evacuation tubes are being put forth, a branch may grow out laterally from the supporting hypha, just below the base of the sporangium (Fig. 9, G; H; I; J, a). At other times, when discharge has been accomplished quickly by means of a short apical evacuation tube, a second sporangium may develop within the emptied envelope (Fig. 9, K), eventually to give rise to zoospores in a vesicle formed a variable distance from the orifice of the first sporangium (Fig. 9, L).
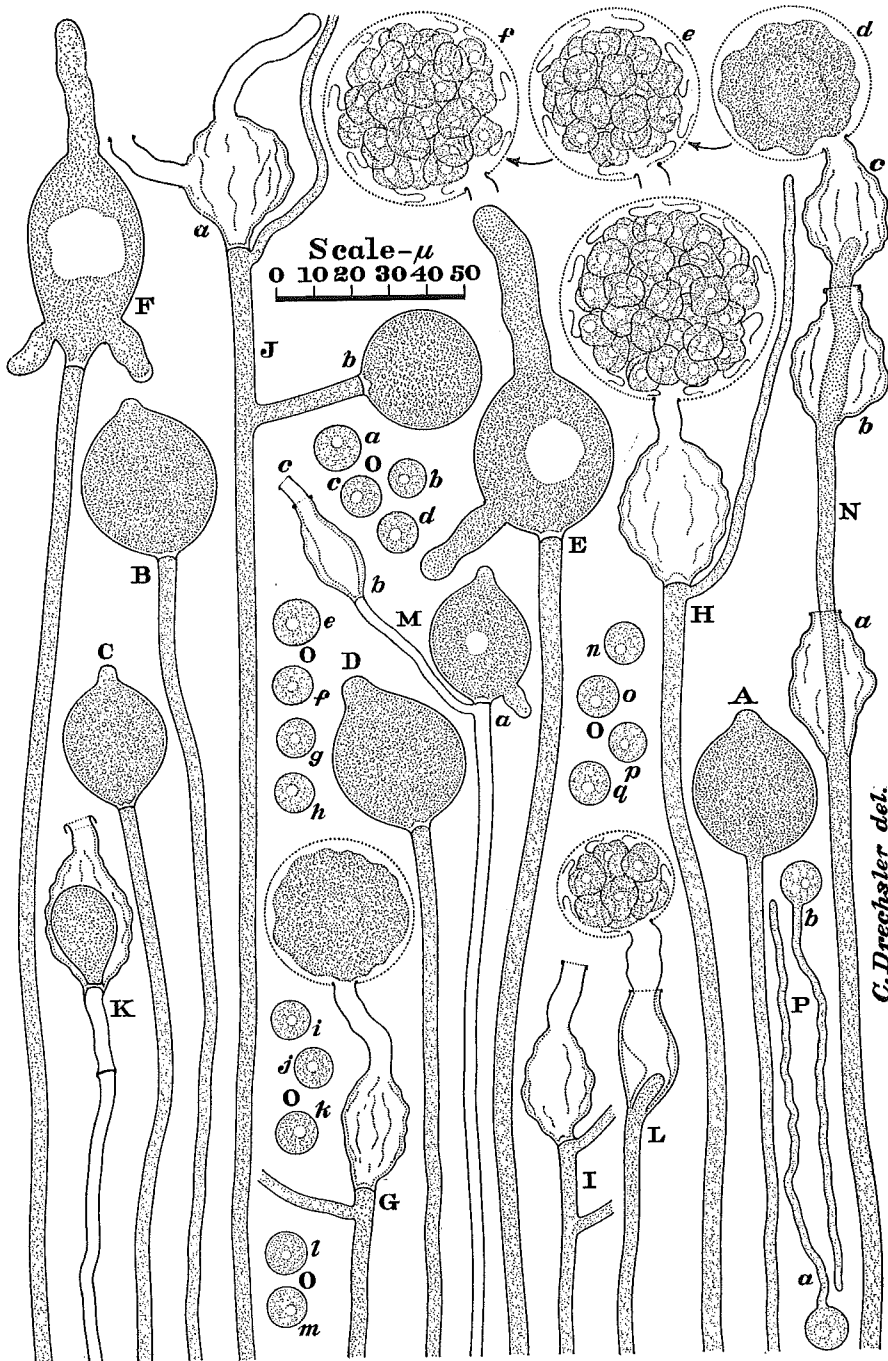
FIG. 9.   Asexual reproductive apparatus of *Pythium ostracodes* produced in irrigated Lima-bean-agar preparations; drawn to a uniform magnification with the aid of a camera lucida; ×500 throughout. A. Young sporangium continuous with supporting hypha. B–D. Full-grown delimited sporangia. E, F. Sporangia with evacuation tubes. G–J. Discharged sporangia. K–M. Proliferous development of sporangia. N. Three uniaxial sporangia, (a–c); same vesicle at successive stages (d–f). O. Encysted zoospores (a–q). P. Germinating zoospores (a, b).

Owing to differences in promptness of discharge a terminal sporangium (Fig. 9, M, a) may sometimes be found still retaining its contents, even when a second and a third sporangium, formed subsequently on a lateral prolongation of the supporting filament, are present only as nested envelopes (Fig. 9, M, b, c). Such nested arrangement betokens, in the main, rather prompt discharge, as does also a seriate uniaxial arrangement of empty sporangial envelopes, brought about by repeated straightforward prolongation of the supporting filament and production of successive sporangia (Fig. 9, N, a, b, c), which, one after another, produce vesicles wherein their undifferentiated contents are transformed into zoospores (Fig. 9, N, d, e, f). After being liberated the zoospores swim about for some time before coming to rest and rounding up into subspherical cysts (Fig. 9, O, a–q) that germinate commonly by the production of a single germ hypha about 1.8 to 2 μ in width (Fig. 9, P, a, b).

Thus with respect to the morphology and development of its asexual reproductive phase, the fungus appears closely similar to the 4 proliferous species I presented earlier (14) as members of the *helicoides* series. It perhaps resembles *Pythium oedochilum* Drechsl. and *P. palingenes* more closely than *P. helicoides*, since its sporangiferous filaments, like those of the former 2 species, are little given to racemose or cymoid branching, even though now and then they may bear an auxiliary sporangium on a short branch arising some distance below the terminal sporangium (Fig. 9, J, b).

The fungus seems unusually reliable in producing normal sexual apparatus (Fig. 10, A–I; Fig. 11, A–G) on different kinds of agar culture media. On maize-meal agar containing in suspension a substantial quantity of finely divided maize-meal, its sexual development is, as a rule, not only very abundant but also to an extraordinary degree free of degeneration. The oogonium often arises as a lateral subspherical enlargement sessile on the parent filament (Fig. 10, A, B, C; Fig. 11, C); or it may be formed terminally either on a short branch (Fig. 10, D; Fig. 11, A, B), or on a longer hypha (Fig. 10, G); or, again, it may develop as a laterally intercalary (Fig. 10, E, F, I; Fig. 11, E, F) or mesially intercalary (Fig. 10, H; Fig. 11, D) body. During its earlier stages of growth it seems usually to have no direct contact with any part that can be distinguished as a male element (Fig. 10, A). On attaining definitive size it is fertilized commonly by a single antheridium (Fig. 10, B–I; Fig. 11, A–F), and only occasionally by 2 antheridia (Fig. 11, G, a, b).

Alongside of the robust oogonium the antheridium looks puny and frail, although actually it is not smaller than the corresponding organs in many of the more familiar species of *Pythium*. It consists usually of a somewhat swollen elongated cell formed terminally on a branch arising most often from the mycelial filament that either bears the oogonium directly (Fig. 10, B, C, E–I; Fig. 11, C, D, E), or gives off a short branch supporting the oogonium (Fig. 10, D; Fig. 11, A, B, G, a). In some units of monoclinous sexual apparatus, where the male branch is short and arises from a position

Scale in μ

0 10 20 30 40 50

C. Drechsler del.

FIG. 10. Sexual reproductive apparatus of *Pythium ostracodes* formed in unfiltered maize-meal-agar medium; drawn to a uniform magnification with the aid of a camera lucida; ×1000 throughout. A. Hypha with a young oogonium growing out laterally. B-E. Complete sexual units in immature condition. F-I. Complete sexual units, each with its mature oospore showing multiplicate internal organization.
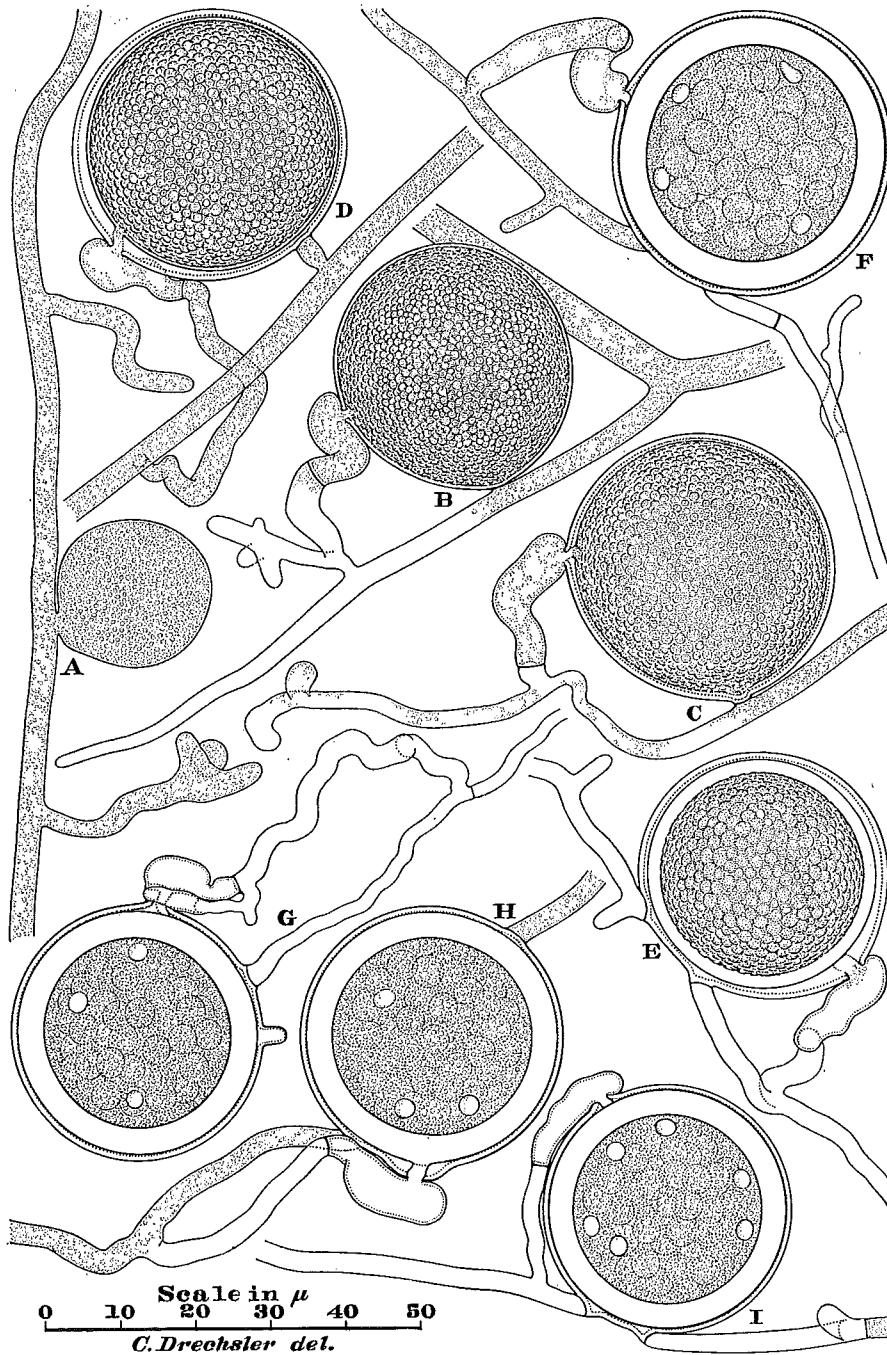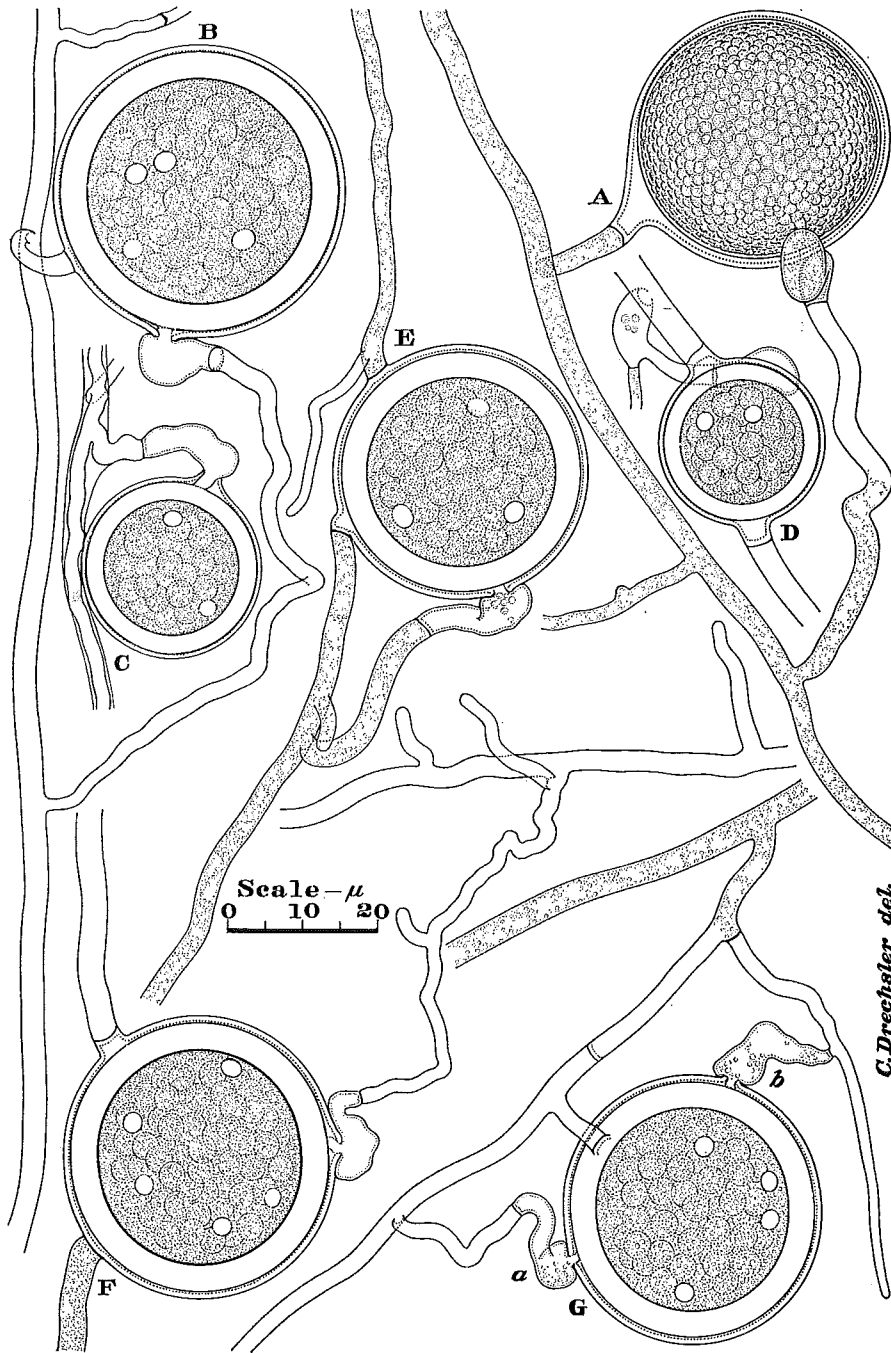
FIG. 11. Sexual reproductive apparatus of *Pythium ostracodes* formed in unfiltered maize-meal-agar medium; drawn to a uniform magnification with the aid of a camera lucida; ×1000 throughout. A. Complete sexual unit in immature condition. B–G. Complete sexual units, each with its mature oospore showing multiplicate internal organization; the parent hypha in C illustrating the ''Durchwachsung'' often found in aging material.

close to the oogonium, the mycelial parts connecting the male and female organs may have an aggregate length of only 20 μ (Fig. 10, I), while in other monoclinous units, where the male branch arises from a more distant position, the mycelial connection between oogonium and antheridium may exceed 125 μ (Fig. 11, A) or even 150 μ (Fig. 11, B) in length. In units of diclinous sexual apparatus the length of the antheridial branch is rarely less than 50 μ (Fig. 11, F), and sometimes exceeds 200 μ. Now and then an antheridium may be found attached laterally (Fig. 11, G, b),—a positional relationship that might come about either from lateral development in the beginning, or from terminal development followed by renewed elongation of the supporting branch.

The antheridium is often found applied to the oogonium laterally almost throughout its length (Fig. 10, D, H, I), much as in *Pythium helicoides*. No less often, however, it is applied laterally only along its distal portion (Fig. 10, B, E; Fig. 11, B, F); and frequently, indeed, it merely makes apical contact (Fig. 10, C, F; Fig. 11, G, a, b) in somewhat the same commonplace manner as the antheridia, for example, of *P. dissotocum* Drechsl. (21, Fig. 2, 3). Although in some instances the oogonium comes to protrude rather markedly toward the antheridium (Fig. 10, E, H; Fig. 11, C) and thus provides obvious parallelism with *P. helicoides* and *P. oedochilum*, more often the wall of the globose body bulges out only slightly where the short fertilization tube is thrust through it (Fig. 10, F, I; Fig. 11, E, G, a, b). At the time of fertilization the oogonial contents appear to consist largely of protoplasmic blocks or lumps (Fig. 10, B, C). The lumpy mass often shrinks away perceptibly from the oogonial membrane (Fig. 10, D; Fig. 11, A) before its envelopment in a thick peripheral wall of its own (Fig. 10, E) heralds the internal changes required for its conversion into an oospore of multiplicate structure (Fig. 10, F–I; Fig. 11, B–G).

Usually only 2 to 6 refringent bodies are discernible in the oospore at early maturity. This number, however, includes only the refringent bodies lying close to the spore surface nearest the observer, since those in deeper positions are at this stage rather effectively obscured by the overlying granular material and reserve globules. The reserve globules, while noticeably larger than the refringent bodies, appear smaller and correspondingly more numerous than the multiple globules in *Pythium helicoides*. As a rule they have a diameter approximately equal to the thickness of the oospore wall, which dimension is even greater here than in the 4 closely related forms previously described. The oospore usually fills the oogonial chamber so nearly completely that its distinctly yellowish wall very often appears extensively in contact with the colorless oogonial membrane; the structural separateness of the 2 envelopes then remaining recognizable chiefly because of their difference in coloration. With respect to its main dimensions the sexual apparatus ordinarily is given only to moderate variation. The relevant metric data submitted in the diagnosis below were derived from 200 measurements of oogonia selected at random in a culture showing abundant

sexual development with virtually no degeneration.   These 200 oogonia gave
values for diameter, expressed to the nearest integral number of microns,
distributable as follows: 14 μ, 1; 18 μ, 1; 21 μ, 1; 24 μ, 1; 25 μ, 1; 26 μ, 1;
29 μ, 1; 30 μ, 1; 31 μ, 2; 32 μ, 5; 33 μ, 22; 34 μ, 21; 35 μ, 32; 36 μ, 40;
37 μ, 27; 38 μ, 17; 39 μ, 10; 40 μ, 10; 41 μ, 2; 42 μ, 2; 43 μ, 2; and the 200
oospores of correct internal structure contained within them gave measure-
ments for diameter distributable thus: 13 μ, 1; 16 μ, 1; 19 μ, 1; 22 μ, 1;
23 μ, 1; 26 μ, 1; 27 μ, 1; 28 μ, 1; 29 μ, 3; 30 μ, 3; 31 μ, 16; 32 μ, 31;
33 μ, 25; 34 μ, 38; 35 μ, 26; 36 μ, 22; 37 μ, 11; 38 μ, 10; 39 μ, 4; 40 μ, 1;
41 μ, 2.

Maize-meal-agar cultures containing numerous oospores of correct inter-
nal organization have, on examination after 2 years of storage at tempera-
tures between 5 and 10° C., shown relatively little degeneration among the
reproductive bodies.   The conspicuously thick-walled structures seem, in-
deed, well designed for conserving life over long periods of time.   Yet
despite their longevity and their strongly indurated appearance the oospores
will not only germinate very promptly, but, what is perhaps more remark-
able, will germinate by producing zoosporangia and zoospores in almost all
instances where liquid water moderately free of nutrients is present.   Thus,
when the oospores produced in 0.03 cc. of unfiltered maize-meal-agar me-
dium are distributed in 3 cc. of water over the floor of a Petri dish 90 mm.
wide, motile zoospores begin to be liberated after 6 or 7 hours, and in 15
hours may usually be found swarming in spectacular abundance throughout
the preparation.   Such behavior suggests that under natural conditions the
fungus may possibly give rise to zoospores on a greater scale in connection
with the germination of its oospores than through development of sporangia
from its mycelium.

The first change unmistakably associated with germination becomes evi-
dent when the oospore wall—or, rather, the thick shell-like layer over the
thin membrane intimately surrounding the protoplast—shows within a re-
gion between 5 μ and 10 μ wide an array of striations extending radially
from its inner contour half way toward its outer contour (Fig. 12, A).
These striations signify apparently that the wall is being progressively
honeycombed with radial pockets.   As the striations elongate and reach the
outer contour, the remnants of wall material along the inner contour vanish,
leaving a space into which the protoplast and the thin pliable membrane
surrounding it soon protrude (Fig. 12, B).   Solution of the honey-combed
fabric continues centrifugally, and soon a fairly wide channel is cleared,
permitting protrusion of the protoplast to the oogonial membrane (Fig.
12, C).   Meanwhile, the portion of this membrane lying in the path of the
channel has become somewhat less distinct optically, and now gives an im-
pression of reduced firmness.   At all events, it yields without much resis-
tance in allowing the protrusion to break through and to elongate externally
as a stout germ tube (Fig. 12, D).   Thereupon radial striations come into
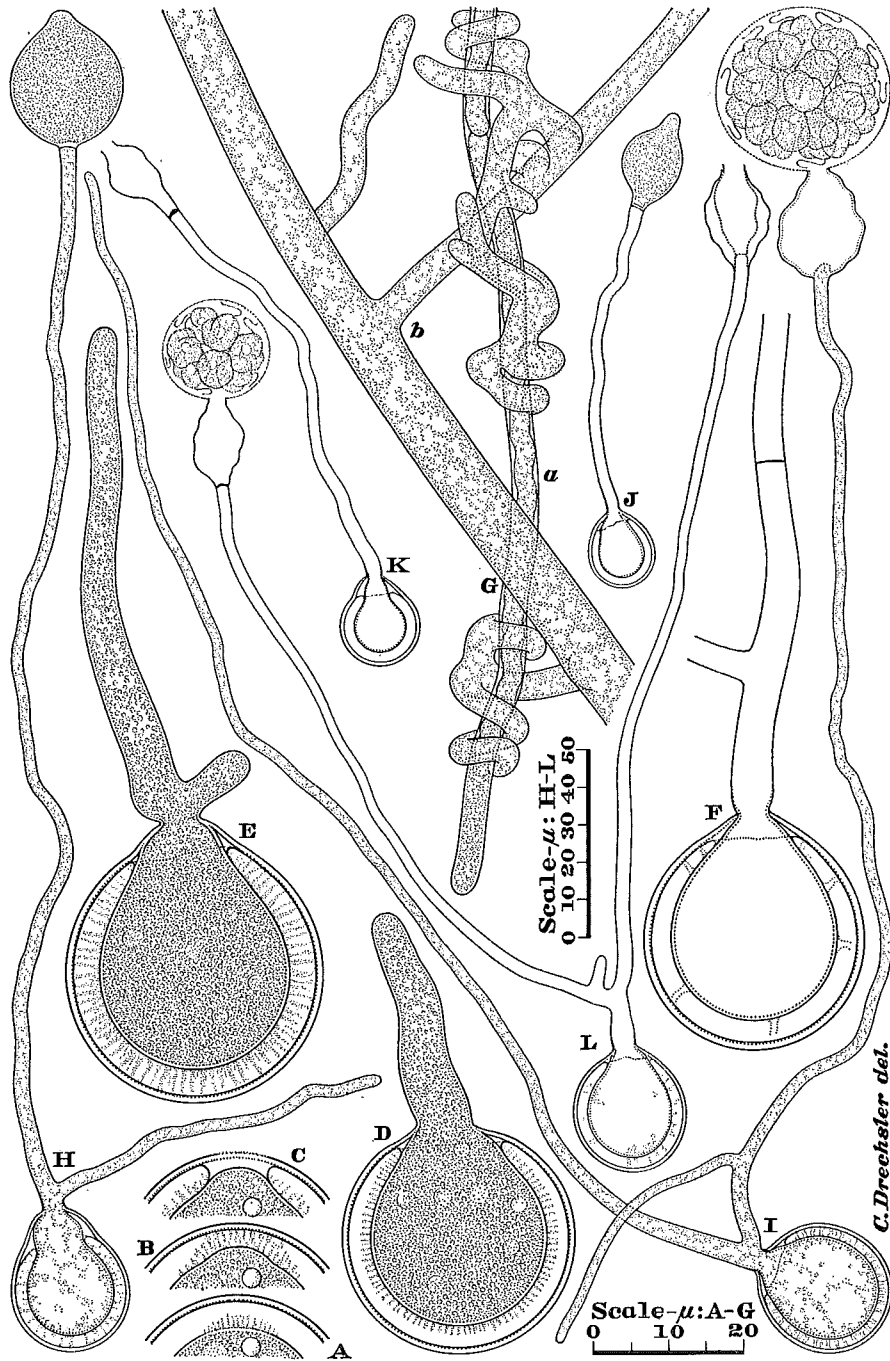view everywhere along the inner contour of the thick oospore wall (Fig.

FIG. 12. *Pythium ostracodes.* A–C. Successive stages in perforation of oospore wall preparatory to germination; ×1000.   D, E. Oospores with elongating germ tubes; ×1000. F. Membranous remains of oospore after germination; ×1000.   G. Hypha of *P. ostracodes* (a) parasitized by spinach strain of *Aphanomyces cladogamus* (b); ×1000.   H, I. Oospores which after forming one sporangium are ready to form other sporangia; ×500. J, K, L. Oospores which have exhausted their contents in forming 1, 1, and 3 zoosporangia, respectively; ×500.

12, D), and are soon extended to its outer contour (Fig. 12, E). Thus honeycombed throughout, the substance of the massive wall undergoes resorption, until finally only some few radial markings may remain as vestiges in the space between the persistent oogonial membrane and the similarly persistent membrane which intimately surrounded the protoplast (Fig. 12, F) ; and very often, again, even such meager vestiges are extinguished.

At about the time the first radial markings can be observed in a localized region of the massive wall surrounding them, the protoplasmic contents of the oospore begin to show significant changes. The reserve globules appear less distinct and are reduced in volume, while simultaneously the granular material is increased in corresponding measure. Temporarily the refringent bodies in the deeper portions of the protoplasmic mass become more clearly visible, so that their total number, usually between 10 and 15, and less often between 5 and 10, or between 15 and 20, can now be determined. During the period when the germ tube grows to a length of 50 to 75 μ, the refringent bodies gradually are lost to view in their densely granular matrix (Fig. 12, D, E). Usually, during this early period, too, a branch is given off by the germ tube close to its origin (Fig. 12, E). The 2 resulting elements commonly grow in rather widely divergent directions (Fig. 12, H), one or the other often putting forth an additional branch (Fig. 12, I). When a filament has attained a length of 75 to 500 μ, or sometimes even a length of 700 μ or 800 μ, it gives rise at its tip to a terminally papillate sporangium (Fig. 12, H) similar in shape to the sporangia produced from vegetative mycelium, and, like them, after development of an evacuation tube with an apex of dehiscence, discharging its contents into a vesicle for transformation into laterally biciliate zoospores (Fig. 12, I). The largest sporangia borne on germ hyphae yield between 20 and 25 zoospores, yet the production of one such organ ordinarily leaves unexpended much of the protoplasm in an oospore of good size (Fig. 12, H, I), even if some undersized oospores are completely exhausted in producing individually a short germ hypha together with a single small sporangium that may yield only between 5 and 10 zoospores (Fig. 12, J, K). Most oospores, consequently, give rise to 1 or 2 additional sporangia, in many instances with a display of proliferous development. Thus, the second sporangium often is produced on a prolongation of the filament that bore the first, while a third may be formed elsewhere at the tip of a branch (Fig. 12, L) ; or proliferous development may be absent, the 2 or 3 sporangia being produced on different filaments of the branching apparatus.

The more commonplace type of germination by production of mycelia ensues, of course, when oospores are transferred to agar media devoid of free water, as also when they are put into rich liquid media or into water containing substantial quantities of nutrients. In the earlier stages of such vegetative germination, digestion of the thick oospore wall proceeds much as in germination under aquatic conditions; but soon after the germ hyphae have emerged they begin to take up food substances, thereby initiat-

ing a new cycle of autonomous growth. Yet a supply of fresh water or fresh substratum is not always necessary for germination. Often, while undergoing microscopic examination, oospores have been found germinating actively within 2 hours after they had been removed, completely dormant, from maize-meal-agar cultures 6 or 8 weeks old, even though the stale ambient in which they were formed and which still surrounded them not only had received no addition of fresh water but was slowly losing water through evaporation.

As the fungus, apart from other diagnostic features, would seem distinguished from all known species of *Pythium* by its remarkably thick oospore wall, it is described as a new member of the genus under a specific name meaning "testaceous."

Pythium ostracodes sp. nov.

Mycelium hyalinum, ramosum, in hyphis 1.8–6.5 μ crassis consistens; zoosporangiis sphaeroideis vel ellipsoideis vel citriformibus, 25–55 μ longis, 16–38 μ crassis, fere terminalibus, pluribus deinceps ex apice unius hyphae repullulantis saepe gignentibus, protoplasma eorum per tubulum in vesiculam fundentibus; tubulo 2–45 μ longo, 4–9 μ crasso, plerumque ex apice sporangii rarius ex latere ejusdem orto; vesicula 5–35 zoosporas gignente; zoosporis primo reniformibus, a latere biciliatis, mox quietis, globosis, plerumque 10–12.5 μ diam. Oogonia terminalia vel intercalaria vel lateralia, globosa, 14–43 μ (saepius circa 35.4 μ) diam., pariete 0.5–1 μ crasso circumdata; antheridiis plerumque singulis rarius binis, vulgo androgynis rarius diclinis, clavatis vel cylindratis, fere 10–20 μ longis, 4–7 μ latis, modo a fronte modo aliquatenus vel omnino a latere ad oogonium appositis, membrana saepius aliquanto undulata tectis; oosporis flavidis, sphaeralibus, vulgo 13–41 μ (saepius circa 33.5 μ) diam., muro 1.5–4.3 μ (saepius circa 3.6 μ) crasso circumdatis, 10–75 pilulas oleosas plerumque 3–4 μ crassas et 5–20 corpuscula nitida 2–3 μ crassa continentibus, prompte germinantibus denique aut mycelium emittentibus aut hypham fertilem simplicem vel saepius aliquantulum ramosam cum 1–3 zoosporangiis proferentibus.

Habitat in radicibus Tritici aestivi in Texas.

Intramatrical mycelium in transparent agar media frequently of somewhat lustrous radiate appearance, capable of approximately 15 μ radial extension in 24 hours at 24° C., composed of hyphae mostly 1.8 to 6.5 μ wide, the more delicate ramifications usually developed only in moderate quantity. Aerial mycelium sometimes absent, but at other times moderately or even rather abundantly developed, and then under conditions not too humid persisting long without collapsing.

Sporangia under aquatic conditions formed terminally on long, simple, or sparingly branched extramatrical hyphae, mostly 2.5 to 4.5 μ wide; subspherical, prolate ellipsoidal, or lemon-shaped, measuring usually from 25 to 55 μ in length and from 16 to 38 μ in transverse diameter; discharging their contents into a vesicle through an evacuation tube often somewhat reflexed, 2 to 45 μ long and 4 to 9 μ wide, usually formed as a prolongation of an apical papilla, but sometimes arising from a lateral position, especially after frustration of an apical tube; proliferous in moderate measure, either through subsporangial branching, or through uniaxial elongation of the supporting filament. Zoospores formed in numbers ranging from 5 to 35, kidney-shaped, laterally biciliate, on rounding up forming cysts mostly 10 to 12.5 μ in diameter, which germinate usually by putting forth a germ tube about 2 μ in width.

Oogonia sometimes formed terminally on lateral branches less than 25 μ in length, but more often borne directly on long filaments in terminal, lateral, laterally intercalary, and mesially intercalary positions; in some instances protruding noticeably toward the antheridium, yet generally subspherical, measuring 14 to 43 μ, mostly 30 to 41 μ (average 35.4 μ) in diameter; provided with a wall 0.5 to 1 μ in thickness. Antheridia usually occurring singly in relation to an oogonium, but sometimes 2 in number; usually formed terminally or somewhat laterally on a branch 2 to 100 μ long arising from the same hypha as the oogonium at a distance 5 to 100 μ from the oogonium, but ocasionally of diclinous origin and then usually borne terminally on a branch 50 to 250 μ long arising from a distant filament; elongated cylindrical, clavate, or saccate in shape, often with somewhat wavy contours; measuring usually 10 to 20 μ in length and 4 to 7 μ in width; sometimes applied apically, but at other times applied lengthwise for some distance below the apex, if not for the entire distance from apex to base; producing a fertilization tube often 1

to 3 μ long and 1 to 1.5 μ wide. Oospore usually distinctly yellowish, subspherical, measuring 13 to 41 μ, mostly 28 to 39 μ (average 33.5 μ) in diameter; provided with a wall
1.5 to 4.3 μ, mostly 3.2 to 4 μ (average 3.6 μ) in thickness; containing 10 to 75 reserve
globules mostly 3 to 4 μ in diameter, and 5 to 20 globose refringent bodies 2 to 3 μ in
diameter; germinating readily by producing a mycelium, or, under aquatic conditions,
by extending a simple or somewhat branched sporangiophore, 75 to 800 μ long, whereon
1 to 3 sporangia are borne terminally, often in part through proliferous development.

Occurring in rootlets of *Triticum aestivum* in Texas.

BIOTIC RELATIONSHIPS WITH SOME OTHER ROOT-ROTTING OOMYCETES

As might be expected, agar plate cultures that have been prepared for
the isolation of root-rotting or damping-off fungi by placing pieces of
affected plant material on sterile medium often afford development of
mycelia belonging to 2 or 3 separate species of *Pythium*. When the several
mycelia present in a culture are all referable to such familiar pathogenic
species as, for example, *P. ultimum* Trow, *P. debaryanum*, *P. irregulare*
Buism. and *P. mammillatum* Meurs, no hyphal relationship expressive of
parasitism comes to light. When, however, a growing mycelium of one
or another of these species has occasion to meet a growing mycelium of
P. oligandrum Drechsl. (14), a species with echinulate oogonia, which occurs
rather frequently in diseased underground parts of many phanerogamic
plants, the former is attacked by the latter in a violently parasitic manner.
Everywhere in the region of encounter delicate ramifications of the spiny
form enwrap the filaments of the other elaborately, soon penetrating in
different places and intruding hyphal processes that grow lengthwise
through the filaments to assimilate the degenerating host materials. Here
and there the internal hyphae give off branches that push their way
out through the enveloping host membrane and, after some elongation,
bring about the destruction of other host filaments nearby. During their
earlier stages of development oogonia, as well as conidia or zoosporangia,
may be invaded, though oospores appear immune from attack once they
are surrounded by their thick wall. Frequently the attack of *P. oligandrum*
on the congeneric forms commonly associated with damping-off is so devastating that in extensive tracts of substratum they can give rise to only a
few reproductive bodies.

The same destructive parasitism came to light again and again when
Pythium oligandrum was intentionally planted in dual culture with the
familiar pathogenic forms found attacked in isolation cultures. Among
more than a score of additional congeneric species that were likewise grown
in dual culture with *P. oligandrum*, a few were parasitized with similar
violence. Most of the others, including *P. myriotylum*, suffered less severe,
though readily appreciable, injury. The remaining forms, of which
P. ostracodes may serve as a convenient example, incurred little or no
injury, even though in some instances their hyphae were intricately enveloped by branches of the spiny form. *P. complens* at times attacked *P. oligandrum* on a small scale.

As *Pythium acanthicum* and *P. periplocum* Drechsl. in their morphology
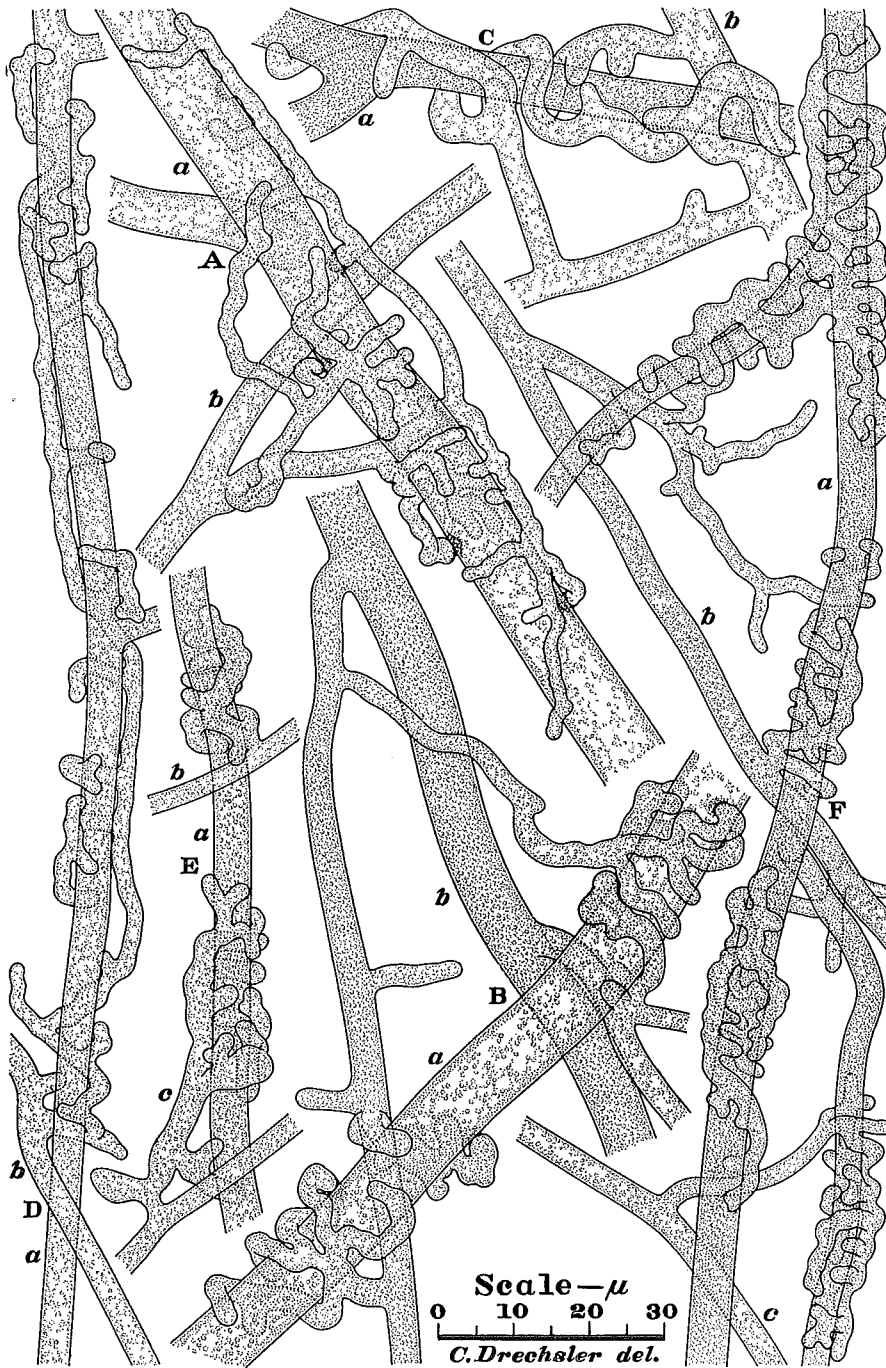show close kinship with *P. oligandrum*, these two species, better known

FIG. 13.   Drawn to a uniform magnification with the aid of a camera lucida; ×1000 throughout.   A. Hypha of *Pythium myriotylum* (a) attacked by *P. acanthicum* (b). B. Hypha of *P. myriotylum* (a) attacked by *P. oligandrum* (b).   C. Hypha of *P. myrio-tylum* (a) attacked by spinach strain of *Aphanomyces cladogamus* (b).   D. Hypha of *P. ostracodes* (a) scantily enwrapped by *P. periplocum* (b).   E. Hypha of *P. ostracodes* (a) more densely enwrapped by *P. periplocum* (b, c).   F. Hypha of *P. ostracodes* (a) enwrapped by *P. oligandrum* (b, c).

to me from their causal connection with blossom-end rot of watermelon than from their association with root decay, were also grown in series of dual cultures with a wide assortment of congeneric forms. In the 2 series of cultures parasitism and antagonism of varying degrees of severity again prevailed; the performance of the fruit-rotting fungi being generally similar to that of *P. oligandrum*. *Pythiogeton autossytum* Drechsl. (15), a member of the Pythiaceae, though alien to the genus *Pythium*, was attacked unmistakably. However, when the 3 echinulate forms were grown in dual culture with a number of saprolegniaceous forms occurring in association with root rot of crop plants, they not only proved innocuous, but like numerous other species of *Pythium* tried out, were themselves subjected to adverse action. In the case of *Aphanomyces cochlioides* Drechsl. (13) this action appeared to be mainly defensive, and caused little positive injury. Elaborate enwrapment of the pythiaceous filaments marked the more aggressive and injurious attack of *Plectospira myriandra* Drechsl. (11) as well as of the 3 strains of *Aphanomyces*, referable apparently to *A. cladogamus* Drechsl. (13) that were isolated, respectively, from diseased roots of pansies, *Viola tricolor* L. (16), spinach, *Spinacia oleracea* L. (18), and flax, *Linum usitatissimum* L. (18).

Pythium myriotylum, as has been intimated, is attacked by *P. acanthicum* (Fig. 13, A; Fig. 14, A), *P. periplocum* (Fig. 8, C; Fig. 14, B), and *P. oligandrum* (Fig. 13, B; Fig. 14, C, D) less severely than *P. ultimum* or *P. debaryanum*. On encountering its mycelium the spiny forms put forth numerous delicate branches that make contact with the filaments of *P. myriotylum*, and often enwrap them more or less extensively. Some of the delicate branches become slightly enlarged at the tip, which then is applied closely to the wall of the *myriotylum* filament, where often it is visibly cemented in place by means of an opaque secretion. The terminations thus present the appearance of appressoria, and, indeed, sometimes operate as such by intruding haustorial threads into the filament to which they are applied (Fig. 8, C; Fig. 14, D). These haustorial threads grow lengthwise within the invaded filament until their further extension in either direction is checked by a septum that the host has in the meantime laid down at some distance from the place of ingress. Since the granular contents of the host disappear locally as invasion proceeds, it may be presumed that they are assimilated by the haustorial threads, and that, accordingly, the relationship present here is an unambiguously parasitic one. Yet, in most cultures the greater proportion of *myriotylum* hyphae invested by branches of the spiny forms are not actually invaded. Often these hyphae show abnormal modification both in somewhat increased thickness of the peripheral wall generally and in much more pronounced local thickening on the inner side of the wall at places where appressoria have become attached. The protoplasm within them often remains in normal condition for a protracted period, but often, too, it migrates to other portions of the mycelium, or undergoes gradual degeneration. Similar migration and
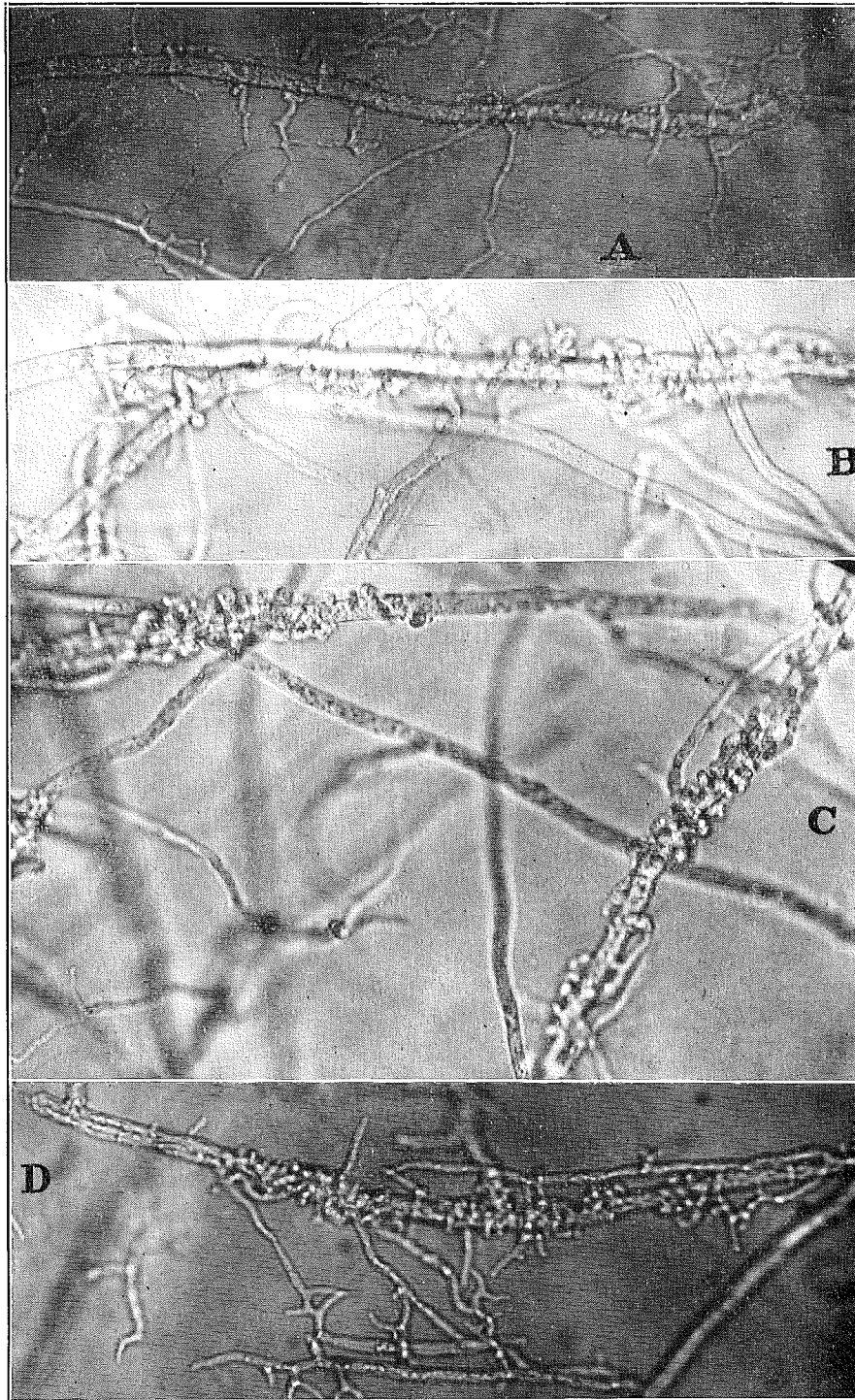
FIG. 14. A. Hypha of *Pythium myriotylum* attacked by *P. acanthicum*. B. Hyphae of *P. myriotylum* attacked by *P. periplocum*. C, D. Hyphae of *P. myriotylum* attacked by *P. oligandrum*.

degeneration of protoplasm takes place in some measure also in vigorous mycelium of *P. myriotylum* that, after being removed to a glass slide, is subjected to microscopical examination; so that the harmful effect of the enveloping branches is not easily ascertainable. Nevertheless the impression usually is gained that the branches are injurious, at least locally. It is not obvious, however, that the spiny forms obtain food materials from the *myriotylum* filaments merely intricated by them, unless, perchance, after the intricated filaments have been made to degenerate internally, their soluble contents may become available in part by diffusing out into the ambient. The expanded terminations that sometimes serve as appressoria seem hardly large enough to operate effectively as cupping organs.

When a growing mycelium of *Pythium myriotylum* encounters a growing mycelium of *Plectospira myriandra* (Fig. 8, E) or meets a growing mycelium of the pansy (Fig. 8, D), the spinach (Fig. 13, C), or the flax strain of *Aphanomyces cladogamus,* its hyphae in the zone of encounter are promptly enveloped in an elaborate manner by short ramifying branches, which, except for their greater width, resemble the branches extended under similar conditions by the 3 echinulate species of *Pythium.* As this envelopment proceeds the protoplasmic contents of the affected hyphae lose their normal appearance and become more or less opaque (Fig. 8, E; Fig. 13, C). Unmistakable disorganization promptly follows. Sometimes the enveloped portions of hyphae are, in addition, invaded internally (Fig. 8, D), but the injury accruing locally to the mycelium of *P. myriotylum* is obvious even where no invasion occurs. In any case the enveloped portions of hyphae, whether undergoing destruction through parasitism or through antagonism, are soon delimited from the adjacent healthy portions by cross walls.

Although *Pythium myriotylum* thus sustains some evident injury on encountering antagonistic oomycetous forms, its general development in dual culture is often impaired less than that of the fungus inflicting the injury. Under certain conditions of culture *P. acanthicum* and the strains of *Aphanomyces cladogamus* from pansies, spinach, and flax, are unable to advance any considerable distance beyond the zone of encounter into the tract of substratum already permeated with mycelium of *P. myriotylum;* whereas *P. myriotylum* will sooner or later spread over the tract of substratum originally occupied only by the opponent fungus. Under similar condition of culture, *Pythium periplocum* and *Pythium oligandrum,* as well as *Plectospira myriandra,* usually will halt the mycelial advance of *P. myriotylum* abruptly at the zone of encounter, while continuing their own advance into the tract already occupied by *P. myriotylum.* In such advance, however, their envelopment and destruction of hyphae diminish markedly, whether because they are adversely affected by the increasing staleness of the substratum, or because the older hyphae of *P. myriotylum* are less subject to attack. Meanwhile, as the older mycelium of the opponent species loses in vegetative vigor through aging and onset of sexual repro-

duction, the mycelium of *P. myriotylum* often recovers initiative and resumes growth by advancing in thinner array beyond the zone of encounter; so that, eventually, its oospores, like those of the opponent species, may often be found distributed in all portions of the culture. The ability of *P. myriotylum* to offset the discomfiture suffered in the zone of encounter seems attributable in part to its more prolonged vegetative period and its ready production of aerial mycelium.

When a growing mycelium of *Pythium ostracodes* (Fig. 15, A, a) encounters a growing mycelium of *P. acanthicum*, *P. periplocum* (Fig. 15, A, b) or *P. oligandrum* in a Petri-plate culture, both usually continue to advance without interference becoming manifest macroscopically in modification of their circular outlines. Indeed, on microscopical examination, sometimes only a few hyphae of *P. ostracodes* may be found enveloped by branches of the opposed spiny form. At other times, however, especially when a rather soft agar medium has been used in the preparation of the dual culture, numerous hyphae of *P. ostracodes* in the zone of encounter are very elaborately enveloped in ramifications of *P. periplocum* (Fig. 13, D, E), of *P. oligandrum* (Fig. 13, F; Fig. 16, A), or of *P. acanthicum*. On either side of the zone of encounter much less envelopment is noticeable; the intrication present in the tract where new hyphae from one of the 3 echinulate species have encroached upon aging hyphae of *P. ostracodes* usually being somewhat more abundant than that present in the tract where new hyphae of *P. ostracodes* have encroached upon old hyphae of the spiny form. It is remarkable that however elaborately the hyphae of *P. ostracodes* may be enveloped by any one of the 3 echinulate species, they commonly show no injury from such envelopment.

A far different result is obtained when *Pythium ostracodes* (Fig. 15, B, a) is grown in dual culture with *Plectospira myriandra* (Fig. 15, B, c; Fig. 16, B) or with any one of the strains of *Aphanomyces cladogamus* isolated from diseased roots of spinach (Fig. 12, G), flax, and pansies. In such dual culture the mycelial advance of *P. ostracodes* is abruptly halted wherever it encounters the mycelium of the saprolegniaceous form, which continues to grow with unabated rapidity. In the zone of encounter and soon afterwards also in tracts closer to their origin the hyphae of *P. ostracodes* are elaborately enveloped by branches of the opponent fungus, and their protoplasmic contents made to degenerate (Fig. 16, B). Here and there a filament may suffer internal invasion (Fig. 12, G). The injury thus sustained by the *Pythium* mycelium often results in noticeable reduction in the quantity of oospores formed by it.

Evidence indicating some sort of biotic relationship between oomycetes associated with damping-off and other diseases destructive to terrestrial phanerogamic plants was brought forward by de Bary (2) more than 60 years ago. In the garden cress (*Lepidium sativum* L.) seedlings that he used for substratum, this investigator never observed his *Pythium artotrogus* to occur alone: in all instances he found it accompanied by a congeneric
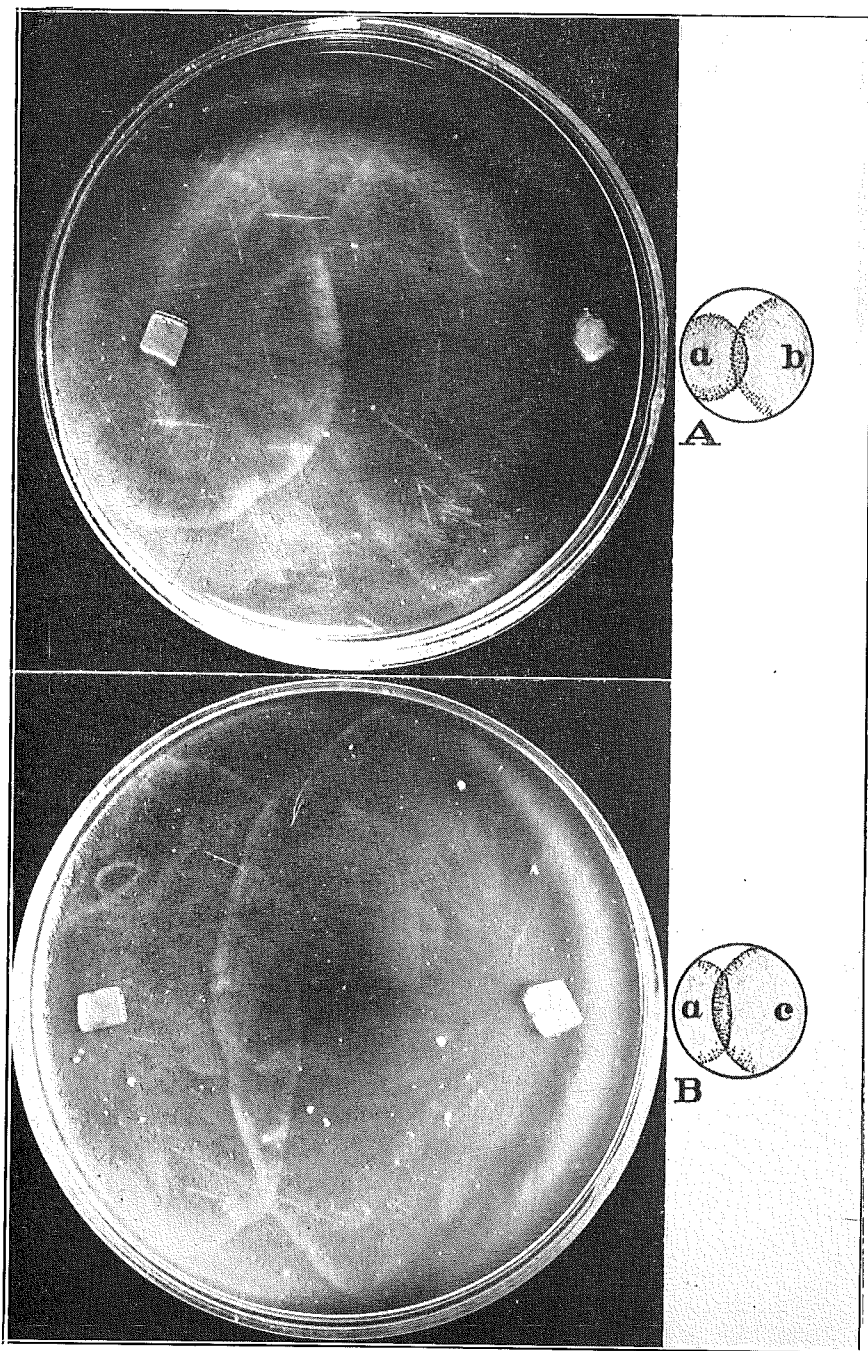
FIG. 15. Growing mycelium of *Pythium ostracodes* (a) encountering growing myce-lium of *P. periplocum* (b), the rounded outlines of both mycelia showing no sign of re-tarded advance. B. Mycelium of *P. ostracodes* (a) encountering growing mycelium of *Plectospira myriandra* (c); the former, as is manifest from the lesser convexity of the portion of its outline within the region of encounter, being retarded or halted in its advance.
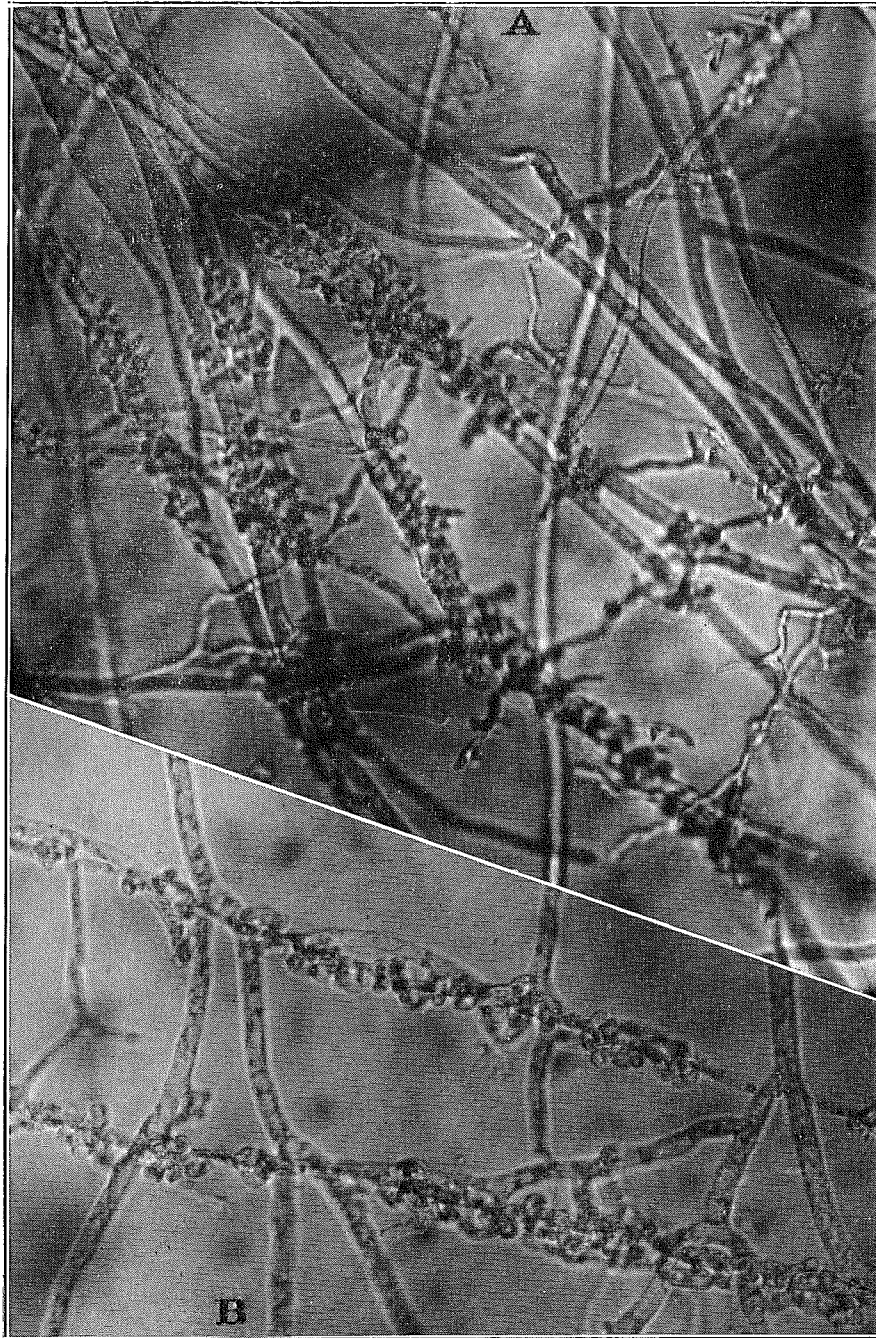
FIG. 16. A. Mycelium of *Pythium ostracodes* attacked by *P. oligandrum*. B. Hyphae of *P. ostracodes* attacked by *Plectospira myriandra*. The enveloped hyphae in A have remained normal, whereas those in B have collapsed.

species, which he identified as the widely pathogenic *P. debaryanum*. The congeneric species always appeared first, or at least became recognizable first through its earlier formation of reproductive organs. De Bary made repeated attempts to propagate *P. artotrogus* separately by removing living pieces of germ hyphae from its germinating oospores and placing them in drops of water to which were then added pieces of living and of scalded cress tissue. Though the germ hyphae branched abundantly in the liquid of the cultures thus prepared, the ramifying filaments consistently failed to infect either the living or the scalded tissue fragments, and consequently perished after a few days. Yet, in similar cultures to which *P. artotrogus* had been added, together with the related fungus recognized as *P. debaryanum*, the former always reproduced, even if at times only meagerly, by giving rise to its distinctive spiny oogonia.

De Bary offered 2 alternative explanations for the curious behavior of *Pythium artotrogus*: the fungus might require substances for growth that are absent in living or scalded cress tissue, but become available when such tissue is acted on by some other fungus, as, for example, *Pythium debaryanum*; or it might subsist as a parasite on *P. debaryanum*, much as species of *Chaetocladium* or *Piptocephalis* subsist as parasites on Mucoraceae. Further, de Bary had noted association of *P. artotrogus* with other fungi in its occurrence elsewhere than on cress seedlings in the laboratory. Thus, he had observed it associated with *Phytophthora infestans* (Mont.) de Bary in brown lesions on potato (*Solanum tuberosum* L.) stems, and with *P. infestans* as well as with *Pythium vexans* in dead tissue of potato vines. Again, in dried potato material in an authentic specimen of Montagne's *Artotrogus hydnosporus*—which species he held identical with his own *P. artotrogus*—he had observed spiny oogonia associated with swollen bodies of some alien fungus that might possibly have represented conidia of *P. debaryanum*.

De Bary's observations on *Pythium artotrogus* have been given some corroboration in several later writings. Butler's (4) report that he found *P. artotrogus* in rotting potato tubers attacked by *Phytophthora infestans* in Calcutta in 1902 tends to support the presumption of a habitual biotic association; though his further statement that for some time he successfully maintained the spiny fungus in cultures apparently free of *Phytophthora* hyphae, as also Mitra's (28) subsequent report that *P. artotrogus* had been found in stored potatoes in India, growing individually or associated with *P. infestans*, would seem at variance with the presumption of an obligate association. Under the binomial *Pythium hydnosporum* (Mont.) Schroet. supposedly the same spiny fungus was reported by Clinton (5) in Connecticut to have been observed not only in rotten potato tubers that had first been injured by *P. infestans*, but also in pea (*Pisum sativum* L.) roots injured by *Phytophthora cactorum* (Leb. and Cohn) Schroet., and especially abundantly in rotting grapes injured by the black-rot fungus and the grape berry moth. Sawada (31), working in Formosa, found *P. hydno-*

sporum in old tissue in a portion of a leaf of *Boehmeria nivea* Hook. and
Arn. that had been attacked by *P. cactorum*. Dissmann (8), in Bohemia,
while studying the relationship of premature decay of water lily (*Nymphaea
candida* Presl.) foliage to the development, more particularly, of the 2
Pythium species he designated as *P. undulatum* Pet. and *P. proliferum*
de Bary, noted that *P. artotrogus* sometimes occurred concomitantly with
these 2 species, though only in older disintegrating portions of affected
leaves,—a manner of occurrence he set forth as illustrating de Bary's view
that the spiny form is adapted to the utilization of nutrient products sup-
plied or modified by other fungi.

There is reason to suspect that among these additional first-hand reports
on the occurrence of *Pythium artotrogus* in association with other fungi,
as, indeed, also among the several first-hand reports mentioning its occur-
rence regardless of any such association, some may have been based on
material not truly referable to de Bary's species. It is not evident that
the different observers, with the exception of Butler (4), gave sufficient
heed to the necessity for ascertaining whether their fungi showed corre-
spondence with de Bary's (1) description in regard more particularly to
fertilization of the oogonium by an antheridium consisting always of an
adjacent hyphal segment, usually not modified externally, about 3 to 6 times
longer than wide, and delimited by deposition of a septum in the supporting
filament. Where determinations were based only on similarities to the
oogonium of *P. artotrogus*—which organ was described originally (2) as
being mostly 18 to 27 μ in diameter, and as being beset with tapering spines
3 to 6 μ long—the specimens might readily have belonged, instead, to
P. acanthicum, P. periplocum, or *P. oligandrum.* In the morphology of
their oogonia my 3 echinulate species show such close agreement with the
illustrations and description of *P. artotrogus* that, from the beginning,
I considered them intimately akin to de Bary's fungus. The agreement in
morphology of oogonium would now seem to be sustained in parallelism of
biotic relationship. This parallelism, it is true, would seem relieved by
some noteworthy divergence. Contrary to de Bary's experience with *P. arto-
trogus*, my 3 species grow and reproduce well on many kinds of substrata,
both natural and artificial, without the intervention of any other fungus;
and 2 of them, moreover, are known to flourish under natural conditions
as autonomous parasites on at least one phanerogamic host.

Attack on other phycomycetes by a saprolegniaceous fungus solely
through external enwrapment of their hyphae was set forth by Couch (6)
in the original descriptive account of his *Aphanomyces exoparasiticus.*
Among the phycomycetes found subject to envelopment and injury was an
unnamed species of *Pythium*. Although in one instance a direct proto-
plasmic connection was seen between a filament of this *Pythium* and a
branch of *A. exoparasiticus*, no interchange of materials was observed.
Apparently, *A. exoparasiticus* never invades the hyphae that it has envel-
oped, and that, as a result of being enveloped, have suffered disorganization

of their protoplasmic contents; so that, like *A. cochlioides* in some combinations, it would seem to lack even such scant internal assimilative apparatus as is formed under analogous conditions by *Plectospira myriandra* and by the 3 strains of *A. cladogamus* isolated from diseased roots of pansies, flax, and spinach. Unless the externally applied ramifications are capable of serving far better as cupping structures than appearances suggest, the elaborate attack of the several saprolegniaceous root-rotting forms here discussed would seem directed more to thwarting the development of competing fungi than to utilizing their substance as nourishment. In fine, the aggressive relationships displayed by these forms would seem to hold a larger measure of antagonism than of parasitism.

SUMMARY

Under warm, moist conditions, *Pythium myriotylum* produces abundant aerial mycelium, which, on encountering solid objects, gives rise to innumerable appressoria in dense clusters. This manner of growth makes possible the aerial parasitism whereby the fungus is enabled, much like *P. butleri*, to destroy bulked vegetables such as cucumbers, summer squash, and string beans. *P. myriotylum* rather closely resembles *P. butleri* also with respect to its asexual reproductive stage, though noticeable differences are present in the less pronounced inflation of its zoosporangial branches and in its somewhat less bountiful production of zoospores. Its sexual reproductive stage, however, differs greatly from that of *P. butleri*, seeming, rather, to betray intimate taxonomic kinship with *P. arrhenomanes*. The oospores, of unitary internal organization, often germinate, when bathed in fresh water, by putting forth a long simple germ hypha, which, after having received the entire protoplasmic contents, dehisces terminally as a zoosporangium.

A species of *Pythium*, isolated in Texas from wheat roots, is described as new under the binomial *P. ostracodes*. Its production of subspherical zoosporangia at the tips of hyphae, together with its development of oospores having multiplicate internal organization, characterizes it as a member of the *helicoides* series. In this series it is distinguished more especially by the very simple make-up of its sexual apparatus; fertilization being accomplished usually by a single antheridium borne terminally on a branch arising from the same hypha that supports the oogonium either directly or on a short stalk. When bathed in fresh water its oospores germinate very readily by producing, often in part through proliferous development, 1 to 3 terminal subspherical zoosporangia. During germination the very thick, shell-like, colored, outer layer of the oospore wall becomes conspicuously honeycombed with radial pockets and canals as its substance undergoes progressive resorption.

Pythium myriotylum, like numerous congeneric forms, is attacked by *P. acanthicum*, *P. periplocum*, and *P. oligandrum*, though less destructively than the familiar pathogenic species *P. ultimum*, *P. debaryanum*, and *P.*

irregulare. In their attack the 3 echinulate fungi send out numerous branches that invest the *myriotylum* hyphae, in some places merely bringing about noticeable abnormality, in some other places inducing local disorganization of protoplasmic contents, and in still other places intruding assimilative elements internally. The 3 echinulate species often envelop filaments of *P. ostracodes* more elaborately without, however, causing any appreciable injury. Further, the hyphae of *P. myriotylum* and *P. ostracodes*, as well as of many other species of *Pythium*, are subject to elaborate envelopment also by such saprolegniaceous root-rotting forms as *Plectospira myriandra* and the 3 strains of *Aphanomyces cladogamus* occurring in diseased roots of pansies, flax, and spinach, respectively. Invasion sometimes follows envelopment, but even when invasion does not occur the enveloped *Pythium* filaments suffer local disorganization of their contents. The seemingly meager utilization of the degenerating materials suggests that the relationship may be antagonistic more largely than parasitic.

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CORRECTIONS

In my paper entitled "Three species of *Pythium* with proliferous sporangia" (Phytopath. 31: 478–507. 1941) the line preceding the last line on page 504, and reading, "sporangia, which often show proliferous development. In an old culture on" should properly follow the second line of text on page 505. A somewhat less bewildering error in line 20 on page 499 of the same paper may be corrected by changing the word "furthering" to "further."—C. D.