

## THREE SPECIES OF PYTHIUM ASSOCIATED WITH ROOT ROTS

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In a paper (9) published nearly 10 years ago I presented as new 15 species of *Pythium* that had been isolated from decaying parts of various phanerogamic host plants originating from different localities in eastern and southern regions of the United States. Aside from some introductory comments, mainly of a comparative nature, the descriptions then accorded to the new forms were limited to diagnostic statements not accompanied either by needful explanatory remarks or by figures illustrative of details and peculiarities difficult to set forth adequately in words. It is hoped that as far as 6 of the species are concerned, these deficiencies of treatment have in a measure been remedied in supplementary accounts (12, 13) that have recently appeared in this journal. Similar supplementary consideration is herein devoted to 3 additional species, *P. dissotocum*, *P. periculum*, and *P. paroecandrum*, all of which I described from pure cultures isolated through procedure elsewhere (7, p. 310-312) recorded, from softened or discolored tissues of roots affected by decay. To facilitate comparison, the accompanying figures were prepared for reproduction at magnifications uniform with those in similar illustrations of the congeneric forms dealt with earlier. As the zoosporangia of *P. paroecandrum* are of the conveniently compact sub-spherical type and appear unaccompanied by significant differentiation of supporting hyphae, they are shown, like some of the less rangy homologous structures (12, p. 399, fig. 3, I, J, K) of *P. acanthicum* Drechsl., at the same magnification (*i.e.*,  $\times 1000$ ) employed in illustrations of sexual apparatus, rather than at the lower magnification (*i.e.*,  $\times 500$ ) resorted to in figures of the more extensive filamentous or lobulate sporangial units of numerous related species.

### PYTHIUM DISSOTOCUM

The diagnosis of *Pythium dissotocum* Drechsl. was based primarily on a culture submitted to me in a varied assortment of fungus cultures isolated by R. D. Rands from diseased roots of sugar cane, *Saccharum officinarum* L., collected near Thibodoux, Louisiana, in April, 1927; some utilization, however, being made also of observations on 5 other cultures in the same assortment that were easily recognized as conspecific from a thoroughgoing similarity of macroscopic appearance, and from a close parallelism in mycelial habit, zoosporangial development, and arrangement of sexual apparatus revealed by each of them under the microscope. On diseased sugarcane roots the fungus would seem to occur with moderate frequency. Under the binomial *P. dissotocum*, Rands and Dopp (19) make mention of 57 cultures that were isolated by them from such roots and subjected to growth measurements and to tests for pathogenicity. They further cite *P. dissoto-*

*cum* among the 3 species that, apart from *P. arrhenomanes* Drechsl., were most frequently obtained by them from rotted sugar-cane roots in 1930. Inoculation experiments of these authors reveal the fungus as only weakly parasitic when environmental conditions are in ordinary degree favorable for the host plant; severe root rot with appreciable reduction in plant weight ensuing, however, under the predisposing influence of a soil toxin, salicylic aldehyde, in dilute concentration.

The fungus is known to occur also on phanerogamic plants other than sugar cane. It was found in several sets of cultures isolated from softened roots of canning peas, *Pisum sativum* L., collected in the course of a survey on which a report (5) was rendered in 1925. The sets of cultures in question were derived from collections made respectively near Easton, Maryland, May 15, 1924; near Centerville, Md., May 16, 1924; near Bridgeville, Delaware, May 27, 1924; near Cedarville, New Jersey, May 29, 1924; and near Westminster, Md., June 11, 1924. *Pythium dissotocum* was likewise represented in 2 cultures isolated by F. R. Jones from pea roots collected in the course of another disease survey (16). One of these cultures was derived from material collected near Templeton, Wisconsin, July 4, 1924; the other from material collected near Gillett, Wis., July 17, 1924. The fungus was obtained later from discolored rootlets of *Pilea pumila* (L.) Gray collected near Cabin John, Md., Oct. 20, 1926. Four cultures isolated from discolored rootlets of the sugar beet, *Beta vulgaris* L., gathered near Saginaw, Michigan, late in June, 1927, have been readily identified as belonging to the species; and a similar determination was made of 3 cultures derived from discolored roots of spinach, *Spinacea oleracea* L., collected near Norfolk, Virginia, late in November, 1932.

In pure culture on a transparent gel substratum not excessively rich in nutrients, such as is available more especially in maize meal agar, *Pythium dissotocum* grows appreciably more slowly than the very familiar congeneric forms causing damping-off in seed-beds. Aerial mycelium is usually altogether absent on this medium, though sometimes developing in meager quantity on substrata that contain food substances in higher concentrations. As was stated in the diagnosis, the submerged mycelium is somewhat lustrous, presenting an appearance, therefore, in a sense median between that of the diffuse intramatrical mycelium of *P. ultimum* Trow on the one hand, and that of the very lustrous mycelium of *P. complens* Fischer on the other. Commensurate with its moderate luster, the thallus of *P. dissotocum*, while consisting more largely than that of *P. ultimum* of rather straightforward axial hyphae arranged nearly parallel with one another, is composed of such hyphae in smaller measure than is the thallus of *P. complens* or of the similarly very lustrous *P. vexans* de Bary (= *P. complectens* Braun). The curious regional variegation with respect to density of hyphal development that becomes apparent to the naked eye in a cumulous effect is not usually evident in cultures of the fungus under consideration. Appressoria of modest

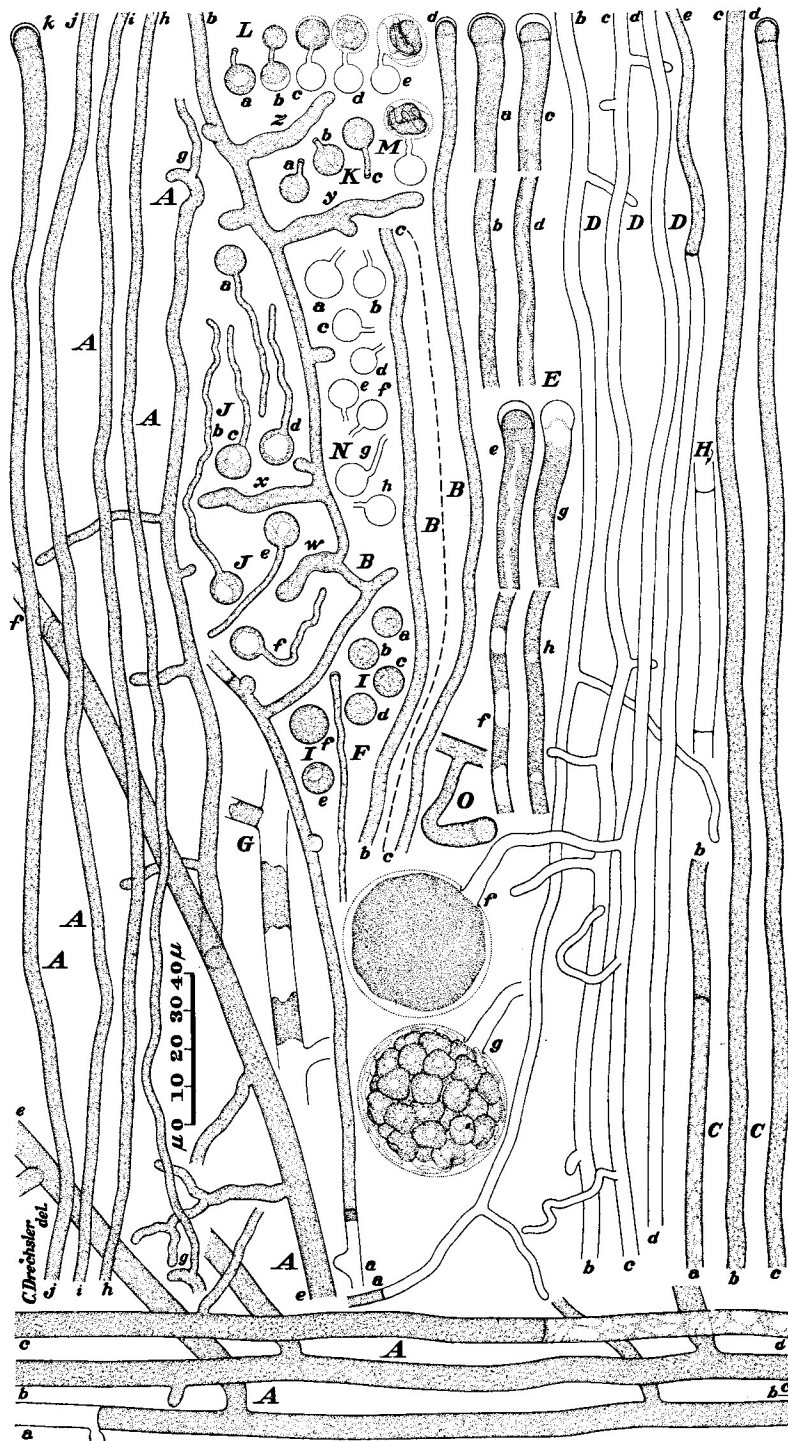


FIG. 1. Asexual reproductive apparatus of *Pythium dissotocum* Drechs. drawn with aid of a camera lucida;  $\times 500$ . From lack of space A is shown in sections connecting at the points b, e, g, h, i, j; B similarly connecting at points b, e; C connecting at points b, e; and D connecting at points b, e, d.

dimensions (Fig. 1, O) are formed in some numbers where hyphae come in contact with the surface of the culture dish or of other hard objects.

Production of zoospores by *Pythium dissotocum* ensues with much regularity when vigorous mycelium of the fungus is bathed in water. Extraordinarily prolific development of swimming spores may be induced conveniently by cutting sizeable slabs well permeated with young mycelium from a vigorously growing Lima-bean agar plate culture, then transferring the excised slabs to an empty sterile Petri dish, and irrigating them by careful addition of well-aerated sterile water until the upper surface of the substratum is barely flooded. Often, on proper manipulation, virtually the entire mycelium becomes converted into sporangial units. In many instances the individual unit is composed of a longish portion of a wide axial filament together not only with contiguous portions of a few main branches but also with perhaps more numerous narrower lateral ramifications, one of which may become prolonged into a rangy evacuation tube more than 1 mm. in length (Fig. 1, A, a-k). Such a large extensive sporangium naturally gives rise to a correspondingly large vesicle wherein from 100 to 125 zoospores may be fashioned. A sporangial unit of more moderate volume consists often of an intercalary portion of filament, 1 to 2 mm. long and 3 to 4  $\mu$  wide, together with a half-dozen subsidiary ultimate branches, and yields between 50 and 75 zoospores (Fig. 1, D, a-e, f, g). Frequently an unbranched terminal portion of filament, from 0.5 mm. to 1 mm. in length, becomes delimited by a basal septum, and, after forming a broad tip of dehiscence (Fig. 1, C, a-d), functions as a sporangium. A small sporangium may be provided with an evacuation tube measuring as little as 1.5  $\mu$  in width below an expanded tip only 2.5  $\mu$  wide (Fig. 1, F).

Under conditions very favorable for zoospore production, conversion of a portion of vegetative mycelium into an asexual reproductive unit is in most instances not preceded or accompanied by development of any specially differentiated elements, apart, of course, from the expanded cap of dehiscence. In less numerous instances (Fig. 1, B, a-d), however, such conversion entails production of several swollen lateral branches (Fig. 1, B, w-z) noticeably wider than the filament bearing them, though frequently not exceeding in width the undifferentiated main axial hyphae of the fungus. The swollen branches attain more conspicuous development ordinarily after an expanse of mycelium has largely exhausted itself in zoospore production and has possibly been affected besides by some accumulation of staling products, as well as by incipient bacterial contamination (Fig. 2, A). Though usually affording only a rather meager display, the dactyloid branches appear truly homologous with the distended digitations, lobulations, and moriform aggregations familiar in certain congeneric forms like *Pythium arrhenomanes*, *P. complens* and *P. periplocum* Drechsl.

The zoospores of *Pythium dissotocum*, after swimming about very actively for a variable period, come to rest and round up, thereby forming spherical cysts slightly smaller than the cysts of *P. debaryanum* Hesse and of most

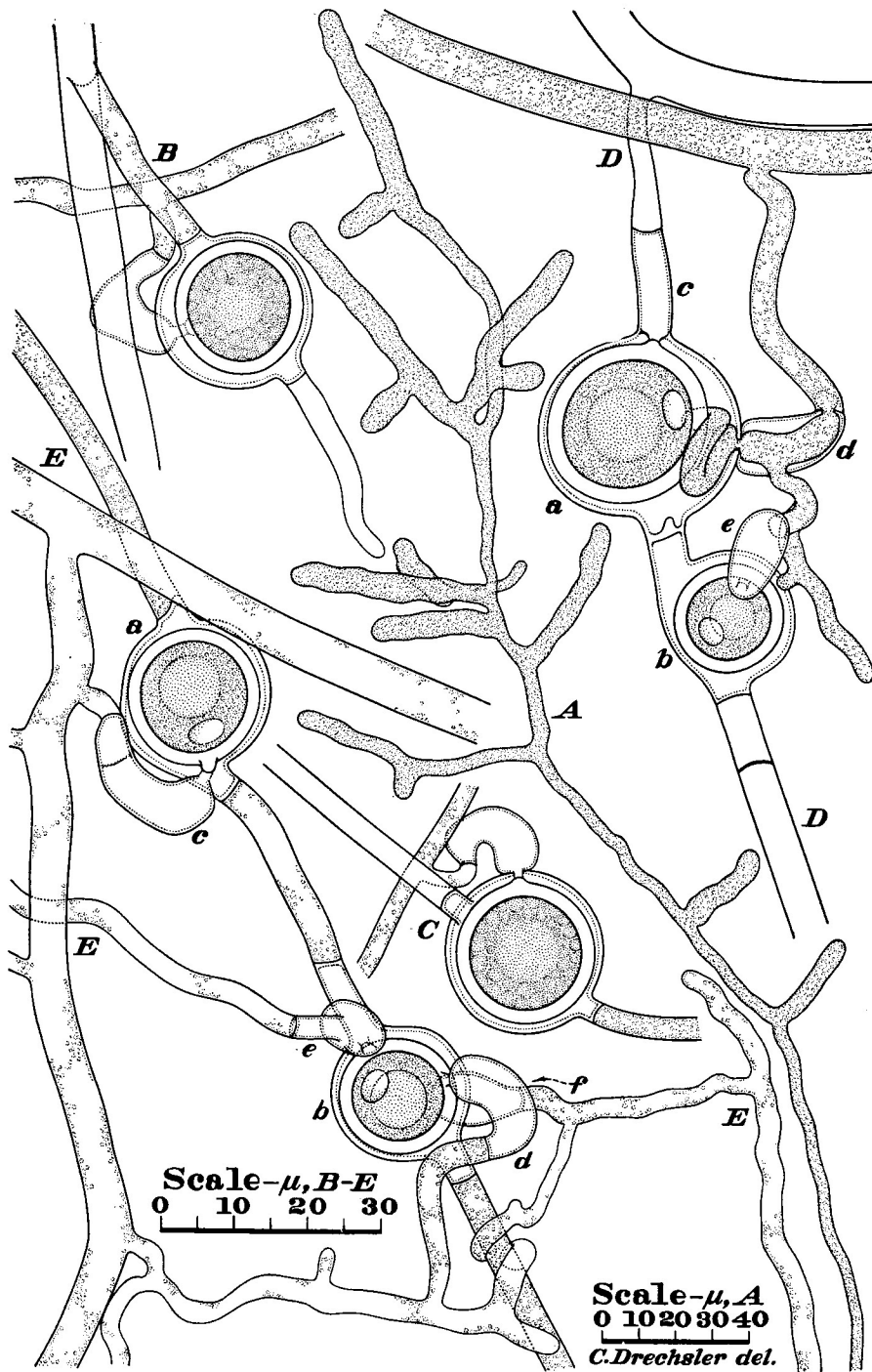


FIG. 2. *Pythium dissotocum* drawn with the aid of a camera lucida. A. Portion of irrigated mycelium, showing zoosporangial differentiation in relatively luxuriant development of inflated lateral branches;  $\times 500$ . B-E. Units of sexual apparatus;  $\times 1000$ .

other allied species familiar to plant pathologists. Often the spherical bodies then germinate in a commonplace manner by the production usually of a single delicate germ hypha (Fig. 1, J, a-f); or, again, they may develop iteratively, each putting forth an evacuation tube (Fig. 1, K, a-c; L, a) and discharging its contents (Fig. 1, L, b) into a small vesicle, there to be fashioned into a new biciliate motile zoospore (Fig. 1, L, c-e; M) of the same type as the one from which it originated. As a result of such iterative development, innumerable empty cyst envelopes with open evacuation tubes of varying lengths (Fig. 1, N, a-h) are often to be observed scattered about everywhere in an irrigated preparation.

The frequency of iterant swarming in zoospores of *Pythium dissotocum*, to which brief allusion was made in an earlier paper (8, p. 569, lines 47 to 50) devoted mainly to similar activity in zoospores of various other pythiaceous forms, suggested the specific epithet, a term meaning "twice-born," subsequently applied to the fungus. Additional instances of iterant swarming following repeated emergence have since been supplied by Sparrow (21) in the descriptions of his *P. adhaerens* and his *P. angustatum*. Höhnk (14) noted that the zoospores of the fungus he described as *P. epigynum* underwent a second swarm period when fresh water was added after a first encystment had occurred. This investigator later took occasion to give details concerning particular examples of repetitional development observed by him (15).

Most strains of *Pythium dissotocum* ordinarily show abundant and prompt sexual reproduction when grown on maize meal agar containing in suspension a moderate quantity of finely ground maize meal. It is true, sexual reproduction occasionally fails to take place, not only in the more refractory strains but also in strains habitually productive of oospores in immense numbers. As the conditions evoking such apparently capricious behavior have not hitherto been determined, it may only be conjectured that possibly some specific nutrient substance, not always available in sufficient quantity, exerts a governing influence. However, once sexual structures have been formed, they are little given to degeneration on a serious scale.

The subspherical oogonia of the fungus may be terminal (Fig. 3, A; D, a; H) or subterminal (Fig. 2, B; Fig. 3, E) on branches of variable length, though more often they are borne on the main hyphae in intercalary positions, sometimes mesially (Fig. 2, C; D, a; E, a, b; Fig. 3, B; C; D, b; F; J; K, b; L, a) or, again, laterally (Fig. 3, G, a, b; I; K, a; L, b). As the delimiting septa are often placed at appreciable distances from the subspherical contour, cylindrical parts, commonly  $2\ \mu$  or  $3\ \mu$  long, but sometimes measuring  $5\ \mu$  (Fig. 3, J),  $6\ \mu$  (Fig. 3, C),  $7\ \mu$  (Fig. 3, D, b) or even  $11\ \mu$  (Fig. 2, E, b) in length, come to be included in the female organ. Not infrequently, 2 intercalary oogonia are formed adjacent to each other (Fig. 2, D, a, b; Fig. 3, G, a, b; L, a, b).

The male complement of the individual oogonium consists usually of 1 to 3 antheridia, which, for the most part, are of the inflated crook-necked type

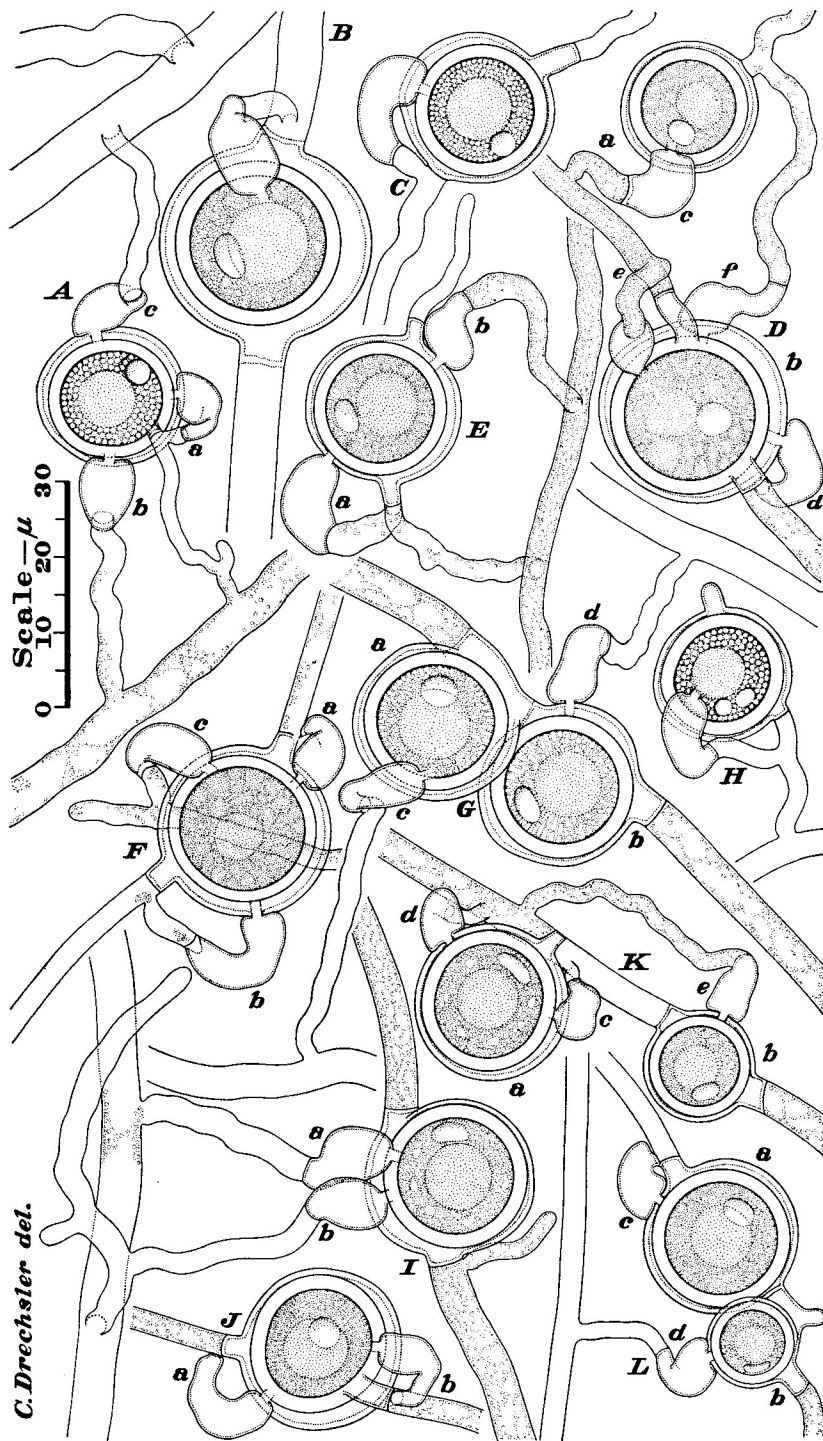


FIG. 3. Sexual apparatus of *Pythium dissotocum* drawn with the aid of a camera lucida to a uniform magnification;  $\times 1000$  throughout.

familiar in many congeneric species. Where plural antheridia are present they appear usually to have arisen without reference to one another, often despite a moderately close mycelial connection between them. As for their other relationships, the antheridia are often borne terminally on branches arising either from neighboring hyphae having no close connection with the oogonial filament (Fig. 2, B; C; D, e; E, e, d, f; Fig. 3, A, c; D, c; F, c; G, c, d; I, a, b; L, d), or from the oogonial filament at variable distances from the oogonium (Fig. 3, A, b; E, b; H; K, c, d, e), or often, again, from the oogonial filament in close proximity to the oogonium (Fig. 3, A, a; B; C; D, e; E, a; F, b). Often, too, an antheridium is borne sessile on the oogonial filament in immediate proximity to the oogonium (Fig. 3, D, d; F, a; J, a, b; L, c). The "hypogynal" type of antheridium, consisting of an outwardly undifferentiated portion of filament adjacent to the oogonium (Fig. 2, D, c) has likewise been observed in cultures of the fungus, though so infrequently that the few instances must be regarded as somewhat exceptional.

Application of the antheridium to the oogonium usually entails some apical flattening, so that contact between the opposed organs is generally wider in this species than in *Pythium peritum*, for example. In units of sexual apparatus with plural antheridia, all ordinarily discharge their contents into the oogonium. As in many other fungi, various irregularities of sexual reproduction occur in *P. dissotocum*, an example being illustrated somewhat incidentally in figure 2, D, where the antheridium "d," which presumably became applied to the oogonium "a" at a relatively late stage, is shown to have resorted to vegetative growth by intruding a hyphal prolongation into the unoccupied portion of the oogonial chamber, and by putting forth laterally a filament that gave rise to a short branch bearing the antheridium "e," which clearly was operative in the fertilization of the oogonium "b."

Following fertilization the oogonium of *Pythium dissotocum* shows a sequence of internal change familiar in most congeneric species. The contracted protoplast surrounds itself with a thick wall. The sizeable lumps of somewhat homogeneous consistency into which the porridge-like granular material has been aggregated, become displaced at the center of the young sexual spore by a homogeneous globule of increasing size (Fig. 2, B; C; Fig. 3, F). The resulting parietal layer diminishes in thickness correspondingly, and often reveals a perceptibly radial orientation as its constituent lumps undergo transformation into smaller granules (Fig. 3, D, a, b; E; G, a, b; J). At early maturity the layer has a densely and rather minutely granular texture, contrasting sharply with the apparently homogeneous structure of the single subspherical or oblate ellipsoidal refringent body (Fig. 2, D, a, b; E, a, b; Fig. 3, B; I; K, a, b; L, a, b). After several weeks of aging it is often found composed of larger subspherical granules measuring about  $.5 \mu$  in thickness (Fig. 3, A, C), and the single refringent body may be replaced by 2 similar bodies of slightly reduced dimensions (Fig. 3, H).



In the texture of its parietal layer the oospore at advanced maturity thus comes to present an engaging resemblance to the oospores of various Saprolegniaceae, including the several terrestrial parasitic species known to cause root rot in phanerogamic crop plants. This resemblance would seem sustained in a transitory arrangement of protoplasm observable in the zoosporangium of *Pythium dissotocum* shortly, though not immediately, preceding its evacuation. During the earlier stages in the development of an apex of dehiscence (Fig. 1, E, a), the contents of the hyphae to be included in the new sporangial unit show little alteration from the granular texture usual in vegetative filaments (Fig. 1, E, b). Later, when the hyaline cap has nearly attained its definitive proportions (Fig. 1, E, c), longitudinal vacuoles make their appearance in the hyphae, and often coalesce into extended axial lacunae of irregular outline (Fig. 1, E, d). In some of the narrower filamentous elements, though not usually throughout the sporangium (Fig. 1, E, e), the longitudinally vacuolate condition may for a brief time be supplanted by a transversely vacuolate one (Fig. 1, E, f), so that cylindrical portions of protoplasm alternate with regularly spaced vacuoles in a manner recalling the arrangement of zoospore protoplasts in *Aphanomyces* sporangia previous to their becoming connected by axial strands. To be sure, the transversely vacuolate condition is rather completely obliterated in the sudden reorganization of contents that precedes sporangial discharge by a few seconds. In this reorganization, which is often accompanied by a visible jolt of the hyphae concerned, the protoplasmic contents are withdrawn a short distance from the hyaline cap (Fig. 1, E, g), and revert throughout the reproductive unit to a granular texture relieved only sparingly by a few small, scattered vacuoles (Fig. 1, E, h).

An interrupted disposition of protoplasm, somewhat similar to that associated transitorily with sporangial development, is observable often in aging vegetative filaments (Fig. 1, G) of the fungus. Although aging of mycelium here entails much less abundant deposition of retaining cross-walls than in *Pythium debaryanum*, for example, successive septa may occasionally be found rather closely spaced in the empty hyphae (Fig. 1, H).

With regard to its principal dimensions, *Pythium dissotocum* shows the rather moderate range of variability prevailing in most members of the genus to which it belongs. The data in the diagnosis relevant to oogonial size were derived from 200 measurements of mature oogonia of obviously wholly normal development selected at random in maize-meal-agar cultures showing very copious sexual reproduction with virtually no degeneration. The 200 values for diameter of oogonium, expressed to the nearest micron, showed a distribution as follows: 12  $\mu$ , 1; 14  $\mu$ , 1; 15  $\mu$ , 3; 16  $\mu$ , 1; 17  $\mu$ , 3; 18  $\mu$ , 12; 19  $\mu$ , 34; 20  $\mu$ , 33; 21  $\mu$ , 43; 22  $\mu$ , 36; 23  $\mu$ , 17; 24  $\mu$ , 6; 25  $\mu$ , 7; 29  $\mu$ , 2; 32  $\mu$ , 1. Measurements of the 200 oospores of correct structure contained within the oogonia, gave the following values for diameter, expressed to the nearest micron: 11  $\mu$ , 2; 12  $\mu$ , 1; 13  $\mu$ , 1; 14  $\mu$ , 2; 15  $\mu$ , 8; 16  $\mu$ , 25; 17  $\mu$ , 48; 18  $\mu$ , 45; 19  $\mu$ , 42; 20  $\mu$ , 14; 21  $\mu$ , 6; 22  $\mu$ , 4; 26  $\mu$ , 1; 27  $\mu$ , 1.

## PYTHIUM PERILUM

The same collection of fungus cultures from sugar-cane roots that supplied the material on which primarily was based the description of *Pythium dissotocum*, included also the single culture from which, after varied treatment and propagation, was drawn the diagnosis of *P. perillum* Drechsl. Subsequently, a number of additional cultures, closely similar to the one in question with respect to macroscopic appearance, as well as with respect to morphology of sporangium and sexual apparatus, were committed to me by R. D. Rands and E. Dopp, who had isolated them likewise from affected roots of sugar cane in Louisiana. These investigators have found the fungus only feebly aggressive as a parasite, for under experimental conditions permitting severe damage by *P. arrhenomanes*, it caused only occasional root tips to become flaccid (19). In commenting on *P. perillum*, Stevenson and Rands (22) characterize the species as a weakly parasitic one, infrequently isolated from rotted rootlets of sugar cane.

On maize meal agar, *Pythium perillum* shows approximately the same rate of hyphal extension as *P. dissotocum*, and produces similarly an intramatrical mycelium with a lustrous radiate appearance expressive of a considerable degree of parallelism in the orientation of the main axial filaments. However, instead of the relatively uniform mycelial distribution usual in cultures of *P. dissotocum*, the vegetative thallus has rather marked local inequalities in the concentration of its hyphae, whereby it offers to the naked eye a patchy effect that from a suggestiveness of banked cumulous clouds was denominated "cumulous" in the diagnosis of the species.<sup>1</sup> Though aerial growth is usually absent in cultures on maize meal agar, some richer substrata as, for example, Lima-bean agar, sometimes afford meager development of aerial mycelium in a somewhat appressed, compact, felty layer.

On microscopic examination of its vegetative mycelium the fungus is revealed as one of the more delicate members of the genus to which it belongs. Knob-like appressoria of relatively small dimensions (Fig. 4, A, B) are formed in moderate numbers terminally on some of the delicate branches that encounter the bottom of a culture dish, or that otherwise come into contact with a hard object.

Asexual reproduction can be induced conveniently in *Pythium perillum* by excising sizeable slabs from a thinly poured Lima-bean-agar plate culture well permeated with young mycelium, and transferring them to a shallow layer of aerated sterile water in a sterile Petri dish. Some reproductive units are formed by direct conversion of undifferentiated filamentous hyphae, with only a rather meager increment accruing through development of an expanded tip of dehiscence. Sporangia of such meager external modification are, however, less frequent in irrigated material of the present fungus than in irrigated preparations of *P. dissotocum*, owing to a more abundant production of swollen digitate elements, here singly, there in some-

<sup>1</sup> Sideris (20) has since made reference to the same macroscopic effect by the descriptive term "rosette."

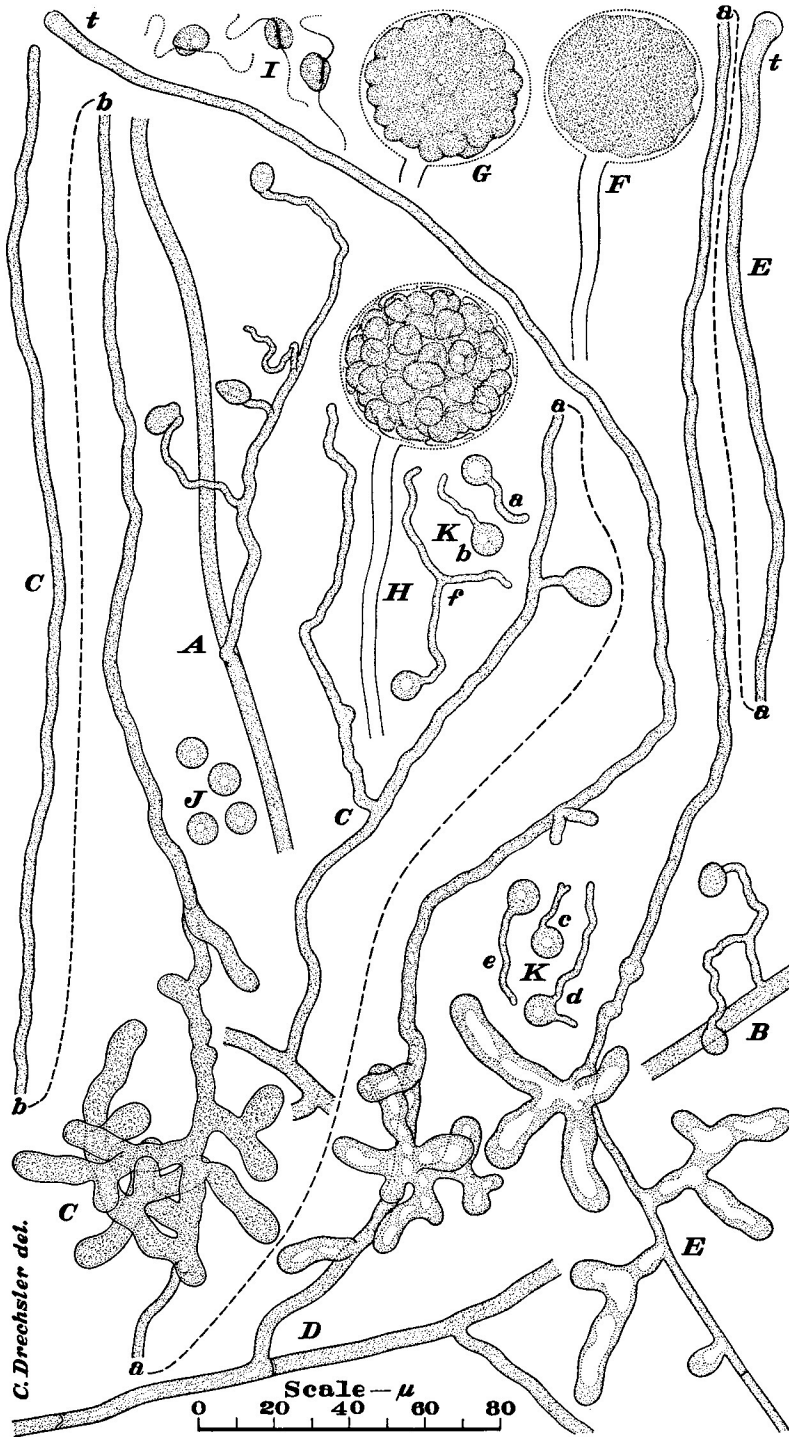


FIG. 4. Asexual reproductive apparatus of *Pythium peritum* Drechs. drawn with aid of a camera lucida;  $\times 500$ . From lack of space C is shown in sections connecting at the points a, b; E similarly connecting at the point a.

what intricately branching systems (Fig. 4, C) comparable, more especially perhaps, with the homologous sporangial complexes of *P. myriotylum* Drechsl. Collectively, the swollen parts included in a sporangium, when it comes to be delimited by deposition of a septum (Fig. 4, E) or of plural septa (Fig. 4, D), often are of a volume equal to (Fig. 4, D) or exceeding that of the unmodified filamentous parts. As in other species vacuolization of the associated filamentous and swollen elements proceeds simultaneously with the development of a widened refringent apex of dehiscence on an evacuation tube frequently of considerable length (Fig. 4, D, t; E, t). Discharge of the sporangial contents into a vesicle resulting from inflation of the refringent cap (Fig. 4, F), transformation of the discharged mass into biciliate zoospores (Fig. 4, G, H), and liberation of the motile bodies (Fig. 4, I) by disintegration of the vesicular membrane, follow in familiar sequence.

With appropriate irrigation *Pythium peritum* produces swarm spores in about the same moderate measure of abundance as *P. myriotylum*; the fungus being in general more prolific asexually than *P. debaryanum*, *P. irregulare* Buism., and *P. mammillatum* Meurs, but appreciably less prolific than *P. butleri* Subr., and decidedly less prolific than *P. dissotocum*. After swimming about for some time the zoospores come to rest and round up into cysts (Fig. 4, J) that like the cysts of *P. dissotocum* would seem to be somewhat smaller than the homologous bodies of most of the congeneric parasites causing damping-off. Germination of the globose structures takes place mostly by production of a delicate germ tube (Fig. 4, K, a-c, e, f) or of 2 such tubes (Fig. 4, K, d).

In maize-meal-agar cultures, containing some finely divided maize-meal, *Pythium peritum* gives rise promptly to sexual apparatus that develops usually with little evidence of degeneration. The subspherical oogonia appear very often in intercalary positions (Fig. 5, A, D-L), less frequently in subterminal (Fig. 5, B) or terminal positions (Fig. 5, C). Generally, while the individual female organ is still continuous with its supporting hypha, it becomes inwrapped rather intimately and extensively by a branching filament (Fig. 5, A). Usually this filament arises from a hypha having no close mycelial connection with the one bearing the oogonium (Fig. 5, A-G, I-K), but occasionally it originates as a branch given off by the oogonial hypha at a distance perhaps not exceeding 50  $\mu$  from the female organ (Fig. 5, H). On the ramifications of this filament are soon borne, mostly terminally, but in some cases approximately laterally, from 2 to 5 antheridia, which become delimited by basal septa at about the same time the oogonium also is demarcated by deposition of one or, more often, 2 cross-walls, now flush with the spherical contour, now at a distance of 1 to 4  $\mu$  from it (Fig. 5, B-L). As a rule all of the antheridia discharge their contents into the oogonium, whereupon, if degeneration does not intervene, an oospore is formed that at early maturity shows the unitary internal organization evident in ripe oospores of most species of *Pythium*,—its single

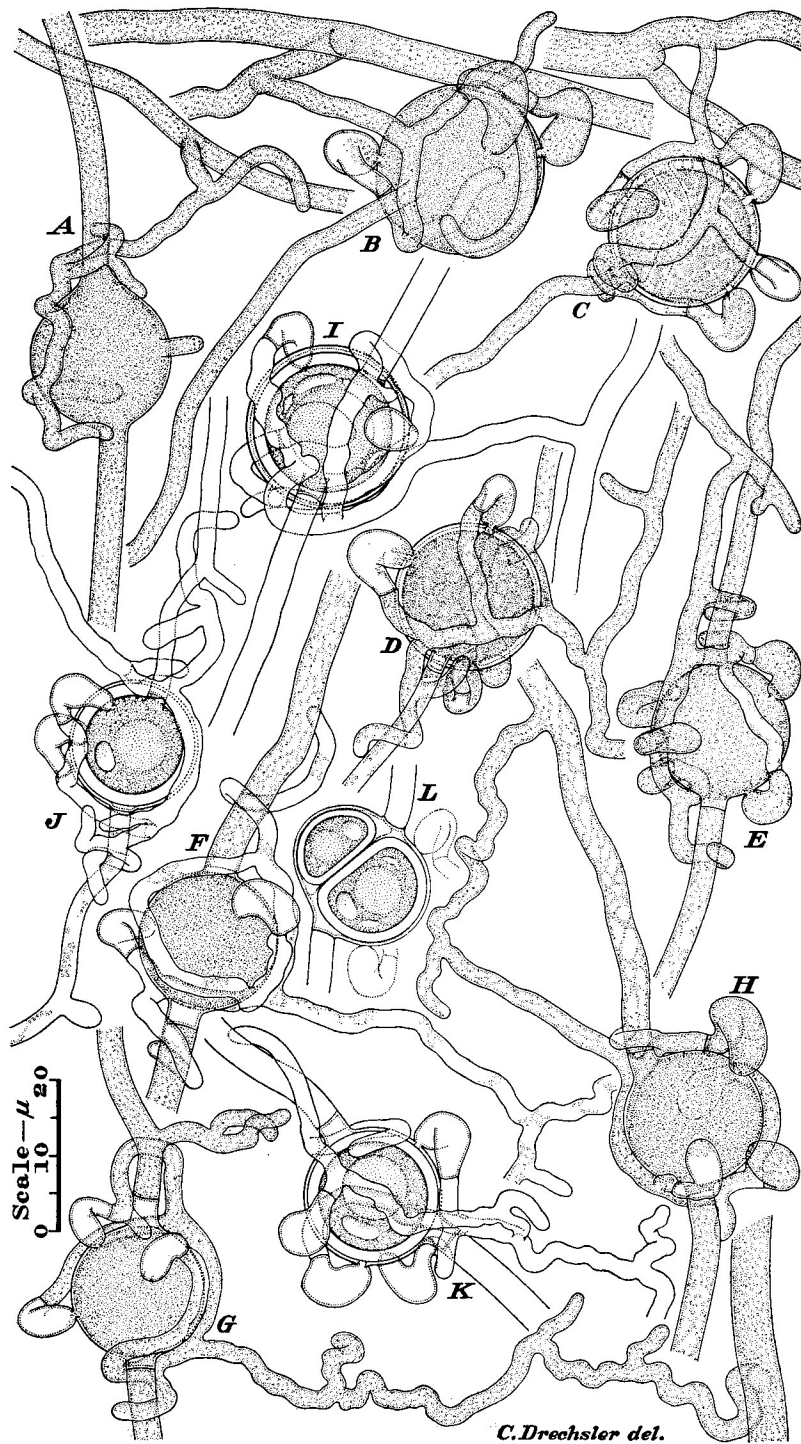


Fig. 5. Sexual reproductive apparatus of *Pythium peritum* drawn with the aid of a camera lucida to a uniform magnification;  $\times 1000$  throughout.

central reserve globule being surrounded by a densely granular parietal layer in which is imbedded a single refringent body, subspherical or oblate-ellipsoidal in shape (Fig. 5, I-K). Developmental irregularity sometimes becomes manifest in the production of 2 oospores within an oogonium (Fig. 5, L). For better comparison with other species, all inordinately fecund units of sexual apparatus were excluded from consideration in the parts of the diagnosis relevant to the main dimensions of oogonium and oospore; the data there given having been derived from measurements of 100 monosporous sexual units selected at random in a maize-meal-agar culture that had produced oospores very abundantly with little evidence of degeneration. The 100 oogonia gave values for diameter, expressed to the nearest micron, with a distribution as follows: 16  $\mu$ , 1; 17  $\mu$ , 13; 18  $\mu$ , 23; 19  $\mu$ , 31; 20  $\mu$ , 22; 21  $\mu$ , 7; 22  $\mu$ , 3; and the oospores of correct unitary internal structure contained within them gave values for diameter, likewise expressed to the nearest micron, with the following distribution: 14  $\mu$ , 1; 15  $\mu$ , 2; 16  $\mu$ , 21; 17  $\mu$ , 32; 18  $\mu$ , 26; 19  $\mu$ , 14; 20  $\mu$ , 4.

Rather little diagnostic value attaches to the sizes of oogonium and oospore in *Pythium peritum*, as the homologous bodies of many congeneric forms, including the several species most commonly implicated in damping-off, reveal approximately similar dimensions. Certainly, much greater distinctiveness is represented in the extensive and close inwrapment of the female organ by the branching antheridial filament,—this feature, indeed, having suggested the epithet applied to the fungus, a term derived from a word meaning “to wrap round.” Inwrapment of equal extent and intimacy, though frequent among terricolous species of *Aphanomyces*, has been encountered elsewhere in the genus *Pythium*, as far as I am aware, only in *P. scleroteichum* Drechsl. (10), the parasite that, with *P. ultimum*, is responsible for mottle necrosis, a curiously labyrinthine decay of the edible roots of the sweet potato, *Ipomoea batatis* (L.) Lam. Similarity to *P. scleroteichum* is recognizable, besides, in a characteristic frail appearance of the rather small, thin-walled antheridia, which, like the thin-walled branches supporting them, become almost indiscernible after being evacuated of contents. However, the transverse dorsal furrowing, often to be seen in the antheridial branches of *P. scleroteichum*, has never been observed in *P. peritum*. Further, in *P. peritum* the oospore so nearly completely fills the oogonial chamber that, often in large part, its relatively thick wall appears more or less closely adnate to the much thinner, somewhat evanescent oogonial membrane; whereas, in *P. scleroteichum*, the oospore is always very loosely contained within a considerably larger oogonium, and its wall only slightly exceeds in thickness the conspicuous and extraordinarily enduring oogonial envelope.

#### PYTHIUM PAROECANDRUM

The diagnosis of *Pythium paroecandrum* Drechsl. was based primarily on a culture isolated from the somewhat blackened tip of a rootlet that alone

seemed to harbor an infection among hundreds of wholly unblemished rootlets on a flourishing clump of field garlic, *Allium vineale* L., originating from near McLean, Virginia, early in May, 1925. The culture in question had been the first one referable to the species to come into my hands. Previous to its description the fungus had been isolated also from several discolored rootlets of the pale touch-me-not, *Impatiens pallida* Nutt., taken from specimens of that plant collected in the District of Columbia early in September, 1926. A few additional conspecific cultures have since been obtained from separate discolored rootlets of the bloodroot, *Sanguinaria canadensis* L., collected in Arlington, Va., in April, 1931.

When planted on maize meal agar, *Pythium paroecandrum* gives rise to a slightly lustrous intramatrical mycelium of rather pronounced radiate appearance that extends itself about half as rapidly as mycelium of *P. ultimum*, *P. debaryanum* or *P. irregulare*. On this medium the fungus, unlike the 3 congeneric forms mentioned, produces usually no aerial hyphae, although on various richer substrata, such as Lima-bean agar, some meager aerial development may take place. When portions of a vigorously growing culture are removed to a shallow layer of water devoid of nutrients, hyphae are put forth into the surrounding liquid only a short distance and in relatively small quantity. In its feeble extramatrical development the fungus differs markedly from the species habitually associated with damping-off,—the difference connoting undoubtedly an incapacity on the part of *P. paroecandrum* to operate destructively as a seed-bed parasite, inasmuch as strong extramatrical development evidently constitutes not an incidental but an essential and necessary attribute of damping-off pathogens, enabling them to span readily the tracts of unnutritious soil separating individual seedlings from one another.

On appropriate irrigation fresh growth of *Pythium paroecandrum* rather promptly gives rise to subspherical zoosporangia. In dimensions and general conformation these bodies resemble approximately the zoosporangia of *P. debaryanum*, *P. irregulare* and *P. mammillatum*, though perhaps they more frequently include at one (Fig. 6, A, a) or both ends (Fig. 6, B) an outwardly unmodified hyphal portion between  $5\ \mu$  and  $50\ \mu$  in length. A sporangium with hyphal prolongations here, like the similarly composite asexual reproductive structures frequent in *P. acanthicum*, very often puts forth the evacuation tube from the cylindrical component (Fig. 6, A, t; B, t), rather than from the subspherical part. Indeed, even in instances where a hyphal extension is relatively short and of small volume, it yet serves frequently as origin of the evacuation tube (Fig. 6, C, t; E, t; F, t). The more purely subspherical sporangia usual in the species show little preference for any particular positional relationship of the evacuation tube (Fig. 6, G, t-O, t). With regard to vacuolization of the protoplasm within a sporangium, formation of a somewhat expanded hyaline cap at the tip of the evacuation tube (Fig. 6, B, t), discharge of the granular contents into a vesicle resulting from inflation of the cap, cleavage of the discharged

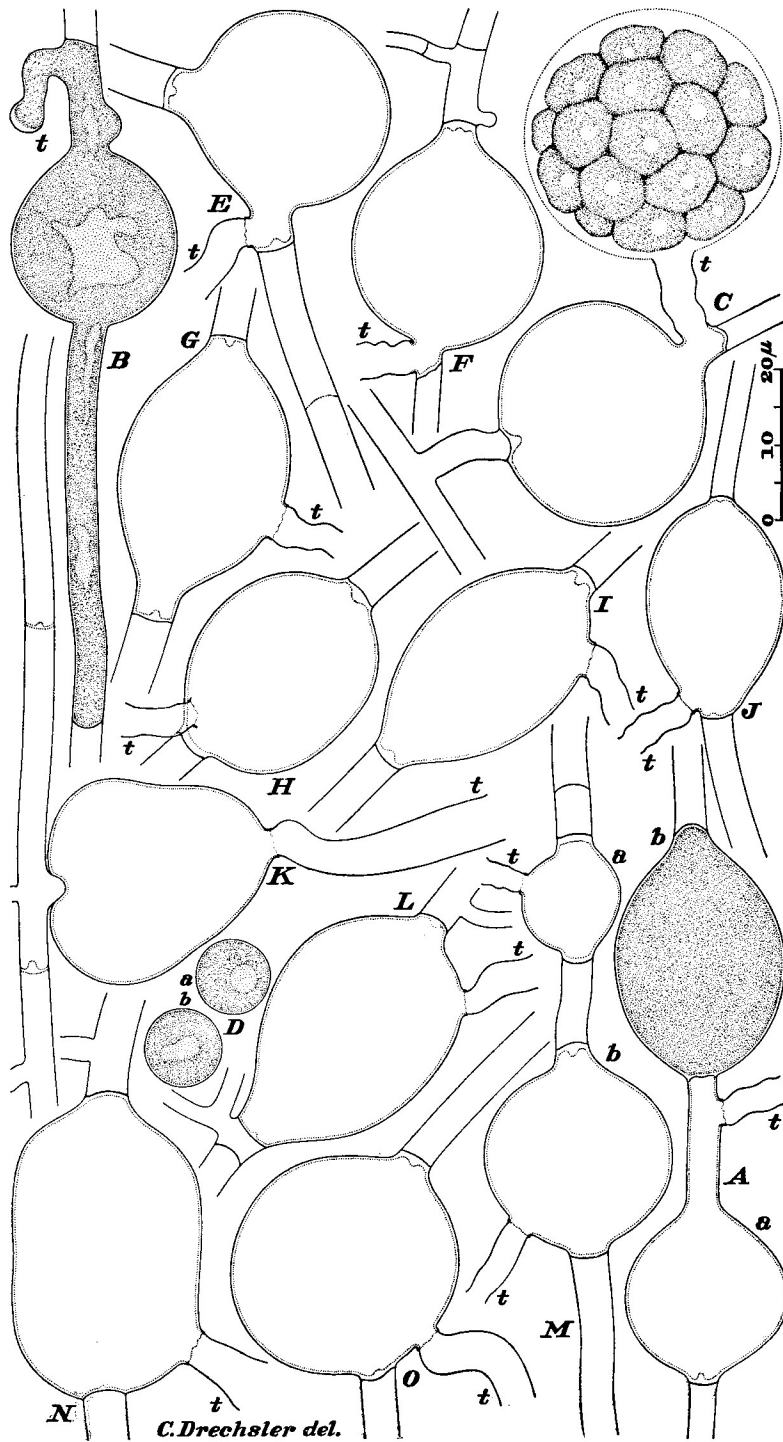


FIG. 6. Asexual reproductive apparatus of *Pythium paroecandrum* Drechs. drawn to a uniform magnification with the aid of a camera lucida;  $\times 1000$  throughout.



protoplasmic mass (Fig. 6, B), and its transformation into motile biciliate zoospores, the fungus shows general parallelism with *P. debaryanum*, *P. irregulare* and *P. mammillatum*. Its zoospores, like those of the 3 congeneric forms mentioned, round up into globose cysts (Fig. 6, D, a, b) slightly larger than the homologous bodies of *P. dissotocum* and *P. perülum*.

In maize-meal-agar cultures *Pythium paroecandrum* forms sexual apparatus promptly and abundantly. The oogonia appear comparable to the asexual zoosporangia in their generally subspherical shape and predominantly intercalary position. They are supplied individually with 1 (Fig. 7, J; Fig. 8, A, f, g; B, d; D; E; F; G; I; O, e) to 5 (Fig. 7, C, e-g) antheridia. An antheridium often arises from a hypha having no close mycelial connection with the oogonial filament; but more frequently it originates from the oogonial hypha in close proximity to the oogonium. When of remote origin the male organ may consist of a saccate cell, borne laterally on an axial hypha (Fig. 8, D, I), or of a crook-necked inflated cell, borne terminally on a branch of varying length (Fig. 7, C, e, f, g); in neither case, however, revealing such variety and distinctiveness as when it arises in proximate relationship to the oogonium. The simplest type of antheridium contributed by the oogonial filament consists merely of an outwardly unmodified portion of the filament adjacent to the female organ (Fig. 7, F, a; Fig. 8, G; J, a; K, a). Such an antheridium, sometimes only 10  $\mu$  (Fig. 7, F, a), at other times over 25  $\mu$  (Fig. 8, J, a) long, of necessity thrusts its fertilization tube directly through the septum delimiting the oogonium, so that the cross-wall together with the tube make up a funnel-like protrusion, which, later, may frequently be seen with narrowed open end touching the oospore. Similar fertilization takes place in instances where, on conversion into an antheridium, the portion of hypha concerned undergoes slight external modification by becoming perceptibly distended at the end immediately adjacent to the oogonium (Fig. 7, B, a; L, a; Fig. 8, O, d; P, b). Further modification in antheridial shape, through the production of a lateral outgrowth arising always from a position close to the oogonium, permits intrusion of the fertilization tube through the spherical wall of the female organ. Depending on the measure of modification the lateral outgrowth may be of small volume in comparison with the cylindrical portion (Fig. 8, C, d; J, b); or, again, in instances where the cylindrical component is reduced to a very short segment, it may provide the main bulk of the antheridium (Fig. 7, D, b; J; Fig. 8, B, e; N, a). The latter condition approaches that represented in the frequent instances where the outgrowth is cut off by a basal septum to form by itself a male organ approximately sessile on the oogonial hypha (Fig. 7, B, b; D, a; E, c, d; H, a, b; I, f, g, h; Fig. 8, A, d; F; H, a, b; N, b; P, a). Often, especially when the outgrowth attains a somewhat greater length, the septum is laid down at an appreciable distance from the parent filament, with the result that the delimited male organ is borne terminally on a stalk of variable length arising, of course, always from a position very close to the oogonium (Fig. 7, C, c, d; G, a, b; H, c; I, i; Fig. 8, A, f, g; B, h; E; K, b).

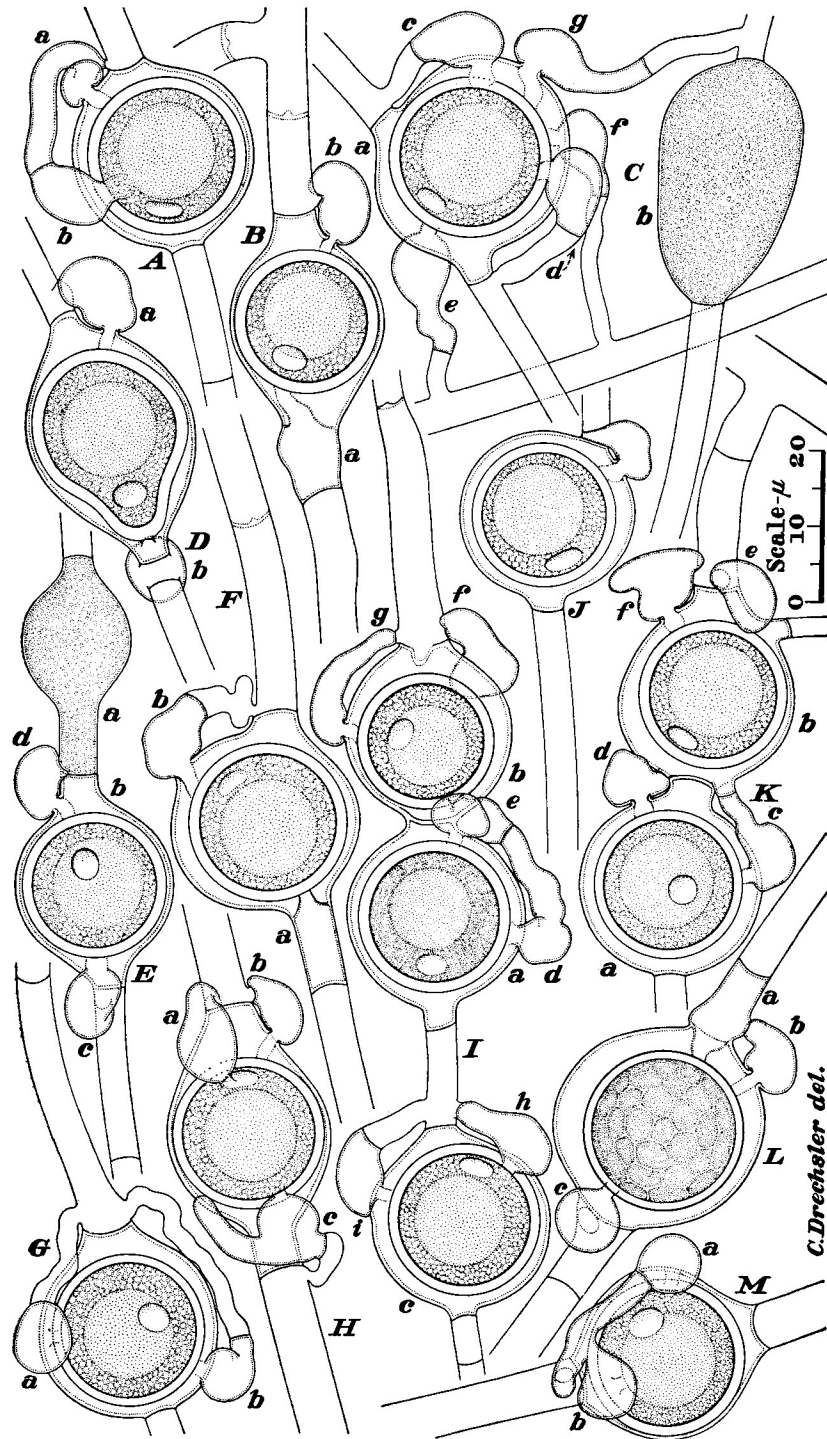


FIG. 7. Sexual reproductive apparatus of the type strain of *Pythium paroecandrum* isolated from field garlic, drawn with aid of a camera lucida;  $\times 1000$  throughout.

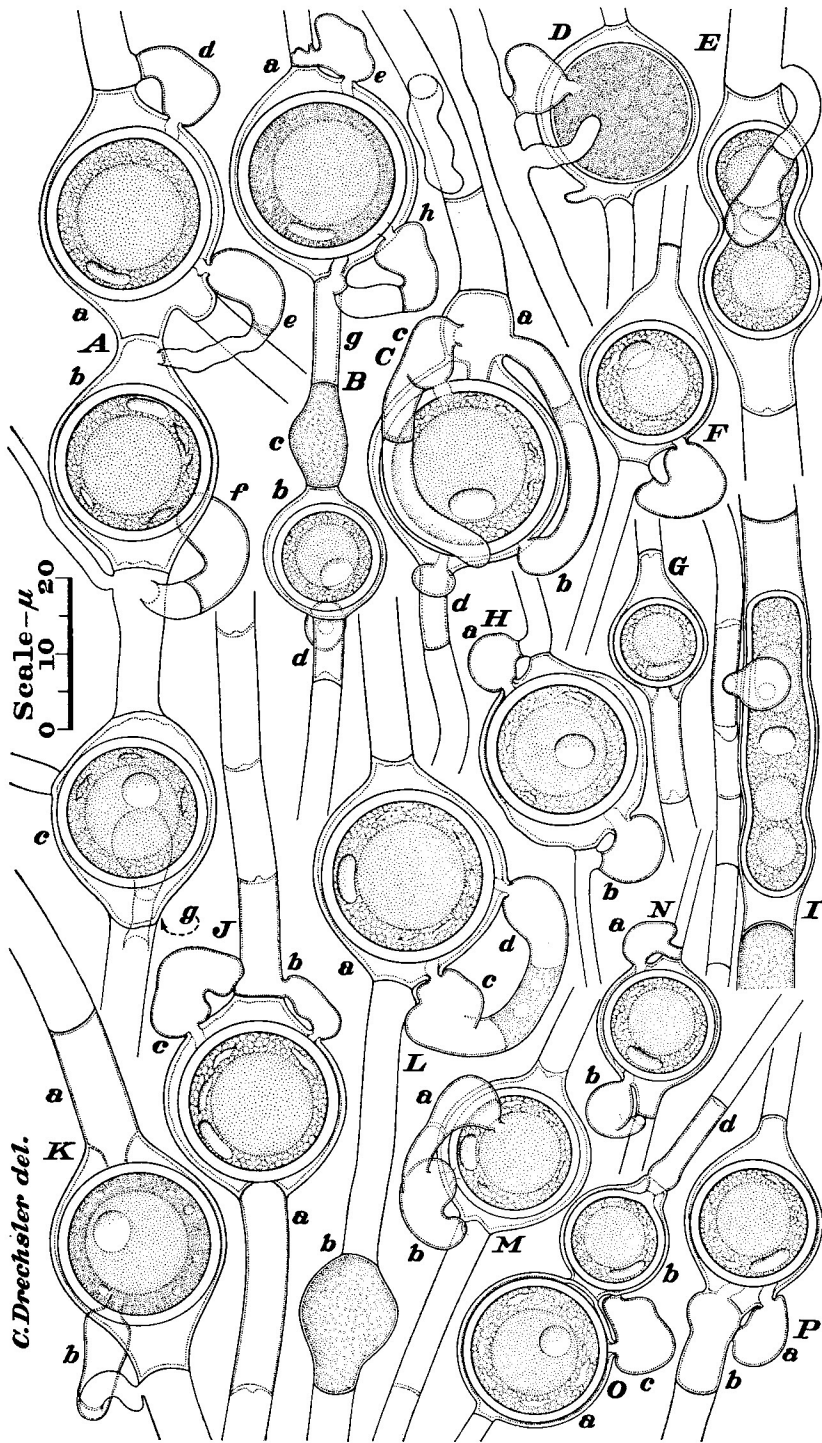


FIG. 8. Sexual reproductive apparatus of a strain of *Pythium paroecandrum* isolated from bloodroot, drawn with aid of a camera lucida;  $\times 1000$  throughout.

Antheridia thus differing considerably in manner of origin are found variously associated in many units of sexual apparatus. Not infrequently a lateral outgrowth, delimited at its base by a cross-wall and divided by a median septum, comes to constitute 2 antheridia arranged in series; the basal portion, sometimes with a pouch-like excrescence of its own, serving as a male organ no less than the distal portion (Fig. 7, A, a, b; I, d, e; M, a, b; Fig. 8, M, a, b). Likewise an antheridium composed wholly or in large part of a segment of hypha adjacent to the oogonium, may be contiguous with a sessile antheridium borne laterally on it (Fig. 7, L, a, b; Fig. 8, J, b, c; P, a, b), or may lack such contiguity only because the lateral antheridium is provided with a stalk (Fig. 8, B, g, h). Occasionally 2 fertilization tubes may be intruded into an oogonium from a more or less rangy antheridial system only rather dubiously divided internally by a protoplasmic plug (Fig. 8, C, a, b; L, c, d). Now and then, too, a sessile lateral antheridium may bear distally an empty sterile hyphal prolongation (Fig. 8, C, c).

After their fertilization the oogonia of *Pythium paroecandrum* show the internal changes familiar in many congeneric species. During the earlier stages in the development of the oospore its contents appear aggregated into somewhat irregular, sizeable lumps of nearly homogeneous consistency (Fig. 7, L; Fig. 8, D). Reserve material of completely homogeneous consistency soon begins to accumulate in a globule at the center of the massed lumps. As the reserve globule increases in size the protoplasmic lumps in the narrowing peripheral layer show indications of radial orientation (Fig. 7, I, a; Fig. 8, B, a; K) before becoming resolved into finer granules. At early maturity the oospore reveals unitary structure, its relatively large single reserve globule being surrounded by a rather narrow parietal granular layer in which is embedded a single refringent body, occasionally subspherical in shape, but more usually rather strongly flattened (Fig. 7, A; B; C, a; D; E, b; F; G; H; I, b, c; K, a, b; M; Fig. 8, A, a, b; B, b; C; F; G; H; J; L, a; M; N; O, a, b; P).

In cultures showing abundant sexual reproduction 2 oogonia may often be found immediately adjoining each other, their chambers separated only by a delimiting cross-wall (Fig. 7, I, a, b; K, a, b; Fig. 8, A, a, b; O, a, b). Frequently in instances of such contiguity one of the female organs is found supplied with antheridia (Fig. 7, I, d, e; K, c, d; Fig. 8, A, e; O, c) coming from its adjacent neighbor, which, therefore, on casual inspection presents the appearance of a bisexual structure. On more careful examination the presumption of bisexuality is not sustained. Evidently the antheridia of apparently anomalous origin arise, individually, like other male organs in the species, from an unmodified portion of hypha adjoining the oogonium they are destined to fertilize, and come to have their curious positional relationship only when the portion of hypha in question is utilized directly in the production of a second oogonium contiguous with the first. Should the portion of hypha be utilized instead in the development of a contiguous sporangium, any antheridium it may have put forth will seem to have arisen from the asexual reproductive body (Fig. 7, E, d).

In *Pythium paroecandrum*, as in many related fungi, oogonia and oospores departing markedly from a spherical shape are occasionally produced. Mycelium that has become largely exhausted in reproduction seems more inclined than young mycelium to afford development of malformed oogonia, wherein may be developed cylindrical oospores measuring perhaps 40  $\mu$  in length and 10  $\mu$  in width (Fig. 8, I), or, again, oospores of shapes suggestive of a dumb-bell (Fig. 8, E). Such oospores are often extensively adnate to the oogonial wall, and internally may reveal 2 (Fig. 8, E) or 3 (Fig. 8, I) reserve globules. The partly multiplicate internal structure represented here is manifestly referable to spatial exigencies, and is therefore not to be confused with the distinctive multiplicate structure characteristic of the oospores of *P. helicoides* Drechsl. and its allies.

Oogonia and oospores of such atypical form were excluded from consideration in the 200 measurements on which were based the statements given in the diagnosis relevant to the main dimensions of the fungus. Apart from this meager discrimination the measurements were made on units of sexual apparatus selected at random in maize-meal-agar cultures of the strain originating from field garlic,—each of the cultures used showing very abundant sexual reproduction with virtually no degeneration. The 200 mature oogonia gave values for diameter that when expressed to the nearest micron were distributed as follows: 11  $\mu$ , 1; 15  $\mu$ , 1; 16  $\mu$ , 1; 17  $\mu$ , 1; 18  $\mu$ , 3; 19  $\mu$ , 11; 20  $\mu$ , 33; 21  $\mu$ , 52; 22  $\mu$ , 51; 23  $\mu$ , 27; 24  $\mu$ , 15; 25  $\mu$ , 3; 27  $\mu$ , 1; and the 200 oospores of correct unitary internal structure contained within the oogonia gave values for diameter that when expressed to the nearest micron showed the following distribution: 10  $\mu$ , 1; 13  $\mu$ , 1; 14  $\mu$ , 1; 15  $\mu$ , 1; 16  $\mu$ , 8; 17  $\mu$ , 39; 18  $\mu$ , 71; 19  $\mu$ , 54; 20  $\mu$ , 18; 21  $\mu$ , 5; 22  $\mu$ , 1.

While in its asexual reproductive phase *Pythium paroecandrum* is closely similar to *P. debaryanum*, *P. irregulare* and *P. mammillatum*, it differs markedly from these species in its sexual phase; for, as has been noted, wherever its oogonia are fertilized by antheridia coming from the same filament, the antheridia in question are never borne, as usually in the 3 congeneric forms mentioned, on branches arising some distance from the female organ, but either consist in whole or in part of an adjacent portion of filament, or constitute the whole or a part of a process arising laterally from the axial filament in immediate proximity to the female organ. To be sure, the antithesis between *P. paroecandrum* and *P. debaryanum* with regard to origin of antheridia in monoecious sexual apparatus is not a complete one, as the latter species also reveals, though only rather sparingly, male organs arising in proximate relationship to the oogonia. The same partial antithesis has been set forth earlier (6, 11) in distinguishing *P. ultimum* from *P. debaryanum*. Certainly, in their antheridial relationships, *P. paroecandrum* and *P. ultimum* present a striking parallelism. However, the mature oospore of *P. paroecandrum* differs rather markedly from that of *P. ultimum* in the greater size, proportionally, of its central reserve globule, in the correspondingly lesser thickness of its parietal granular

layer, and in the frequently much flattened shape of its refringent body. Further, of course, *P. paroecandrum* like *P. debaryanum*, *P. irregulare* and *P. mammillatum*, is separated from *P. ultimum* by its ready production of zoospores.

Before *Pythium paroecandrum* was described as new its morphological features were considered in comparison more especially with the morphological details given by Butler (3) in the original account of his *P. rostratum*. Although the measurements for diameter of zoosporangium given by Butler somewhat exceed those of my fungus, the difference could not be regarded as sufficient for the separation of 2 species. Even less disparity was evident with respect to size of oogonium. If the antheridium of *P. rostratum*, described as being generally single, and as consisting often of a short hyphal segment adjacent to the oogonium, or of such a segment together with a short lateral process arising therefrom, fails to embody the whole range of variability revealed by antheridia in monoclinal sexual apparatus of my fungus, it yet could be recognized as unmistakably embodying an important part of that range. The chief difference impelling separation was much the same as that on which Butler based the separation of his species from *P. debaryanum*. For, in *P. paroecandrum*, as in *P. debaryanum*, and also as in *P. ultimum*, during a long period assimilated to *P. debaryanum*, the oospore is considerably smaller than the oogonium, and is, therefore, loosely contained within the oogonial chamber; whereas, in *P. rostratum*, the oospore completely or nearly completely fills the oogonium. The distinction appears all the more valid from the circumstance that Butler studied his fungus in water cultures, where oospores of many species of *Pythium* tend to become smaller in proportion to the oogonium than on firm agar substrata. According to Butler, moreover, the tube of discharge in the sporangium of *P. rostratum* is characteristically thickened about midway in its length. By way of contrast the evacuation tube produced by the sporangium of *P. paroecandrum* does not show consistently any distinctive localized modification; its tendency toward occasional crookedness and toward distal widening being shared by the homologous elements of many related species.

From de Bary's publications (1, 2) on his *Pythium proliferum* and *P. ferax*, it seems clear that these fungi produce antheridia in proximate relationship to the oogonium. However, the terminal proliferous sporangium, characteristic of *P. proliferum* and found presumptively also in *P. ferax*, represents a type of asexual reproductive body differing rather widely from the more commonplace, usually intercalary, nonproliferous sporangium of the present form.

In *Pythium pulchrum*, which, according to its original description by von Minden (18), also produces antheridia adjacent to oogonia, the oogonia and oospores have average diameters of 28  $\mu$  and 24  $\mu$  respectively, and are, therefore, considerably larger than the corresponding structures of *P. paroecandrum*. The clustered basipetal development of sporangia, figured

by von Minden, has never been observed in irrigated preparation of my fungus. Several cultures that I have isolated from aquatic material and that show satisfactory agreement with the description of *P. pulchrum*, are assuredly alien to *P. paroecandrum*.

Höhnk (14), in his original account of *Pythium epigynum*, dealt with a form wherein, again, mostly intercalary, subspherical zoosporangia comparable in size to the zoosporangia of *P. paroecandrum* are associated with oogonia that only slightly exceed those of my fungus in average diameter and that apparently are regularly fertilized by 1 or 2 antheridia consisting of somewhat swollen adjacent hyphal segments. From the discussion and illustrations given by him it is not apparent that antheridia individually consisting in whole or part of a lateral outgrowth arising in immediate proximity to the oogonium were ever observed, or that fertilization tubes ever entered the oogonial chamber except by penetration of the delimiting septum. The oospores of *P. epigynum*, judging from their range in measurements of diameter, 14 to 22  $\mu$ , mostly 18  $\mu$ , appear closely similar in size to those of *P. paroecandrum*, and, incidentally, would seem to have been more plausibly described in Höhnk's arresting phrase "oogone not filling" than in the deduction he drew from an overlapping of plotted curves representing values for diameters of oogonium and oospore. The rather meager dimensional overlap suggests not so much that some oospores fill the oogonia wherein they are produced, as that some of the larger oospores produced by the species, if they could be transferred, would fill some of the smaller oogonia produced by the species, and would, indeed, more than fill other still smaller oogonia produced by it.

Matthews (17), in illustrating *Pythium pulchrum*, gives figures among which some show much resemblance to Höhnk's figures of *P. epigynum* with regard to position and origin of antheridia. If the magnifications indicated for Matthews' figures are correct, the oogonia and oospores drawn by her would appear, besides, to have had dimensions approximately equal to the homologous dimensions of *P. epigynum*, despite an implication in her account that the drawings were prepared from a culture producing oogonia even somewhat larger than the oogonia of von Minden's fairly robust fungus. However, the catenate arrangement of zoosporangia set forth by Matthews received no mention in Höhnk's account. It is not apparent that either of the 2 fungi studied by these authors had a range of variations in antheridial relationship comparable with that revealed by *P. paroecandrum*.

In describing his *Pythium piperinum* as a new species causing root rot of pan, *Piper betle* L., and of pipri, *P. longum* L., Dastur (4) mentioned that its antheridia may consist of a branch from the oogonial hypha, or may develop directly from this hypha. All 3 of Dastur's drawings of sexual apparatus show plural antheridia in positions near an attachment of the oogonium to the supporting filament, suggesting that the male organs were derived from lateral outgrowths put forth by the filament close to the oogonium. In their measurements the oogonia and oospores of *P. piperinum*

appear only slightly smaller than those of *P. paroecandrum*. However, the zoospores of the Indian fungus, measuring only 3.4 to 5.1  $\mu$  would seem not only smaller than the swarm spores of *P. paroecandrum*, but smaller than any normal swarm spores I have seen produced by any species of *Pythium*.

## SUMMARY

Many zoosporangia of *Pythium dissotocum* consist of completely undifferentiated filaments, while others include a number of somewhat distended lateral branches. They yield enormous numbers of zoospores, which often are much given to iterant swarming. In the sexual apparatus of the species are revealed some antheridial relationships familiarly exemplified in *P. debaryanum* and *P. ultimum*.

*Pythium peritum* displays swollen elements more abundantly in its zoosporangia than *P. dissotocum*. Its oogonium is extensively and closely inwrapped by a branching antheridial filament, much like the oogonia of *P. scleroteichum* and various terrestrial species of *Aphanomyces*.

*Pythium paroecandrum* produces subspherical zoosporangia like those of *P. debaryanum*. As is implied in the specific epithet, a term compounded of 2 words meaning "neighbor" and "man" respectively, its antheridia often arise in close proximity to the oogonium. Thus, in arrangement of sexual apparatus, the species greatly resembles *P. ultimum*, although the oospore with its relatively large reserve globule has an internal organization more suggestive of *P. debaryanum*.

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