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SEVERAL SPECIES OF PYTHIUM PECULIAR IN THEIR SEXUAL DEVELOPMENT

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Of the 15 fungi which in 1930 I briefly described (17) as new species of *Pythium* 12 have since been set forth more fully through illustrations and supplementary discussion (20, 21, 22, 23, 25). Similar discussion, together with figures at magnifications (×500; ×1000) for the most part uniform with those of previous papers, is herein supplied for 2 of the remaining species, *P. oligandrum* and *P. salpingophorum*. As certain of the features characteristic of these forms can perhaps be better understood if opportunity is afforded for ready comparison with congeneric species, attention is devoted herein also to the morphology and development of *P. vexans* de Bary and of *P. undulatum* Petersen *sensu* Dissmann. Occasion is taken besides to amplify the earlier accounts of my *P. anandrum* and my *P. periplocum*, especially with respect to antagonistic relationships and oospore germination.

MORPHOLOGY AND DEVELOPMENT OF PYTHIUM OLIGANDRUM

Pythium oligandrum has been found in a wide variety of phanerogamic host plants over an extensive range of latitude in the eastern half of the United States. Its diagnosis was drawn from a culture derived from a diseased pea (*Pisum sativum* L.) root mainly because in my earlier experience I encountered the species in impressive quantity among numerous cultures isolated from underground parts of canning peas affected with root rot. Thus, it was recognized in more than 20 cultures prepared from separate individual plants collected in the course of a pea-root-rot survey made during the unusually cold wet spring of 1924 in Maryland, Delaware, and New Jersey (14). It was found present also in more than a dozen cultures isolated somewhat later in the same season from softened pea roots sent to me by workers in Pennsylvania, New York, and Connecticut; and subsequently was identified likewise in 4 cultures among a more numerous collection contributed by F. R. Jones as being representative of the fungi found in a pea-root-rot survey carried out that year in Wisconsin (30). That the species is not restricted to peas soon became evident from its frequent appearance among a large assortment of *Pythium* cultures obtained in 1924 from blackened rootlets of sweet-potato (*Ipomoea batatas* (L.) Lam.) slips taken by L. L. Harter (27) from large roots planted in hotbeds near Rosslyn, Va. Isolation of the fungus from several assortments of decaying bean (*Phaseolus vulgaris* L.) roots collected near Pompano, Fla., in March and April, 1926, gave testimony to its existence in the South. During the exceptionally wet period beginning in the middle of August, 1926, it was iso-

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lated repeatedly from tomato (*Lycopersicon esculentum* Mill.) roots collected near Rosslyn, Va., as well as from roots of the giant ragweed (*Ambrosia trifida* L.) and of the pale touch-me-not (*Impatiens pallida* Nutt.) gathered in Washington, D. C. It made its appearance with considerable frequency among the fungi found developing in maizemeal-agar plates planted with discolored sugar-beet (*Beta vulgaris* L.) roots collected in fields near East Lansing, Mich., and near Saginaw, Mich., late in June, 1927. In 1928 it was recognized in cultures isolated from discolored sweet-pea (*Lathyrus odoratus* L.) rootlets originating from Long Island, N. Y.; and also in a culture derived from a candytuft (*Iberis sp.*) root from Maine. Before its description in 1930 the fungus had been received from Florida a second time; the second accession coming in cultures isolated from diseased tomato seedlings. In 1939 its occurrence in another southern State was made evident through receipt of a culture which according to a letter from A. A. Dunlap had been isolated from a diseased wheat (*Triticum aestivum* L.) root gathered in the Panhandle region of Texas.

Very often, as has been set forth earlier (24), *Pythium oligandrum* is encountered in root rot and damping-off in association with congeneric species familiar as causal agents of these diseases; the frequency of such association and the behavior of the fungus in dual culture with congeneric forms giving reason to believe that the species occurs in diseased roots less as a primary parasite of the various host plants affected than as a secondary invader subsisting partly on mycelia of primary invaders and partly on host tissues freshly killed by these mycelia. Nevertheless the species now and then occurs under circumstances indicating that it may not be wholly lacking in pathogenicity to higher plants. Thus, a number of bean pods found affected with watery decay in a garden near Delaplane, Va., late in August, 1926, after 2 weeks of rainy weather, promptly yielded *P. oligandrum* unaccompanied by any other fungus likely to have caused the decay. Again, among 64 *Pythium* cultures derived from separate cucumber (*Cucumis sativus* L.) fruits found affected with watery rot in a wet field near Beltsville, Md., in August, 1938, one culture clearly belonged to *P. oligandrum;* 3 of the others being identified as *P. ultimum* Trow, and the remaining 60 as *P. Butleri* Subr. The discovery of *P. oligandrum,* unaccompanied by any other likely pathogenic organism, in a cucumber fruit was contrary to expectations as the fungus has never been isolated from watermelon (*Citrullus vulgaris* Schrad.) fruits affected with blossom-end rot, though watermelon fruits are spontaneously attacked in the field by the closely related *P. acanthicum* and *P. periplocum,* which usually fail to develop in green cucumbers when inoculated by incision. When the cucumber strain of *P. oligandrum* was inoculated by incision into watermelon fruits left attached on the vine, it caused a progressive brownish decay (Fig. 1, A, B) very similar to the natural blossom-end rot due to *P. acanthicum.* On inoculation into healthy green cucumbers it caused watery decay (Fig. 1, C) in most though not in all instances. Several other cultures of *P. oligandrum*

when inoculated into watermelons gave results (Fig. 1, D) very similar to those obtained with the cucumber strain. However, their inoculation into cucumbers brought about infection less frequently, and their rate of advance in the invasion of infected specimens was appreciably slower (Fig. 1, E). Failure of the species to attack watermelon fruits spontaneously in the field would seem attributable mainly to the slow germination of its oospores—a character plainly adverse to ready establishment of a foothold in the tissues of the flower scar (20, p. 383). The cucumber strain apparently has greater capacity for infecting cucumber fruits than most cultures referable to the species. Because of its relatively unambiguous pathogenicity this strain has been used in preparing some of the accompanying figures; the other illustrations of the species being made mainly from 2 strains isolated from separate specimens of diseased pea roots collected in June, 1924, near Hamburg, N. Y., and near Mount Morris, N. Y., respectively.

When a random assortment of separately isolated cultures belonging to *Pythium oligandrum* are grown under similar conditions side by side the differences that come to light with respect to such macroscopic features as rapidity of mycelial extension, luster, and cumulous variegation, are ordinarily more pronounced than the differences evident in a comparable assortment of cultures belonging to *P. acanthicum*. On maizemeal agar the submerged mycelium of *P. oligandrum*, unlike that of *P. acanthicum*, is from the start clearly visible to the naked eye. If the agar contains some finely divided maizemeal in suspension, an arachnoid aerial mycelium will usually develop during the first 2 days; and after 4 or 5 or 6 days the medium often turns yellowish and in places may take on a somewhat crustose appearance owing to the maturation of oospores in enormous numbers. Under microscopical examination the mycelium gives the general impression of being much more delicate than the mycelium, for example, of *P. ultimum* or of *P. debaryanum* Hesse. The greater delicateness here is attributable, as in *P. acanthicum* and *P. periplocum*, to extensive development of the finer ramifications, since the main axial filaments are not markedly narrower than in the coarse-looking damping-off species. The operation of the delicate branches in effecting parasitic attack on congeneric forms has been set forth earlier (24, 25).

Asexual reproduction may be conveniently brought about by cutting out sizable slabs from a thinly poured Lima-bean-agar plate culture permeated with young mycelium of the fungus, and transferring them to a shallow layer of sterile water in a sterilized Petri dish.[2] At temperatures between 15° and 18° C. the irrigated tracts of mycelium give rise here and there to subspherical enlargements filled with densely granular protoplasm. These enlargements occur in varied relationship to the parent hyphae. Several of them may sometimes be found clustered near the tip of a short branch

[2] In order that the water may spread out thinly and not collect in thick unmanageable pools, the floor of any Petri dish intended to be used for zoosporangial development or for oospore germination should be freed of all greasy film by scouring it thoroughly with an abrasive cleanser.

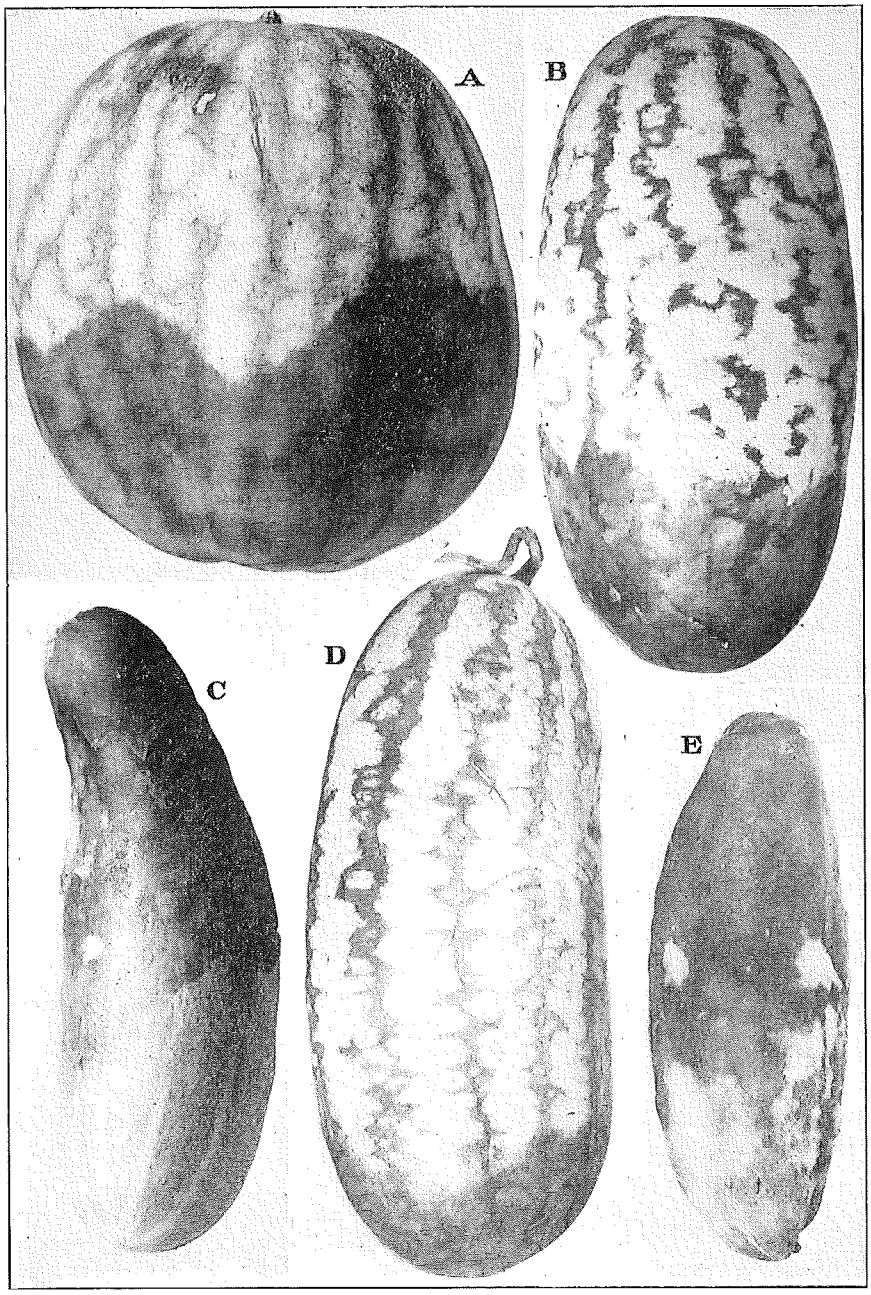

FIG. 1. Cucurbitaceous fruits attacked by *Pythium oligandrum*. A. Watermelon fruit 5 days after inoculation with the culture isolated from a Beltsville (Md.) cucumber; $\times \frac{3}{7}$. B. Watermelon fruit 9 days after inoculation with Beltsville cucumber strain; $\times \frac{2}{7}$. C. Cucumber fruit 5 days after inoculation with Beltsville cucumber strain; $\times \frac{1}{2}$. D. Watermelon fruit 9 days after inoculation with Hamburg (N. Y.) pea-root-rot strain; $\times \frac{3}{10}$. E. Cucumber fruit 9 days after inoculation with Hamburg pea-root-rot strain; $\times \frac{2}{5}$.

(Fig. 2, A), or again, may be compounded in a linear series which when delimited by cross-walls appears as a lobulate sporangial unit (Fig. 2, B). Often a sporangium may consist of a single globose enlargement together with an adjacent portion of hypha (Fig. 2, C, D), or of a conidium-like subspherical enlargement alone (Fig. 2, E, a), or of 2 or more globose enlargements together with connecting and adjoining hyphal parts (Fig. 2, E, b). Soon after it has been delimited the sporangial unit (Fig. 2, F) puts forth an evacuation tube (Fig. 2, F, t) which on attaining definitive length yields at the tip to permit migration of the protoplasmic contents into a terminal vesicle (Fig. 2, G) that often is only faintly discernible. When its migration is completed, the mass of granular material undergoes transformation into laterally biciliate zoospores after the manner usual in the genus *Pythium:* the motile spores (Fig. 2, H) being fashioned in the course of 15 to 25 minutes, and then escaping on disintegration of the vesicular film.

If left undisturbed the moderately thick wall of the evacuated sporangium (Fig. 2, G–W) retains its shape for some time, as does also the membrane of the evacuation tube (Fig. 2, G–W: t). The general make-up of the empty sporangial unit offers parallelism especially with *Pythium acanthicum.* In instances where the sporangium consists of a single globose part together with a relatively short cylindrical portion of the parent hypha, the evacuation tube more often arises from the cylindrical part (Fig. 2, F, G, I, J, L) than from the subspherical component (Fig. 2, K, M). Similar preference is evident likewise where the swollen component is bilobed (Fig. 2, H, P), or where the hyphal part is of considerable volume (Fig. 2, N), or where 2 globose parts are included (Fig. 2, Q–T: a, b). Occasionally 4 (Fig. 2, U, a–d; V, a–d) or 5 (Fig. 2, W, a–e) subspherical enlargements are found united into sporangial units somewhat more complex than can ordinarily be found in *P. acanthicum;* the evacuation tubes of such voluminous units arising sometimes from an expanded part (Fig. 2, U, t) and sometimes from a hyphal component (Fig. 2, V–W: t). While the tubes put forth by small sporangia (Fig. 2, L, t; M, t) may measure only about 15 μ in length, those extended from the more massive sporangia may attain lengths of 200 or 225 μ (Fig. 2, P, t; T, t). The empty tubes as a rule terminate abruptly with little apical modification: they rarely widen out markedly near the tip, are usually not lipped at the mouth, and apparently never are reflexed.

Sexual reproduction starts earlier and proceeds more rapidly in *Pythium oligandrum* than in most congeneric forms. In maizemeal-agar plate cultures kept at temperatures between 25° and 30° C. oogonia often begin developing abundantly within 30 hours after inoculation. They arise as globose hyphal enlargements in subterminal (Fig. 3, A) or intercalary positions (Fig. 3, B–D). Their growth is often directed toward one side with the result that their attachment may become more or less lateral (Fig. 3, E–I). During the later stages of expansion the individual oogonium puts forth protuberances from all portions of its surface. These protuberances

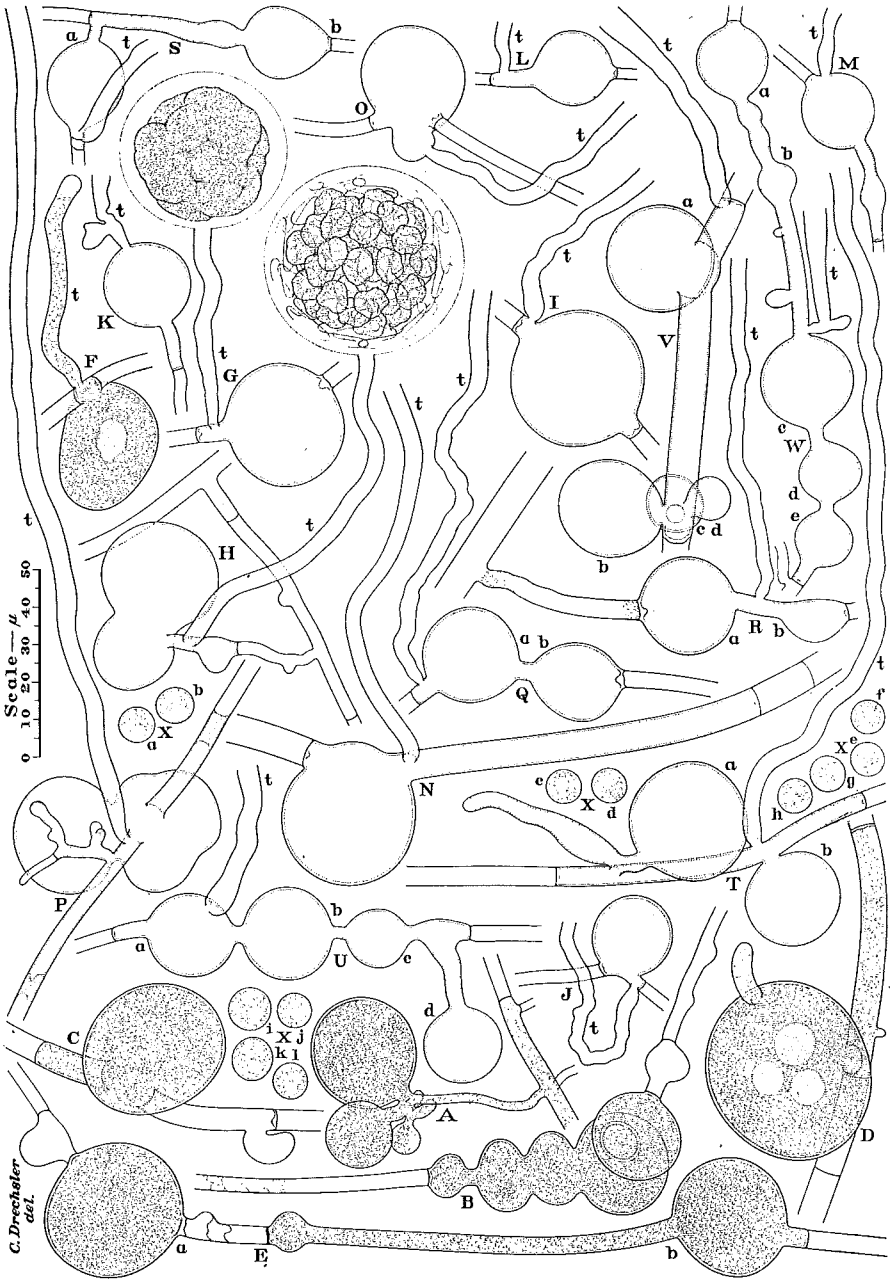

FIG. 2. Asexual reproduction in *Pythium oligandrum;* drawn to a uniform magnification with the aid of a camera lucida, from irrigated Lima-bean-agar preparations; all parts drawn from the Hamburg culture except K, L, M, S, and W, which were drawn from the Eden (N. Y.) pea-root-rot strain; ×500 throughout. A. Globose enlargement developing terminally on a hyphal branch. B–D; E, a, b. Sporangial units delimited by septa. F–H. Sporangia showing successive steps in zoospore production. I–P. Empty sporangia, each with a single expanded component. Q–T. Empty sporangia, each with 2 subspherical components, a and b. U, V. Empty sporangia, each with 4 globose components, a–d. W. Empty sporangium with 5 globose components, a–e. X. Encysted zoospores, a–l, showing variations in size and shape. (t, evacuation tube.)

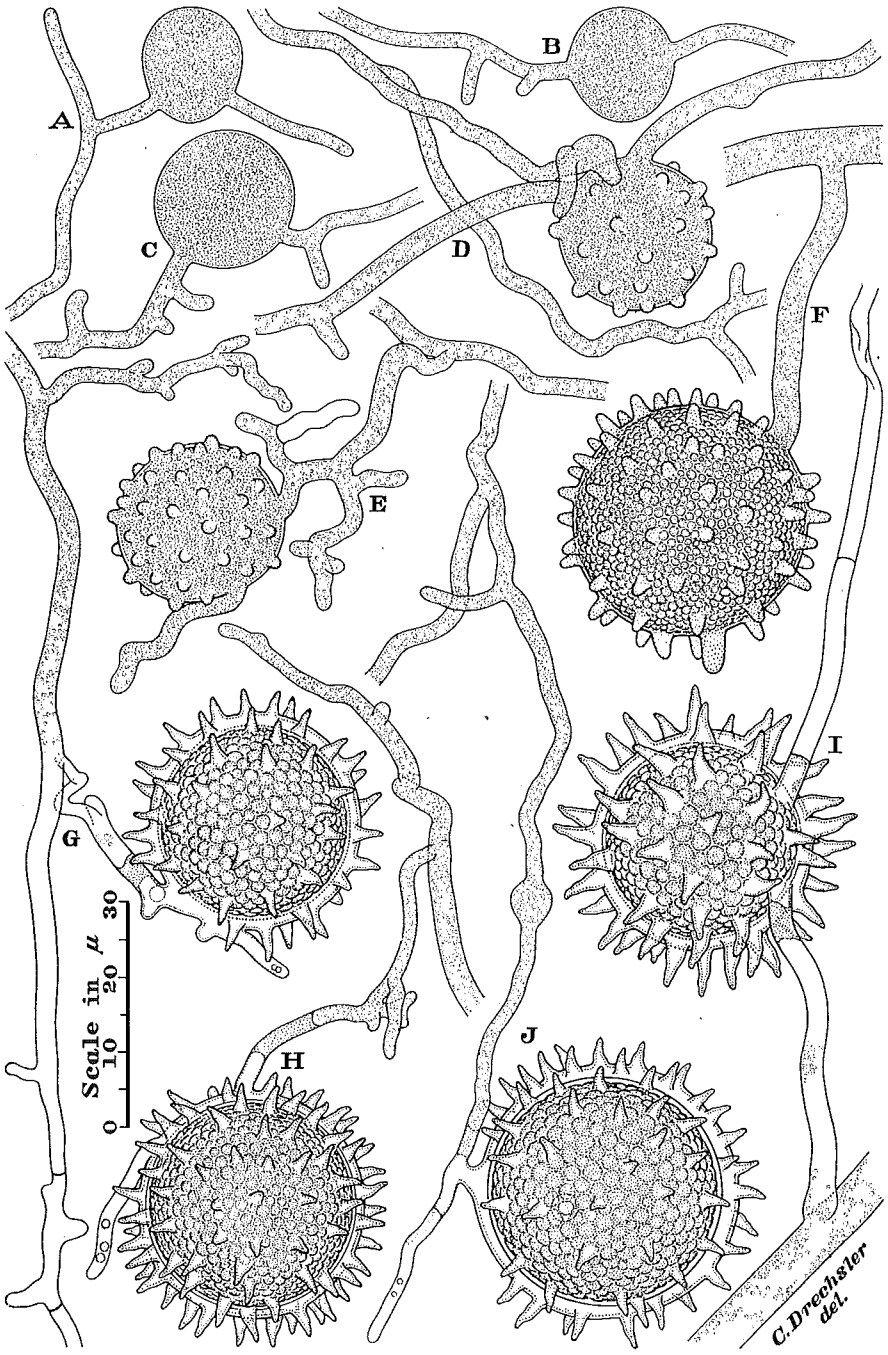

FIG. 3. Sexual reproductive apparatus of *Pythium oligandrum* (Hamburg strain); drawn with the aid of a camera lucida from maizemeal-agar cultures; ×1000 throughout. A–C. Young growing oogonia. D. Nearly full-grown oogonium supplied with a young antheridium. E. Nearly full-grown oogonium. F. Full-grown oogonium shortly before deposition of basal septum; its contents consisting of small lumps. G–J. Parthenogenetic units in stage shortly before thickening of oospore wall.

are at first of a rounded, wartlike conformation (Fig. 3, D, E), but through apical elongation they soon acquire an irregularly conical, tapering shape. About at the time the spines are attaining their definitive length, the protoplasmic contents of the oogonium change from a densely granular to a coarse lumpy texture (Fig. 3, F). Soon afterwards the oogonium becomes delimited by deposition of 1 (Fig. 3, G, H) or 2 (Fig. 3, I, J) partitions, and its contents shrink away from its spiny envelope as the protoplasmic lumps composing them increase rather markedly in size. At this stage, wherever an antheridium (Fig. 4, A) or possibly 2 antheridia (Fig. 4, B) are present—application and development of a male complement takes place usually while the oogonial spines are being formed—fertilization is accomplished much as in the generality of related species. In any case, whether an antheridium is present (Fig. 4, C) or not (Fig. 4, E), the spherical protoplast soon secretes a thick wall, a homogeneous reserve globule gradually collects in the center, and the large protoplasmic lumps disintegrate to furnish material for the finely granular parietal layer through which plural refringent bodies of comparatively small size become distributed. Manifestly the oosphere undergoes conversion into an oospore of distinctive internal structure equally well by parthenogenesis (Fig. 4, F, G; Fig. 5, A, a, b; B–I) as after fertilization (Fig. 4, D).

The prevalence of parthenogenesis in *Pythium oligandrum* varies considerably among different strains of the species, and is, besides, subject to great variation from environmental causes. In the original diagnosis it was indicated that approximately 4 out of 5 oogonia developed parthenogenetically—this being the proportion most usually found when the strain used as type (one isolated from a discolored pea rootlet gathered near Eden, N. Y., in June, 1924) was grown on maizemeal agar at 24° C. Under like conditions the generally very similar Hamburg pea-root-rot strain (Fig. 3, 4, 5) shows virtually the same proportion of parthenogenetic reproductive apparatus. In the Beltsville cucumber-rot strain antheridia are commonly produced 2 or 3 times more abundantly (Fig. 6, A, B, C; Fig. 7, A–D), and as a result parthenogenesis occurs there in correspondingly lesser measure (Fig. 6, D–K). On the other hand, in the Mount Morris pea-root-rot strain 15 or 20 oogonia may often be found developing parthenogenetically (Fig. 8, A, B, D–Q) to every oogonium supplied with an antheridium (Fig. 7, E; Fig. 8, C). A striking illustration of variability from environmental causes was once provided by the Mount Morris strain when occasion was taken to compare a maizemeal-agar plate culture grown in the laboratory at about 27° C., with an irrigated Lima-bean-agar preparation stored in a refrigerator at 18° C. In the former scarcely one oogonium in a hundred was found supplied with an antheridium; whereas in the latter every oogonium was supplied with at least one antheridium, and many were supplied with two.

Whether formed in some abundance (Fig. 4, A–D; Fig. 6, A–C) or in relatively meager number (Fig. 8, C) the antheridia are mostly borne terminally on branches arising from a filament having no close mycelial

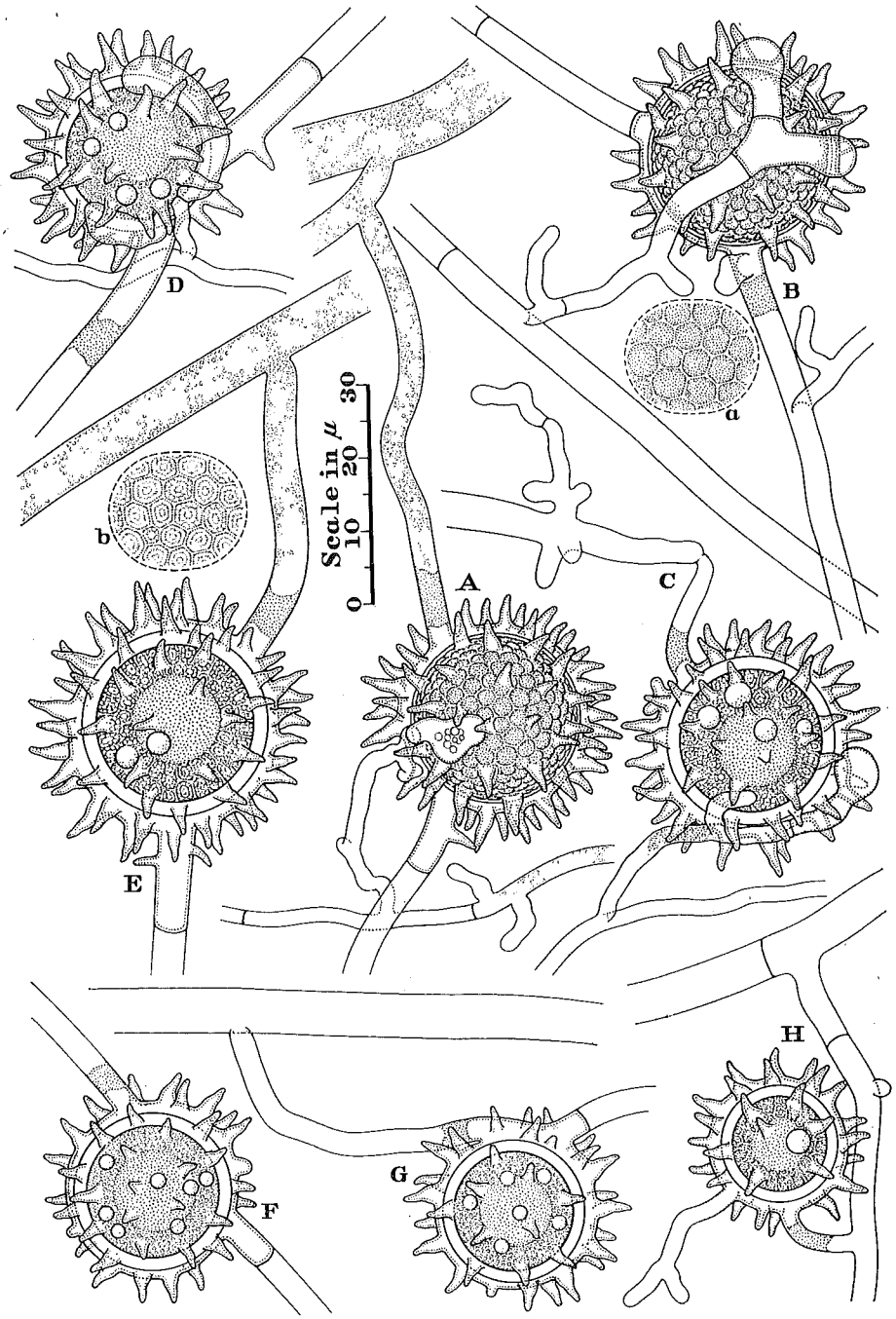

FIG. 4. Sexual reproductive apparatus of *Pythium oligandrum* (Hamburg strain); drawn with the aid of a camera lucida from maizemeal-agar plate cultures; ×1000 throughout except in B, a, and E, b, where magnification is ×2000. A–H. Six intercalary (A, B, D, E, F, G) and 2 terminal (C, H) oogonia, 4 among them (E–H) parthenogenetic, the others fertilized by 1 (A, C) or 2 (B, D) antheridia borne on a single branch arising from a neighboring hypha; the oospores in A and B in early lumpy stage, with lumps appearing in surface view as shown in B, a; oospores in C, E, H thick-walled, with fissured lumps appearing in surface aspect as shown in E, b; oospores D, F, G, fully mature.

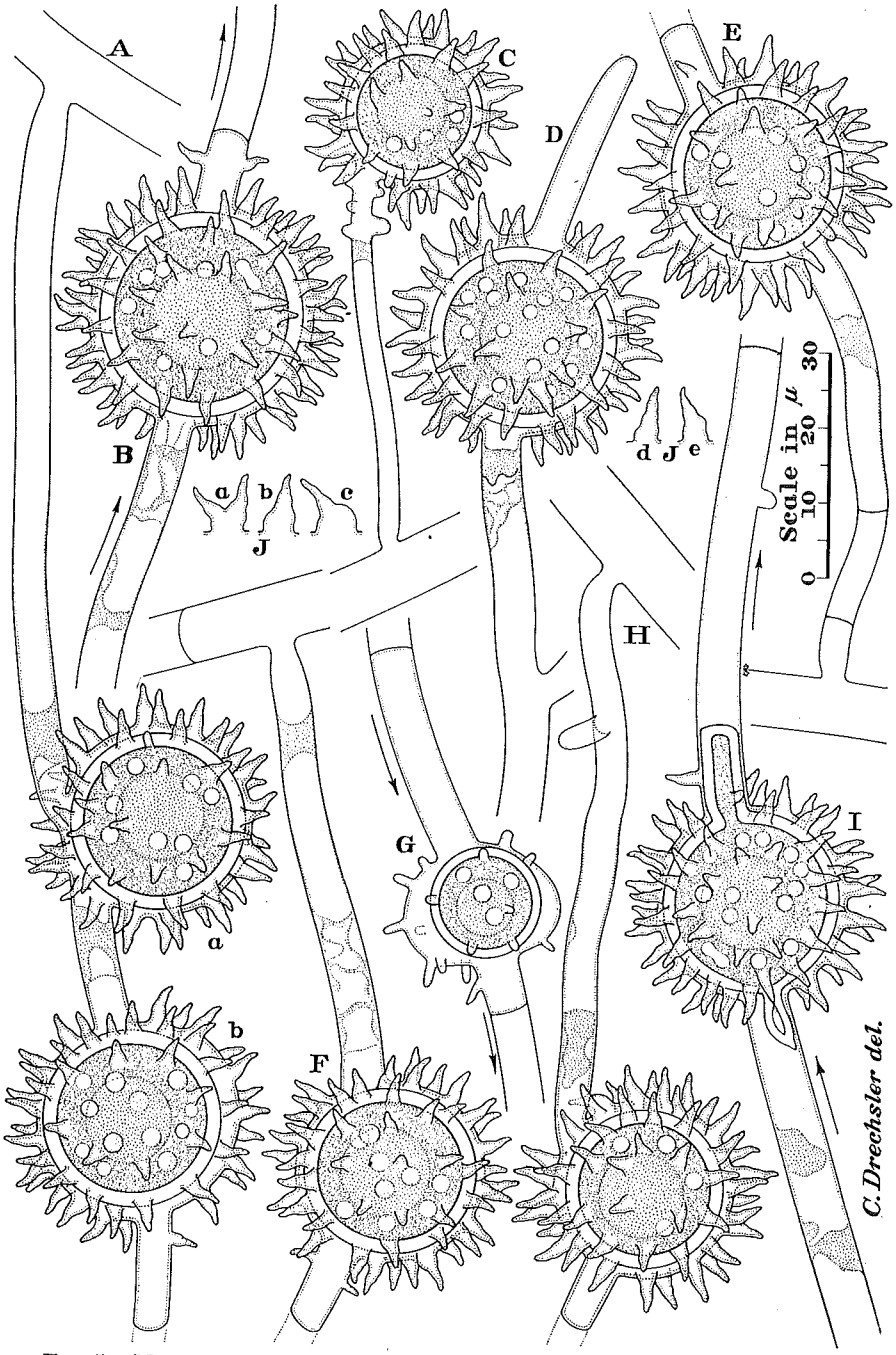

FIG. 5. Mature sexual reproductive apparatus of *Pythium oligandrum* (Hamburg strain); drawn with the aid of a camera lucida from maizemeal-agar plate cultures; ×1000 throughout. A. Two adjacent intercalary oogonia, a and b, the proximal one delimited by a plug on both sides. B–I. Eight oogonia varying in size and shape, 6 among them (B, E–I) intercalary and 2 terminal (C, D) in position; G being an undersized specimen beset with poorly developed protuberances and delimited by crosswalls both proximally and distally; the others (B–F, H, I) being delimited proximally by a plug which in some instances (B, D, F, H, I) is shrinking or disintegrating. J. Well-developed oogonial protuberances, a–e, showing irregularly tapering shape and somewhat blunt tip.

connection with the hyphal element bearing the oogonium. However, with some search in agar cultures rather soon after sexual reproduction has begun, and before too many filaments and branches have faded from view, instances can usually be found where the mycelial connection between antheridium and oogonium can be traced with certainty. The unit of sexual apparatus shown in figure 7, A, where the combined length of oogonial branch (Fig. 7, A, a), antheridial branch (Fig. 7, A, b), and intervening hyphal elements is approximately 225 μ, may be taken as illustrating an unusually close mycelial connection; though an even closer connection, where the total length of the communicating elements is only about 125 μ, is shown in figure 7, B. In most instances where a mycelial connection between the conjugating organs can be followed successfully amid the confusion of hyphae, the aggregate length of the communicating parts varies from 250 to 600 μ (Fig. 7, C–E).

As might be expected in view of their usually somewhat extensive application to oogonia rather closely beset with spines, the antheridia of *Pythium oligandrum* vary considerably in size and shape. Some of the smaller specimens, consisting merely of a slightly crook-necked inflated terminal segment (Fig. 4, A), are not greatly different from the sessile monoclinous antheridia familiar in *P. ultimum*. More often, however, the delimiting septum is laid down some distance backward from the expanded tip, so that the male cell comes to include a tubular portion tapering gradually toward the base (Fig. 4, C, D; Fig. 6, A, B). Frequently, owing to terminal branching or to local constriction, an antheridium may be distinctly lobate (Fig. 7, A, D). Where ramification of the antheridial hypha has led to the formation of 2 fairly massive branches, cross-walls may be laid down to delimit each as a separate antheridium (Fig. 4, B, D; Fig. 6, C).

The production of terminal branch antheridia by *Pythium oligandrum* might perhaps be held to distinguish this species adequately from *P. artotrogus,* in which according to de Bary's (3, 4) original descriptive statements fertilization was seen to be accomplished by an antheridium consisting always of a hyphal segment adjacent to the oogonium. However, since in many cultures of my fungus branch antheridia are often only sparingly produced, and indeed are sometimes almost wholly lacking, the absence of such easily recognized male cells in de Bary's material could hardly be considered a fully decisive distinguishing feature should *P. oligandrum* be found to produce adjacent cylindrical antheridia as well as branch antheridia. Much material was examined, therefore, to determine whether in the species oogonia not supplied with branch antheridia develop parthenogenetically or at times, if not always, are fertilized by an adjacent male segment. Fertilization of the oogonium by passage of protoplasmic materials through the partition delimiting it at the proximal end should be especially subject to observation, for as a rule this partition is not an ordinary membranous cross-wall but a massive plug, usually 3 to 10 μ long, composed of a gelatinous substance (Fig. 3, G–I; Fig. 4, A–H; Fig. 5, A, C, E; Fig. 6, A–G, I;

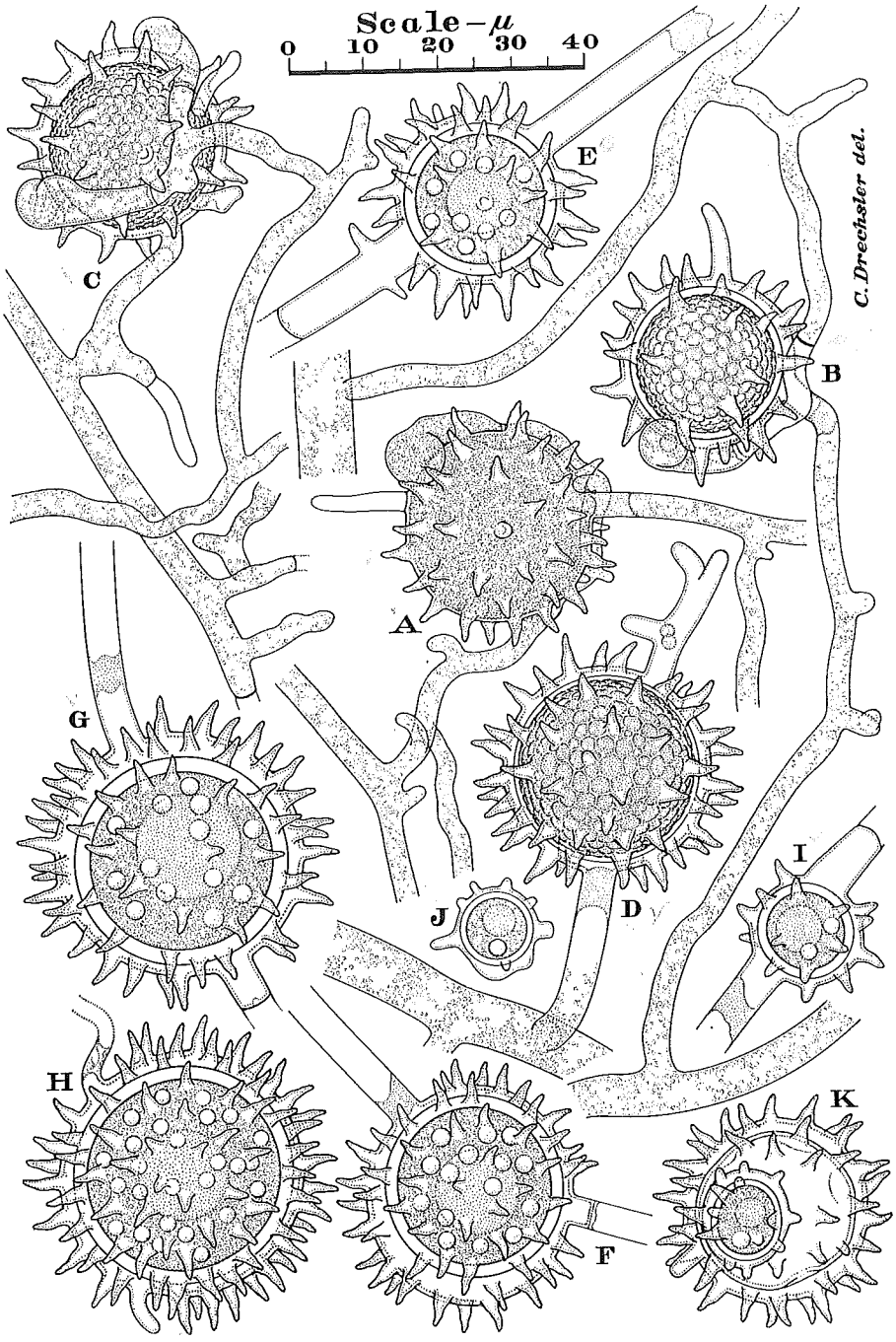

FIG. 6. Sexual reproductive apparatus of *Pythium oligandrum* (Beltsville cucumber strain); drawn from maizemeal-agar plate cultures with the aid of a camera lucida; ×1000 throughout. A–H. Eight well-developed oogonia, each delimited proximally by a plug; two (A, B) being supplied with 1 antheridium, one (C) with 2 antheridia, the others (D–H) lacking antheridia; in A the contents are still granular, in C the lumpy oosphere has contracted, in B and D the oospore wall is being deposited, in E–H the parthenospore is fully mature. I. Small intercalary oogonium with mature parthenospore showing only 2 refringent bodies. J. Very small terminal oogonium with a small parthenospore of unitary internal make-up. K. Oogonium whose envelope surrounds an oospore wall that encloses a very small secondary oogonium wherein is contained a mature parthenospore of unitary organization.

Fig. 7, A–D; Fig. 8, A, C, E) apparently similar to the substance secreted where hyphae are cut or wounded. This plug remains intact not only during the period in which fertilization might take place, but for many days after the oospore is fully mature, until eventually it undergoes gradual shrinkage and alveolar disintegration (Fig. 5, B, F, H, I; Fig. 6, H; Fig. 8, D). When 2 oogonia are formed adjacent to each other (Fig. 5, A, a, b) the proximal one (Fig. 5, A, a) is commonly bounded by a plug on each side; and occasionally a solitary oogonium (Fig. 8, A, F) is likewise delimited by plugs both proximally and distally. As passage of protoplasmic material through a plug never has come under observation, it can hardly be doubted that development of intercalary oogonia bounded by plugs at both ends and of terminal oogonia bounded proximally by a plug (Fig. 3, G, H; Fig. 4, C, H; Fig. 5, C, D; Fig. 6, D) must be parthenogenetic wherever a branch antheridium is lacking.

The commonplace membranous cross-walls usually delimiting intercalary oogonia at the distal end—such cross-walls are found bounding at both ends some intercalary oogonia (Fig. 5, G; Fig. 8, G) presumably formed after the mycelium had become too largely exhausted for the isolated living remnants to retain much polarity—offer greater difficulty in determining presence or absence of fertilization like that ascribed to *Pythium artotrogus.* Since in that species de Bary could see the empty fertilization tube only in favorable instances, it may be inferred that in most units of mature sexual apparatus the antheridial character of an adjacent hyphal segment was evidenced only by an aperture in the delimiting septum. Such an aperture, if present in *P. oligandrum,* would almost certainly be obscured beyond all recognition in the many instances where several oogonial spines are found projecting out in positions directly above and below the septum. Fortunately, in my fungus the distal delimiting wall is frequently placed well beyond the tips of all distally projecting spines, and thus can often be scrutinized to good effect. Yet in no instance has any likely aperture been discovered, nor has any tubular or funnel-shaped modification been seen that could be held to have derived from antheridial activity. Furthermore, oogonia with filamentous hyphal prolongations, often 25 to 50 μ long (Fig. 5, D, G, I; Fig. 8, D), were often taken under observation, especially during the contraction of the protoplast, to determine whether an antheridium might then be present, contributing its contents after complete dissolution of a temporary partition. So far no good evidence of such broad conjugation has been uncovered. Consequently the view that adjacent antheridia are wholly absent in *P. oligandrum,* and that wherever an oogonium is not supplied with a branch antheridium its development takes place parthenogenetically—a view incorporated into the diagnosis of the species—still seems well justified.

However the portion of the diagnosis setting forth the refringent body in the oospore as "often not clearly in evidence, when visible often subspherical 3 to 4.5 μ in diameter" has required emendation (26). The un-

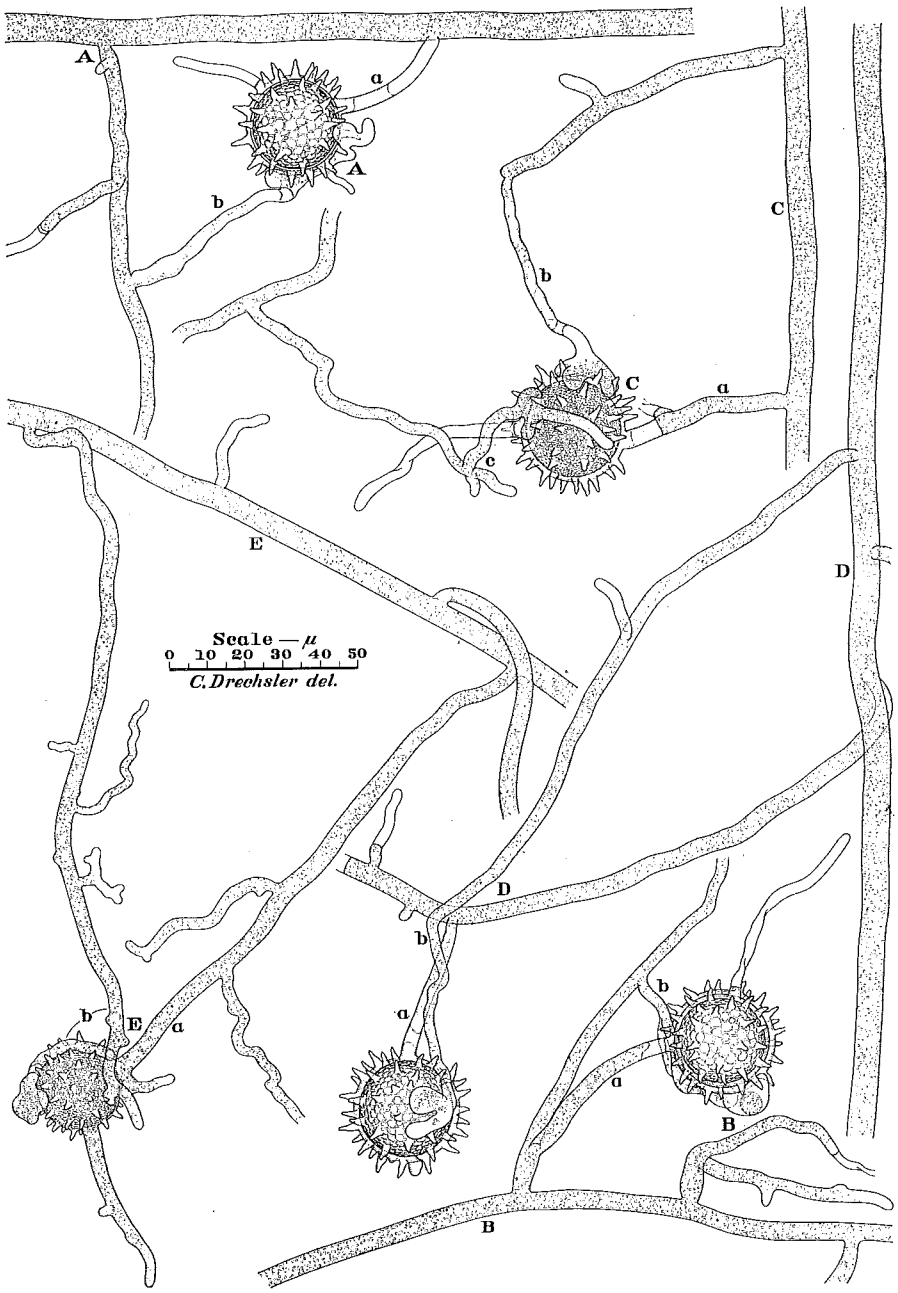

FIG. 7. Sexual reproductive apparatus of *Pythium oligandrum* showing mycelial connection between oogonium and antheridium; drawn from maizemeal-agar plate cultures at a uniform magnification with the aid of a camera lucida; ×500 throughout. A, B. Units of sexual apparatus of the Beltsville cucumber strain, showing unusually close mycelial connection of the paired organs. C, D. Units of sexual apparatus of the Beltsville cucumber strain, showing the rather remote mycelial connection more usually found; in C an additional antheridium is evidently being supplied by a branch, c, from a neighboring hypha. E. Unit of sexual apparatus from Mount Morris (N. Y.) pea-root-rot strain; oogonium full grown but not yet delimited from supporting branch; showing the rather remote mycelial connection usually found. (a, oogonial branch; b, antheridial branch.)

happy phrasing of the passage cited came from perplexity as to what constituted correct internal organization in oospores of the species; the refringent bodies here being not especially hard to see, but assuredly difficult to recognize in their true character if the observer is strongly expectant of the unitary organization common to most pythiaceous fungi, including the very intimately related *Pythium acanthicum* and *P. periplocum*. At the early stage in development of the oospore, when in most congeneric forms the single refringent body first becomes visible among the protoplasmic lumps surrounding it, *P. oligandrum* likewise often shows only one such body. Indeed, sometimes in rather small oospores (Fig. 4, H) only a single refringent body may be present in the somewhat later stage when the protoplasmic lumps have begun to show the minute concentric and radial fissuring whereby they become resolved into minute granules (Fig. 4, E, b); though in most cases 2 to 4 of these cellular components are then revealed (Fig. 4, E, C). As maturation proceeds the refringent bodies increase to their definitive number, which for oospores of usual dimensions, formed at temperatures between 25° and 30° C., varies commonly from 4 to 15 (Fig. 4, D, F, G; Fig. 5, A–I; Fig. 6, E–G; Fig. 8, D–F). In some exceptionally large oospores (Fig. 6, H) as many as 25, 26, or 27 refringent bodies have been counted, while some decidedly small specimens (Fig. 6, I; Fig. 8, G, H) have shown only two. After diligent search very small oospores have even been found that at maturity contained only a single refringent body and thus displayed the unitary organization familiar in related fungi. These minute spores, only about 10 μ in diameter, were apparently formed late from small isolated remnants of living mycelium (Fig. 6, J), or occasionally were produced in a secondary sporangium within primary reproductive apparatus whose contents for the most part had suffered degeneration (Fig. 6, K).

Although the multiplication of the refringent bodies entails reduction of their diameter to about 2 or 2.5 μ, they remain rather conspicuous in the finely granular parietal layer of the mature spore. Once the unusual association of plural refringent bodies with a single reserve globule has become familiar as a distinctive feature of the species, *Pythium oligandrum* can be recognized solely by its resting oospores; its identification then being possible even in old isolation cultures, heavily contaminated with bacteria, where all membranous vestiges of hyphae, antheridia, and spiny oogonia have long disappeared from view. Although old oospores of congeneric forms often show multiple spherical vacuoles in the parietal layer, these vacuoles can usually be distinguished from the plural refringent bodies of *P. oligandrum* by their larger and frequently more variable size. Larger size likewise pertains to the 2, 3, or 4 refringent bodies found in oospores of some congeneric species after their unitary structure has undergone modification through prolonged aging. The multiplication of refringent bodies in *P. oligandrum* cannot similarly be ascribed to after-ripening, as it is usually accomplished in maizemeal-agar throughout the expanse of a Petri-plate culture 100 mm. in diameter within 5 or 6 days after inoculation—at a time

when in parallel cultures *P. ultimum* and *P. debaryanum* have often not yet begun to produce sexual apparatus.

Among the oogonia that in addition to a subspherical spiny part include a rather extensive filamentous prolongation at one end or at both ends, some fail to collect their contents wholly within the subspherical part; so that the oospores will bear a projection, often more or less cylindrical, at one or at both poles (Fig. 5, I). Under certain conditions of development many oogonia may be formed consisting of a relatively small globose part together with much more voluminous prolongations, which sometimes are smooth (Fig. 8, I, J) and sometimes are elaborately beset with spiny protuberances (Fig. 8, K). Such rangy oogonia commonly produce an elongated oospore whose irregularly cylindrical shape is modified by a bulbous enlargement (Fig. 8, I; J, a; K, a), and frequently, in addition, give rise to a second cylindrical oospore having no bulbous modification (Fig. 8, J, b; K, b). Occasionally hyphal segments, wholly devoid of outward differentiation, function as oogonia in giving rise endogenously to elongated cylindrical oospores (Fig. 8, L–Q), or in extreme instances to filamentous oospores 100 to 300 μ long and 3 to 4 μ wide. The reserve material in cylindrical and filamentous oospores is divided among a variable number of globules which like the plural refringent bodies are arranged longitudinally at moderate intervals (Fig. 8, I–P). Obviously the organization here imposed by spatial necessities is not equal in descriptive merit to the multiplicate organization characteristic of the ordinary subspherical oospores of my *Pythium helicoides* and its close allies. Some cylindrical oospores reveal plural reserve globules and a single refringent body (Fig. 8, K, b; Q), thus reversing the normal numerical relationship of these cellular components.

The metrical data given in the original diagnosis relative to size of oogonium and oospore were based on 200 measurements of the Eden pea-root-rot strain grown in maizemeal-agar plate cultures under the same conditions as the cultures of *Pythium acanthicum* and *P. periplocum* utilized for measurements previously reported (20, p. 402, 406). The 200 oogonia chosen at random gave values for diameter, expressed in the nearest integral number of microns, distributable as follows: 17 μ, 2; 19 μ, 2; 20 μ, 1; 21 μ, 1; 22 μ, 3; 23 μ, 8; 24 μ, 23; 25 μ, 27; 26 μ, 31; 27 μ, 36; 28 μ, 31; 29 μ, 14; 30 μ, 11; 31 μ, 5; 32 μ, 2; 33 μ, 2; 35 μ, 1. And the oospores, all of correct internal organization, that were contained in these oogonia, gave values for diameter distributed as follows: 15 μ, 2; 17 μ, 2; 18 μ, 1; 19 μ, 2; 20 μ, 8; 21 μ, 24; 22 μ, 33; 23 μ, 44; 24 μ, 36; 25 μ, 22; 26 μ, 15; 27 μ, 6; 28 μ, 3; 29 μ, 1; 30 μ, 1. While the 3 closely related echinulate species are thus rather similar in their main dimensions, the generally smaller size of *P. acanthicum* is nearly always directly recognizable when microscopical comparison is made between representative cultures grown under similar conditions or between assortments of such cultures. In such comparison *P. periplocum* is not found consistently smaller than *P. oligandrum*, since its oogonia and oospores are virtually of the same size as those of numerous strains of the latter species,

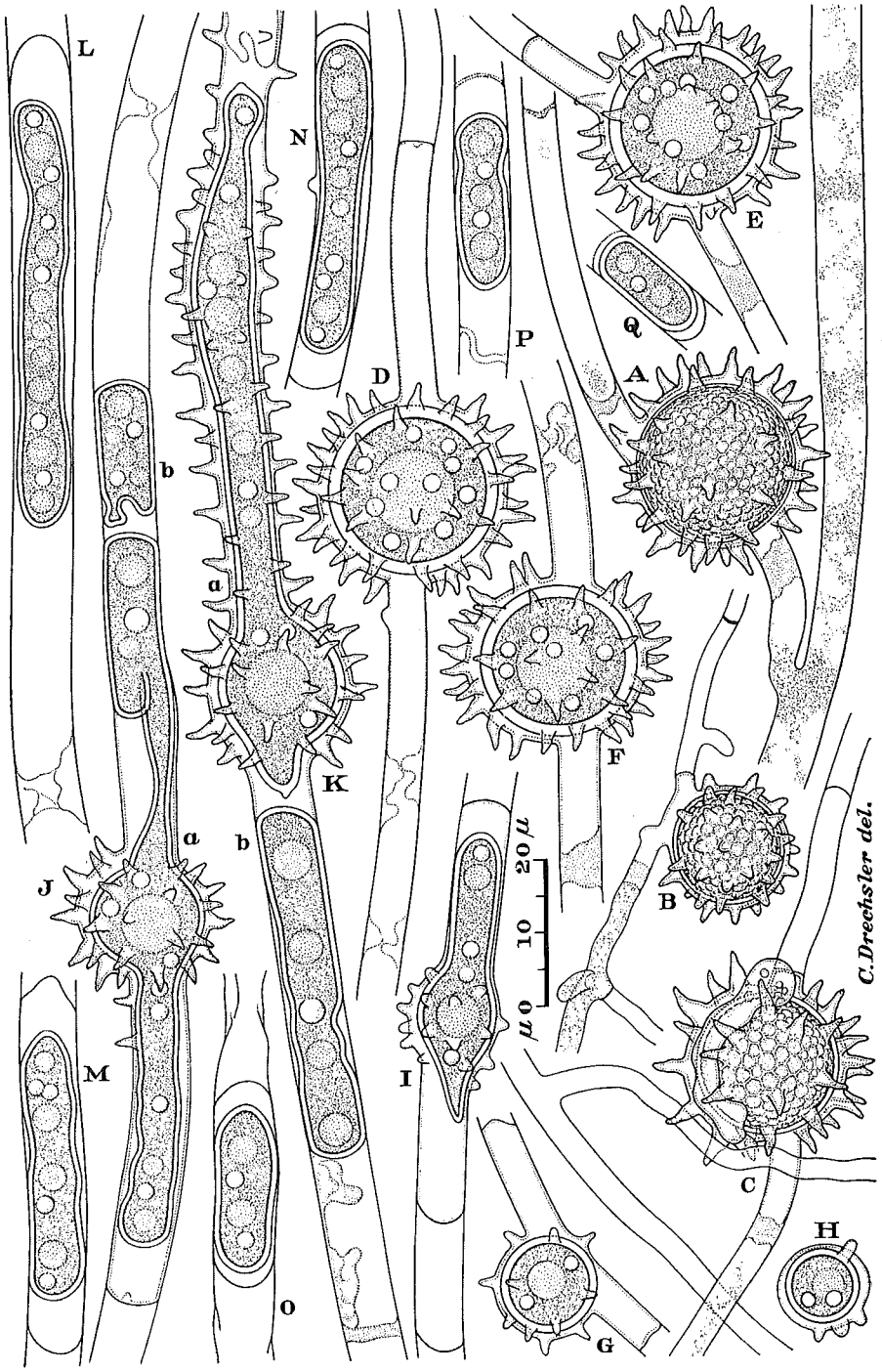

FIG. 8. Sexual reproductive apparatus of *Pythium oligandrum* (Mount Morris pea-root-rot strain) drawn from maizemeal-agar plate cultures with a camera lucida; × 1000 throughout. A, B. Intercalary parthenogenetic oogonia, each with a parthenospore in early stage of development. C. Intercalary oogonium supplied with an antheridium. D–F. Intercalary oogonia of ordinary size, each with a mature parthenospore. G, H. Small oogonia, each with a small parthenospore that contains only 2 refringent bodies. I–Q. Oogonia of aberrant shapes, each with a mature parthenospore, except J and K, which contains 2 parthenospores, a and b.

including, for example, the Mount Morris strain. Nevertheless, owing to the larger dimensions of many other of its strains, such as the Hamburg pea-root-rot strain and the Beltsville cucumber-rot strain, *P. oligandrum* may rightly be considered the somewhat more robust species. Its oogonial protuberances (Fig. 5, J, a–e), on the whole, appear longer, more irregularly contoured, more pronouncedly tapered, and more numerous than those of *P. acanthicum* and *P. periplocum*.

Under natural conditions oospores of the soil-inhabiting Pythiaceae presumably germinate for the most part in water containing no nutrient materials either in solution or in suspension. When bathed in sterilized distilled water newly matured oospores of *Pythium oligandrum* give no sign of germination. After resting for 40 or 50 days a small proportion of oospores will usually germinate when immersed in a shallow layer of water. Practically all oospores in maizemeal-agar culture become capable of germination after aging for 150 to 200 days. Onset of germinative development is manifested by change of the reserve globule from a spherical to an irregular shape. The refringent bodies gradually become less distinctly visible, while at the same time radial markings appear in the oospore wall, or rather in the somewhat darker inner layer making up about two-thirds of the thickness of this wall (Fig. 9, A). Soon the refringent bodies are wholly lost to view, and the radially striate darkish layer merges indistinguishably with the granular protoplasmic mass, which thereby comes to extend to the persistent thin clear outer layer of the oospore wall (Fig. 9, B). The spherical cell, now thin-walled, buds forth a protrusion that on penetrating the oogonial envelope continues growth outside as a germ hypha (Fig. 9, B, t; C, t). On abrupt yielding of the hyphal tip (Fig. 9, D, t) the protoplasmic contents flow into a terminal vesicle to be fashioned into laterally biciliate zoospores; the number of the spores produced varying usually from 5 to 15. The empty evacuation tube here commonly ranges from 10 to 50 μ in length, and from 3 to 4.5 μ in greatest width (Fig. 9, E–L: t). In most instances it terminates abruptly without distal modification, though occasionally its mouth is minutely lipped (Fig. 9, D, t; I, t). Most frequently the evacuation tube is found coming out directly from the spherical envelope of the oogonium, yet now and then it erupts from a filamentous prolongation (Fig. 9, L, t). Wherever germ hyphae attain lengths much in excess of 50 μ without functioning as evacuation tubes, and begin to ramify (Fig. 9, M), zoosporangial reproduction has obviously been abandoned in favor of mycelial growth. Instances of such abandonment are not frequent if the water layer is shallow and is left undisturbed; so that in carefully managed preparations well after-ripened oospores will begin to liberate zoospores within 4 hours, and for about 6 or 8 hours longer will continue producing additional motile spores to provide often a far livelier display of active swarmers than can be obtained by irrigation of young mycelium. After a period of motility the zoospores round up (Fig. 9, N–X) much like those (Fig. 2, X, a–l) produced from sporangia of asexual origin. Later, as a

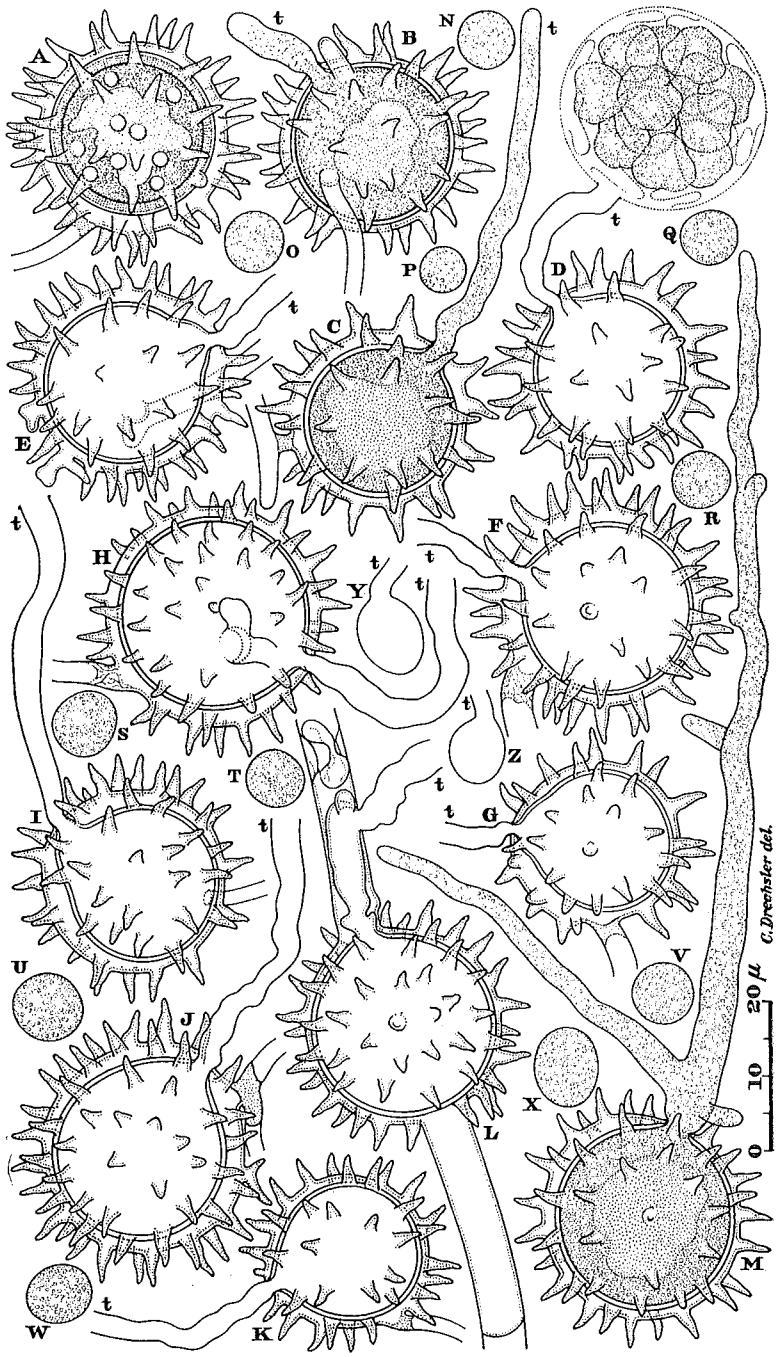

FIG. 9. Germination of oospores and parthenospores of *Pythium oligandrum* (Hamburg pea-root-rot strain) from maizemeal-agar plate cultures 6 months old; drawn with the aid of a camera lucida; ×1000 throughout. A–D. Oospores showing, respectively, (A) assimilation of thick inner layer of wall by protoplast, (B, C) extension of germ hypha, (D) individualization of zoospores in a vesicle formed terminally on the germ hypha. E–L. Empty membranous envelopes left after escape of swarmers from vesicle. M. Oospore germinating by production of a branching mycelial hypha. N–X. Encysted zoospores. Y, Z. Empty cyst envelopes after escape of a secondary zoospore from each. (t, evacuation tube.)

rule, they germinate vegetatively, though some few individuals usually develop iteratively, each discharging its contents through an evacuation tube (Fig. 9, Y, Z), 2 to 5 μ long and 2.5 μ wide, for conversion into a secondary laterally biciliate zoospore. The behavior of the fungus in the laboratory suggests that in nature it produces zoospores more abundantly from its germinating oospores and parthenospores than from zoosporangia formed by its mycelium.

Aside from the eastern United States, where my material of *Pythium oligandrum* originated, the species has been recorded from several other regions. Nattrass (36) in 1937 reported having isolated it 2 years earlier in Cyprus from immature fruits of *Prunus amygdalus* Batsch. Wager (42), who in 1931 cited "*Pythium* sp. cf. *P. artotrogus* (Mont.) de Bary" as having been found associated with wilting of a Shirley poppy (*Papaver rhoeas* L.) and of a snapdragon (*Antirrhinum majus* L.) in South Africa, subsequently (43) referred the fungus to *P. oligandrum,* at the same time making known that it had further been isolated in South Africa from wilting plants of marrow (*Cucurbita pepo* L.) and of cabbage (*Brassica oleracea* L. var. *capitata* L.). For the most part the descriptive particulars given by Wager agree with the morphology revealed in my cultures; yet it may be noted that one of his drawings (43: fig. 9, a) wherein an oogonium is shown fertilized by an antheridium borne terminally on a branch, about 37 μ long, arising from the oogonial stalk only about 7 μ from the female organ, pictures a closer mycelial connection between conjugating sex elements than has ever come under my observation in material of the species. The oogonial spines shown by Wager seem somewhat too sharply pointed at the tip, though they are otherwise of shape and stature usual in my cultures. Very sharply pointed spines are also shown in a figure of *P. oligandrum* recently published by Chesters and Hickman (12), who in England several years earlier (11) isolated from diseased roots of a cultivated violet (*Viola sp.*) some cultures held referable to the species. These authors depict an androgynous antheridium (12: fig. 3, D) that apparently has its origin even closer to the oogonium than the androgynous antheridium figured by Wager. No comment is made by Wager or by Chesters and Hickman concerning the presence of refringent bodies in the mature oospore, nor are such components recognizable in their illustrations. Middleton (34, p. 114, 115), who reported having found the species on the carrot (*Daucus carota* L.), the Christmas flower (*Euphorbia pulcherrima* Willd.), needlegrass (*Stipa sp.*), and wheat (*Triticum aestivum* L.) in the United States, described the oospore as containing a single reserve globule and refringent body.

During several decades preceding its description *Pythium oligandrum* could hardly have failed being encountered from time to time by investigators dealing with root rot and damping-off of the higher plants. It may be presumed that the usual disposition of the fungus in this earlier period is correctly suggested in Butler's report (42, p. 39) on the identity of the cultures isolated by Wager from snapdragon and Shirley poppy. Although

Butler failed to make out antheridia in these cultures and did not discover any evidence of hypogynal male cells such as he (10, p. 100) like de Bary (3, 4) before him had ascribed to *P. artotrogus,* and though he thought the cultures alien to the fungus he had figured in his monograph as well as to the fungus to which de Bary had originally applied the epithet, he nevertheless referred them "for the present in the collective species *artotrogus.*" That the species *artotrogus* had in its application acquired a strongly collective character seems fairly certain. Most of the reports on its occurrence give little suggestion that details relating to antheridial morphology had received appropriate attention when the determinations were made; agreement with respect to size of oogonium, presence of oogonial protuberances, and size of oospore being apparently deemed sufficient to establish identity as long as no other species embodying somewhat similar features had been described in the genus. If in accordance with usage so broad, *P. acanthicum* and *P. periplocum* should, like *P. oligandrum,* have happened to be recorded under the binomial *P. artotrogus,* the error would have concerned species that presumably are intimately related to the one with which they were confused—all being distinguished by capacity for attacking congeneric forms, and by the tapering shape of their oogonial protuberances. Much wider of the mark would have been the almost equally probable application of de Bary's binomial to *P. spinosum* Saw. (39) and *P. mamillatum* Meurs (33), which through their more typically digitate oogonial protuberances, their rather copious production of aerial mycelium, and their inability to parasitize congeneric forms, are at once estranged from the *oligandrum* series and brought into alignment with the familiar damping-off pathogens *P. irregulare* Buism. (9), *P. debaryanum,* and *P. ultimum.*

Since several species of *Pythium* with spiny oogonia mostly 18 to 27 μ in diameter are now known to exist it can no longer be considered wholly certain that the fungus found by de Bary in dead tissues of herbaceous plant parts and described by him mainly from cress-seedling (*Lepidium sativum* L.) cultures as *P. artotrogus* was the same as the fungus which produced in a sprouted potato tuber the pronouncedly echinulate reproductive bodies whereon Montagne (5, 6, 35) more than 30 years earlier had based the generic and specific characterizations of *Artotrogus hydnosporus.* Accordingly, disagreement with de Bary's description can no longer be held necessarily to imply separateness from Montagne's species. The spines figured by Montagne (6: fig. 29) seem much narrower, more acutely pointed and more thickly crowded than any oogonial protuberances I have ever observed in cultures of *Pythium;* though in such particulars allowance must be made for wide differences in habits of draughtsmanship. Failure to mention or to depict antheridia might perhaps be held especially suggestive either of the consistently parthenogenetic *P. anandrum* Drechsl. (17, 20) or of the frequently parthenogenetic *P. oligandrum;* but the oogonia of these 2 species rather markedly exceed in diameter the 1/50 mm. indicated for this dimension by Montagne. Actually Montagne's fungus might have been a form

well supplied with antheridia; for if the male elements of *P. acanthicum* and *P. periplocum,* for example, soon become indiscernible even in a transparent substratum and under a very good modern microscope with excellent illumination, it seems at least probable that similarly evanescent elements could have remained undetected in more or less opaque potato tissue under a microscope of the sort used a century ago. In view of the attending difficulties it is not surprising that de Bary (4, p. 576), who later examined authentic material of *A. hydnosporus* in a dry permanent mount, was not able to give much further information with respect to the mycelial relationships and possible antheridial supply of Montagne's spiny bodies. Although de Bary held as unquestionably identical with these spiny bodies some echinulate structures which he had found in potato (*Solanum tuberosum* L.) tubers affected with the late blight fungus, *Phytophthora infestans* (Mont.) de Bary, and which he subsequently (4, p. 576) recognized as oogonia of his *P. artotrogus* containing oospores in mature or maturing condition, it is worthy of note that one (2, fig. 1) of his three early figures illustrating the echinulate structures seems to show 4 cellular components corresponding remarkably well in size to the plural refringent bodies of *P. oligandrum* while another figure (2, fig. 3) seems to show 2 such components. Possibly the comment (4, p. 576) in his definitive account of *P. artotrogus* to the effect that his earlier description had not adequately set forth the constitution of the oospore contents may have been intended to disparage the accuracy of these figures in showing plurally the cellular components under discussion. He stated (4, p. 624) at all events that at full maturity the condition of the oospore in *P. artotrogus* was like the condition illustrated in a ripe oospore of his *P. megalacanthum,* or in a ripe oospore of his *P. proliferum*—both revealing unmistakably a single refringent body in the parietal layer surrounding the single reserve globule. Likewise in another treatise (3, p. 61), devoted more especially to sexual reproduction in the oomycetes, he included all species of *Pythium* therein described by him—and *P. artotrogus* was one of these species—among representatives of various genera whose ripe oospore he found to contain a single "heller Fleck" (refringent body) in the parietal granular layer surrounding the "Fettkugel" (reserve globule). While these statements by de Bary must be held to establish unitary organization of the oospore as a specific character of *P. artotrogus,* his curious failure to supply in his two later publications any figure showing unitary organization in a ripe oospore of the species invites speculation whether some difficulty may not have intervened such as could have been occasioned by intrusion now and then of material referable to *P. oligandrum.* Although Butler (10, p. 100–101) gave little attention to internal organization of oospores, his characterization of the antheridia in *P. artotrogus* as being "always hypogynal," together with his first-hand delineation of consistently hypogynal antheridia presumably from material of a spiny form he found in Calcutta in decaying potato tubers affected with *Phytophthora infestans,* would seem to provide the only record rather un-

ambiguously setting forth an association of the host relationship of *A. hydnosporus* with the antheridial morphology characteristic of *P. artotrogus*. Indeed, Butler's account would seem to provide also about the only first-hand record, apart from de Bary's description of *P. artotrogus,* wherein spiny oogonia are set forth as being fertilized exclusively by hypogynal antheridia. In recent times, however, Matthews (32, p. 101–104) described as *P. echinulatum* a fungus with spiny oogonia usually fertilized by hypogynal antheridia, rarely by branch antheridia. Though held to be similar to *P. artotrogus* in general appearance and in size of oogonia and oospores—the oospores individually containing a single refringent body—the fungus was separated from that species because it produced numerous conidia and zoosporangia. From the description given of them, these conidia and sporangia resemble rather closely the homologous reproductive bodies of *P. debaryanum* as well as the conidia of *P. ultimum,* which species de Bary evidently included under the one named in his honor. The resemblance seems of some moment, since in all of de Bary's cress-seedling cultures *P. artotrogus* never occurred except in admixture with *P. debaryanum;* the spiny form, according to his account, appearing tardily in somewhat meager quantity after the smooth damping-off parasite had produced a fairly luxuriant mycelium and numerous reproductive bodies. Under these circumstances should the spiny fungus have produced asexual reproductive bodies indistinguishable from those of *P. debaryanum*—and in this connection the difficulty of distinguishing generally similar bodies when seen only in mixture, together with the inferior capabilities of the microscopes in use 65 or 70 years ago, needs to be considered—de Bary might not, he rather clearly intimates (4, p. 574, lines 43 to 49), have been able to refer them to *P. artotrogus.* If seen only in mixed cultures of the kind studied by de Bary, even the sporangia of *P. oligandrum* and of *P. acanthicum,* though differing more pronouncedly from those of *P. debaryanum* than the sporangia described and figured by Matthews, might perhaps not be distinguished successfully from the more numerous alien reproductive bodies present with them. Wherefore, indeed, in separating these 2 spiny forms, and for that matter also *P. periplocum,* from *P. artotrogus,* it was deemed advisable to rely almost wholly on differences relating to morphology of sexual reproductive apparatus.

GERMINATION OF OOSPORES OF PYTHIUM PERIPLOCUM

Oospores of *Pythium periplocum* produced in maizemeal-agar plate cultures seem, like those of *P. oligandrum,* to require a fairly prolonged resting period before they will germinate readily in pure water devoid of nutrient substances. After some cultures had been stored in the laboratory for 165 days at temperatures fluctuating mostly between 28° and 32° C., about a third of the oospores germinated promptly on shallow irrigation. When storage was continued 45 days longer at slightly lower temperatures, nearly all the oospores germinated, some of them undergoing transformation into zoosporangia so rapidly that zoospores began swimming about within 2 hours.

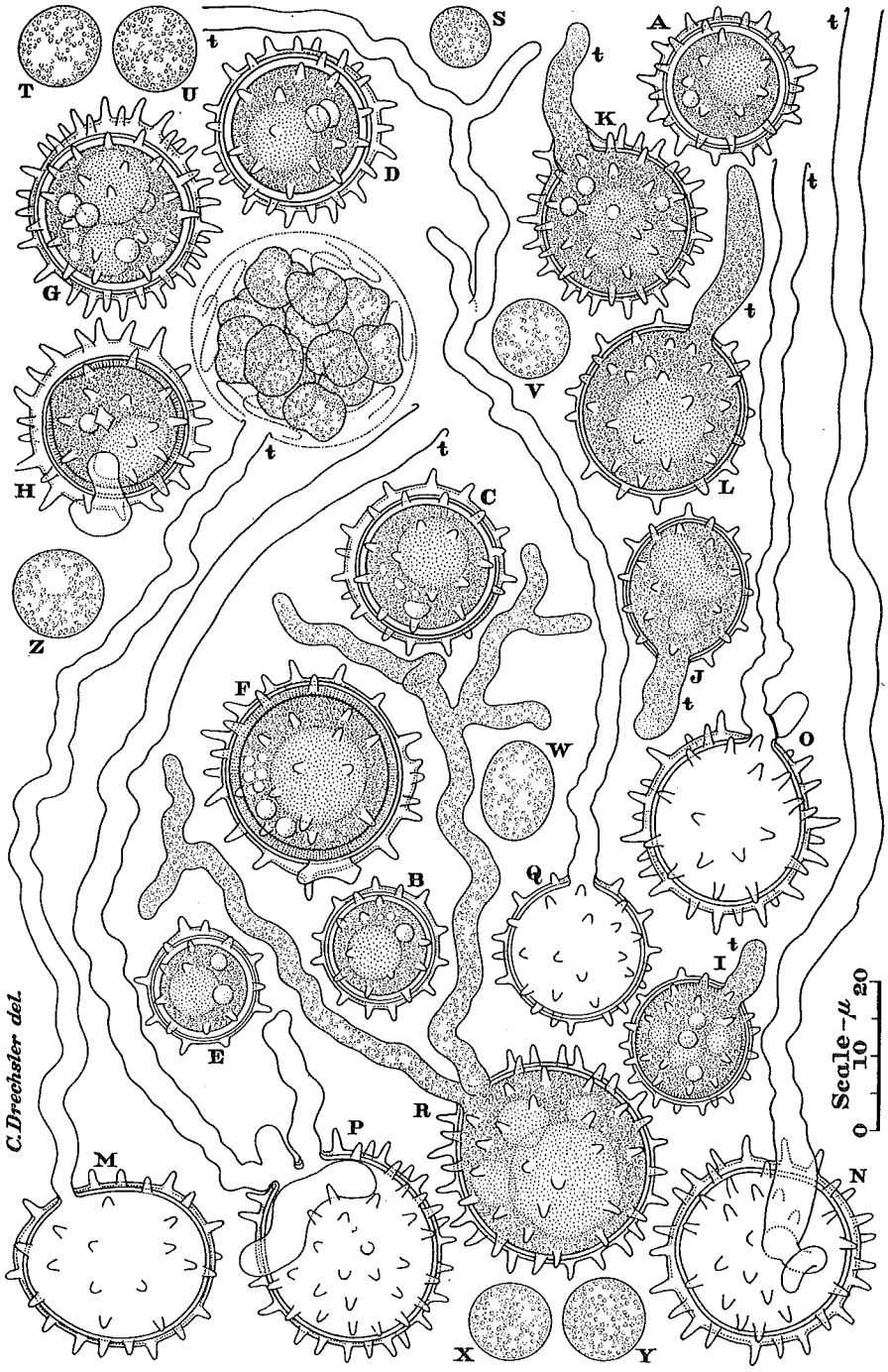

FIG. 10. Germination of oospores of *Pythium periplocum* taken from maizemeal-agar plate cultures 7 months old; ×1000 throughout. A–E. After-ripened oospores showing two layers in wall. F–H. Oospores about 30 minutes after transfer to water. I–L. Oospores with elongating germ hyphae, t. M. Oospore whose contents have been converted into zoospores in the vesicle at the tip of the evacuation tube t. N–Q. Empty oospore envelopes, each with an empty evacuation tube, t. R. Ooospore germinating by production of a mycelium. S–Z. Encysted zoospores produced through germination of oospores.

During the resting period the oospore shows very gradually increasing contrast between a relatively thin colorless outer layer and a thicker yellowish inner layer (Fig. 10, A–G). In many instances the typically unitary organization of the protoplast (Fig. 10, A–C) becomes modified through the presence of 2 (Fig. 10, D, E, F) to 4 (Fig. 10, G) refringent bodies; the increased number of these bodies being often found associated with a noticeably vacuolate condition of the parietal granular layer (Fig. 10, F, G). Soon after an oospore is immersed in fresh water the thicker yellowish inner layer of its wall reveals innumerable closely arranged radial markings (Fig. 10, F). The striate layer now dissolves away in a localized region about 2.5 to 5 μ wide, permitting the protoplast to protrude against the thin outer layer (Fig. 10, H). Before long the outer layer yields in the region of contact as the protrusion presses forward into the oogonial chamber and then forces its way through the oogonial envelope to elongate externally as a germ tube (Fig. 10, I–L: t). In the meantime the striate inner layer of the oospore wall undergoes gradual obliteration throughout its circumference (Fig. 10, I), and soon merges indistinguishably with the protoplasmic mass, which thus is expanded to make contact everywhere with the persistent outer layer of the wall (Fig. 10, J–L); the reserve globule during the same period changing from a globose to a more irregular shape (Fig. 10, H, L), or often dividing into 2 or 3 vacuole-like parts (Fig. 10, J, K) as the refringent bodies are lost to view in their granular matrix (Fig. 10, J, L). Eventually the whole protoplasmic mass streams through the germ hypha into a terminal vesicle, where it is fashioned into laterally biciliate zoospores (Fig. 10, M). The empty evacuation tube here commonly measures from 50 to 200 μ in length (Fig. 10, M–Q: t), thus, with respect to this dimension, generally exceeding the corresponding element in *P. oligandrum*. Very often the membrane of the tube is abruptly reflexed at the orifice (Fig. 10, M–P: t), though instances are never lacking where the mouth has no lipped modification (Fig. 10, Q, t). Rather frequently an evacuation tube is found bearing a short branch near its base (Fig. 10, N–P: t), and occasionally 1 or even 2 branches may be found attached farther upward (Fig. 10, Q, t). However, more abundant branching of a germ hypha (Fig. 10, R) usually betokens here, as in allied species, that direct zoosporangial reproduction is no longer possible, and that, instead, the substance of the oospore will be used for mycelial growth. The zoospores produced through oospore germination swim about for some time before they come to rest and round up (Fig. 10, S–Z). In all respects they behave much like the zoospores produced from sporangia of mycelial origin.

MORPHOLOGY AND DEVELOPMENT OF PYTHIUM SALPINGOPHORUM

Although *Pythium salpingophorum* gives rise to vegetative hyphae as wide as 7 μ, it produces such stout hyphae less abundantly than *P. ultimum*, *P. debaryanum*, or *P. irregulare*, with the result that its mycelium, on the whole, looks considerably less coarse than mycelium of any one of the 3 most

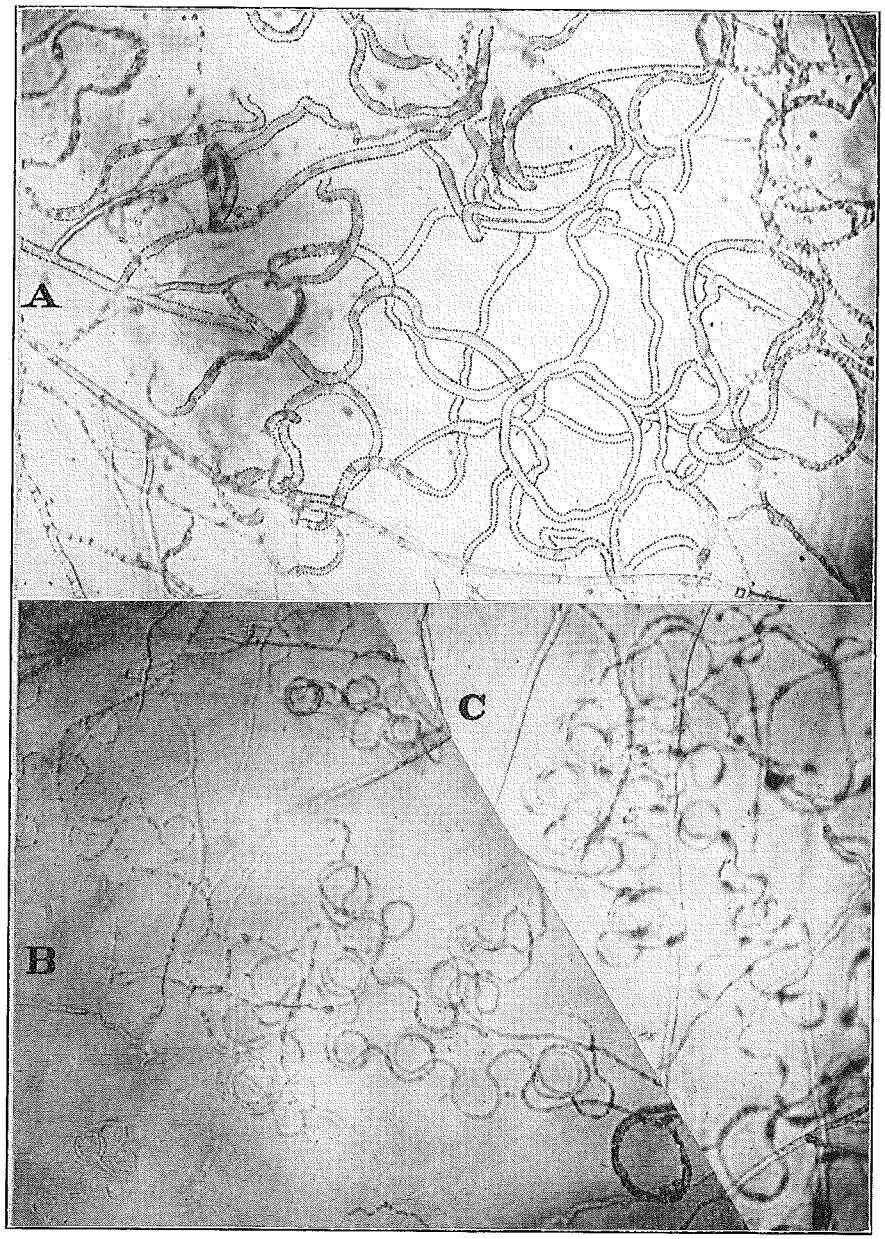

FIG. 11. *Pythium salpingophorum.* A. Mycelium from bottom of a maizemeal-agar plate culture, showing thick curving hyphal elements possibly resulting from elongation of functionally frustrated appressoria; the alveolate protoplasmic structure revealed by the hyphal elements being rather usual in aging mycelium of the species. B, C. Tracts of mycelium from upper surface of a maizemeal-agar plate culture, displaying elaborate systems of small hyphal coils. Photomicrographs, all approximately × 300. Photomicrograph in A has been retouched.

familiar damping-off species. In maizemeal-agar plate cultures the fungus after several days often displays numerous rather coarse irregular hyphal loops in contact with the glass floor of the Petri dish (Fig. 11, A). Since these loops have about the same width as the appressoria which during somewhat earlier stages of vegetative growth are recognizable as swollen knobs borne terminally on branches of variable lengths (Fig. 12, A, a–d; B, a–d; C, a–g), they might readily be presumed to arise by prolongation of frustrated appressoria. Such a presumption, however, is open to some doubt, inasmuch as the submerged hyphal loops are noticeably coarser than the chains of sickle-shaped structures that can often be observed in meagerly irrigated preparations, and that very obviously come into being through repeated renewal of growth by appressoria unsuccessful in penetrating the glass dish. Further ground for doubt is offered in the usually rather copious production of elaborate hyphal coils (Fig. 11, B, C) on the upper surface of agar cultures, where all development of penetrative organs would be excluded. Although in the main the coils formed on the upper surface are more delicate as well as more intricate than those formed on the glass floor, scattered examples seem to provide transition from one type to the other; thereby suggesting that the submerged coils may in some degree derive from growth tendencies not directly connected with development of appressoria. Whatever their nature may be, the curious hyphal coils, above and below, are often helpful in identifying the species when means are lacking for inducing asexual reproduction.

Like most species of *Pythium* adapted to a terrestrial habitat *Pythium salpingophorum* is capable of producing some zoosporangia under cultural conditions unsuitable for zoospore formation. Thus when grown at 25° C. on maizemeal-agar devoid of liquid water it usually gives rise in the course of 15 to 20 days to a fairly generous scattering of subspherical asexual reproductive bodies (Fig. 13, A, a) resembling with respect to size the familiar conidia of *P. ultimum*. Such globose reproductive bodies, if transferred to a shallow layer of water kept at a temperature near 15° C., soon reveal themselves as zoosporangia by putting forth an evacuation tube (Fig. 13, A, t; B, t) individually. Far more abundant development of sporangia ensues, however, when young well-nourished mycelium is placed under conditions suitable for immediate formation of zoospores. When, for example, slabs are excised from a thin plate culture of maizemeal or Lima bean agar well permeated with actively growing mycelium, and are placed in a shallow layer of water at 15° C., asexual reproductive apparatus (Fig. 13, C–R) will usually be found present in enormous quantity after 10 to 20 hours; the innumerable sporangia then produced, intermingled amid a confusion of active and encysted zoospores, not only blanketing the surface of the slabs but extending out over the narrow fringe of extramatrical mycelium.

Probably the most distinctive morphological character associated with asexual reproduction in *Pythium salpingophorum* is the very pronounced distal widening of the evacuation tube (Fig. 13, C, t; D, t). After the apex

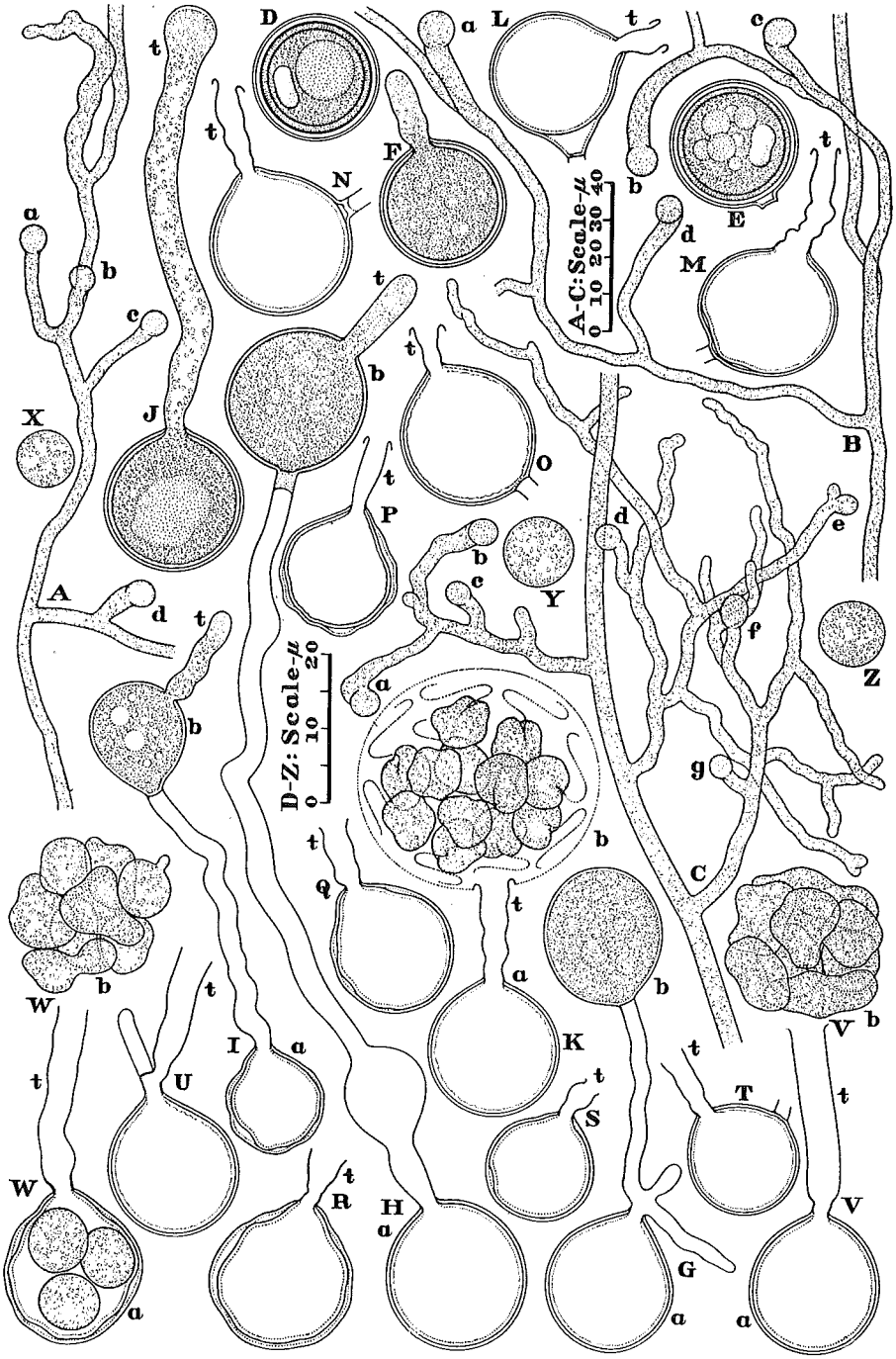

FIG. 12. *Pythium salpingophorum.* A–C. Portions of mycelium at bottom of maize-meal-agar plate culture, showing development of appressoria (a–d, a–d, a–g, respectively) in contact with glass floor of Petri dish; ×500. D–Z. Germination of oospores from maizemeal-agar plate cultures 8 months old; drawn with aid of a camera lucida; ×1000. D, E. Oospores shortly after immersion in water, each showing darkening of inner layer of wall preparatory to germination. F. Oospore whose protoplast has assimilated the inner layer of the wall, and is extending a germ hypha, t. G. Oospore, a, that has produced a terminal sporangium, b. H, I. Oospores, a, that have each produced a terminal sporangium, b, from which an evacuation tube, t, has been extended. J. Oospore that has put

of the tube has yielded to permit the sporangial contents to flow out, the flaring terminal portion of the membranous envelope folds backward after the manner of a trumpet with reflexed bell—a feature signalized in the epithet chosen for the species. Where the empty tube is less than 5 μ in length, the envelope in its entirety offers a bell-like contour (Fig. 13, E, t; N, t; O, t; P, t). Since the vesicular film is attached, as in other species, to the very rim of the membrane, the rolled anterior portion of the reflexed tube extends perceptibly into the chamber of the vesicle (Fig. 13, G, t; H, t). Generally the vesicle here can be seen more readily than in *P. oligandrum*, being nearly always discernible with good illumination. Even at the time sporangial discharge has just been completed it is usually considerably larger than the mass of loosely enclosed protoplasm. It grows in size as zoospore formation proceeds; so that before the zoospores are ready for liberation its diameter is usually twice the diameter of the empty sporangium (Fig. 13, H, a). In most instances the sporangial envelope, on being evacuated, contracts appreciably in volume, and at the same time takes on the haphazard irregularities of contour frequent in the shrinkage of emptied membranes (Fig. 13, F, a; G–I; K, b; L–N; P); though in some instances the membrane is sturdy enough to maintain its smooth outline after evacuation (Fig. 13, O; J, a, b; K, a). Many of the largest and sturdiest sporangial envelopes (Fig. 13, I; J, a, b; K, a, b) are found in intercalary positions in hyphae lying directly on irrigated portions of substratum. However in irrigated material the most usual position for a sporangium is a subterminal position 3 to 20 μ below the tip of a simple or meagerly branched filament; the terminal hyphal part being borne distally on the reproductive body somewhat like an appendage (Fig. 13, D; E; F, b; G; H; M–P). Occasionally where the terminal hyphal part is very short, it is not cut off by a septum; so that the sporangium comes to have a beaked shape, and occupies a terminal position (Fig. 13, F, a). A similar beaked prolongation may at times likewise modify the shape of a sporangium borne more or less laterally (Fig. 13, C). Now and then after a terminal or subterminal sporangium has been evacuated, renewed growth from the basal septum leads to the production of a second sporangium within the emptied envelope (Fig. 13, E); or the supporting hypha may grow out laterally just below the basal septum of the first sporangium (Fig. 13, F, a) to bear a second sporangium (Fig. 13, F, b) on an oblique branch of variable length. In my irrigated preparations of the fungus, proliferous develop-

forth directly an evacuation tube, t, which is about ready to yield at its expanded tip. K. Oospore, a, that has discharged its contents through the evacuation tube, t, into the vesicle, b, where they have been fashioned into 12 zoospores. L–U. Oogonial envelopes, each containing the empty outer layer of the oospore wall, from which the protoplasmic contents have migrated through the evacuation tube, t, to be transformed into zoospores in a vesicle. V. Oogonial envelope, a, surrounding the thin outer layer of the oospore wall which is prolonged into the evacuation tube, t, near the mouth of which 8 immature zoospores have encysted irregularly to form a cluster, b. W. Oogonial envelope, a, surrounding outer layer of oospore wall which encloses 3 well-encysted zoospores; near mouth of evacuation tube, t, is a cluster of 8 irregularly encysted zoospores, b. X–Z. Zoospores that have encysted after period of motility following liberation from vesicle.

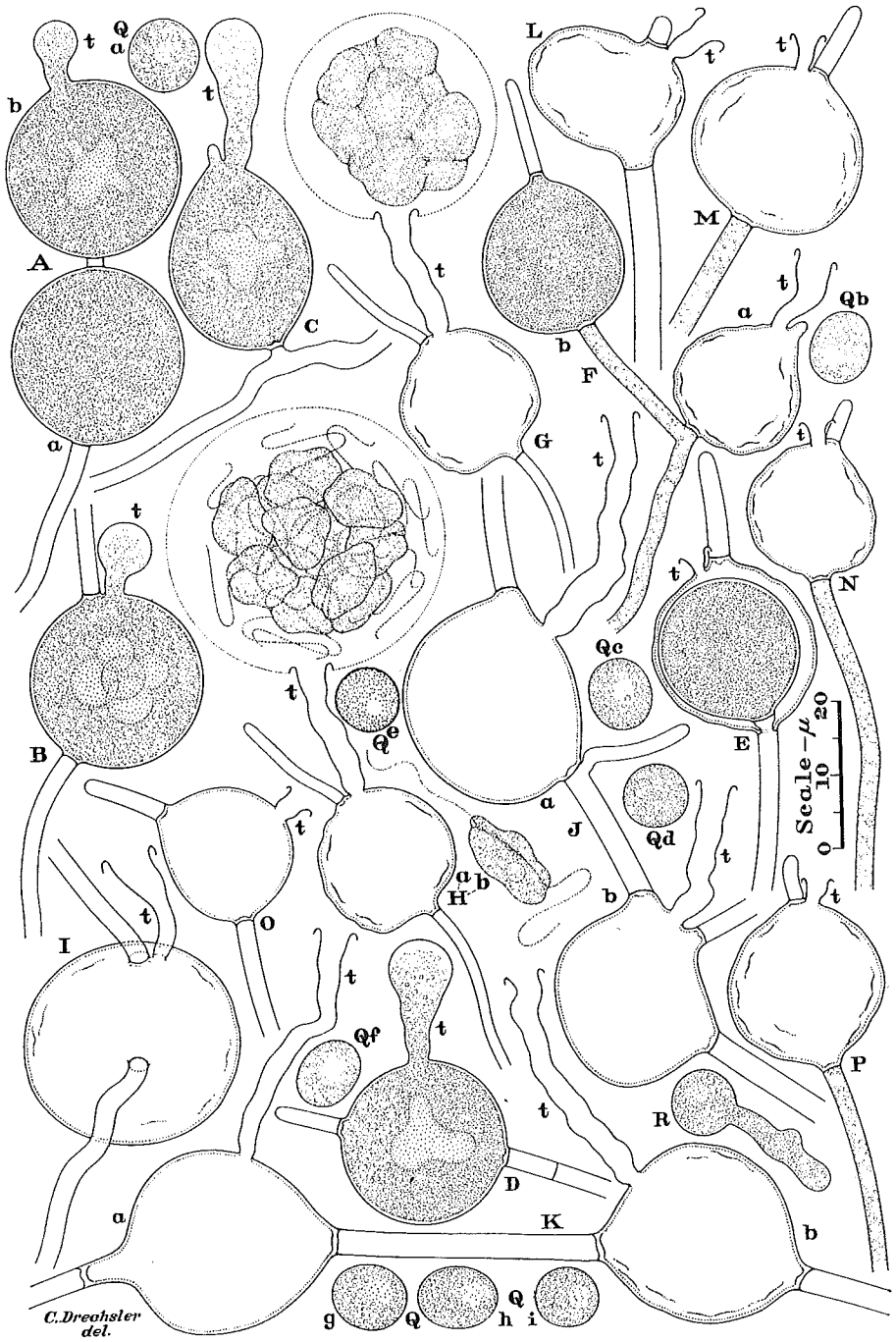

FIG. 13. Asexual reproductive apparatus of *Pythium salpingophorum*, drawn with the aid of a camera lucida from irrigated maizemeal-agar (A, B) and irrigated Lima-bean-agar; ×1000 throughout. A. Two zoosporangia formed close together, one of them, a, in an inert conidial condition, the other, b, actively extending an evacuation tube. B. Intercalary sporangium actively putting forth an evacuation tube. C. Lateral sporangium with an evacuation tube nearly ready for dehiscence. D. Subterminal sporangium with an evacuation tube ready for dehiscence. E. Subterminal sporangial envelope that after being emptied has become largely occupied by a secondary sporangium. F. Hypha which after producing a terminal sporangium, a, branched distally to bear subterminally a second sporangium, b. G. Subterminal sporangium about 2 minutes after

ment, whether by uniaxial elongation or by subsporangial branching, has always been relatively infrequent. Since, further, such development has been found almost exclusively among the less rangily attached sporangia formed in crowded arrangement on the upper surface of irrigated agar slabs, where observation is far more difficult than in the surrounding extramatrical fringe, it can hardly be regarded as a feature promising much usefulness in the recognition of the species.

In some irrigated preparations of *Pythium salpingophorum* the actively swimming zoospores (Fig. 13, H, b) have appeared to be slightly longer in proportion to their width than the zoospores of most members of the genus. The difference in shape has, however, not always been clearly observable, and accordingly is not to be urged as a distinguishing feature. On coming to rest the zoospores round up to form subspherical or prolate ellipsoidal cysts (Fig. 13, Q, a–i). Although these cysts usually remain submerged, they have at times been found floating on the surface of the water in countless numbers. They germinate rather readily by putting forth a commonplace germ hypha (Fig. 13, R).

Sexual reproduction takes place freely both in irrigated Lima-bean-agar preparations and in cultures of maizemeal agar containing in suspension a substantial quantity of finely divided maizemeal. As in *Pythium oligandrum,* parthenogenetic development is frequent. The young oogonia make their appearance here and there on the mycelium as subspherical, prolate ellipsoidal, or oblate ellipsoidal enlargements. Often when two or three are formed adjacent to one another, and no antheridium is present, they look at first much like conidia; their character as oogonia, however, soon becomes evident when, after they have attained definitive size, their protoplasmic contents assume a coarsely lumpy texture (Fig. 14, A, a–c). A thick, spherical oospore wall is then laid down in intimate contact with the inflated portion of oogonial envelope (Fig. 14, B, a, b); the wall as a rule being physically separated from the envelope only where the oogonium is extended at either end. A number of reserve globules thereupon become visible in the midst of the protoplasmic lumps (Fig. 14, B, a, b). Later these are united into a single reserve globule; and the surrounding lumps are resolved into minute granules to be distributed as components of the parietal layer in which a single refringent body of orbicular or oblate ellipsoidal shape emerges clearly into view. The resulting parthenospore when fully mature (Fig. 14, C; D, a–c; E, a–c; F, a–c; G, a–c; H, a–d) thus reveals the unitary internal organization characteristic of oospores in most members of the genus.

Although parthenogenesis often predominates over conjugative development in *Pythium salpingophorum,* it is common for 1 in 3 or 4 oogonia to be

discharge of contents into vesicle. H. Same sporangium about 15 minutes after discharge, showing 14 zoospores within vesicle nearly ready for liberation; b, zoospore after liberation. I; J, a, b; K, a, b. Empty envelopes of intercalary sporangia, each bearing a rather long, empty evacuation tube. L–P. Empty envelopes of subterminal sporangia, each bearing a rather short, empty evacuation tube. Q. Encysted zoospores, a–i, showing variation in size and shape. R. Germinating zoospore. (t, evacuation tube.)

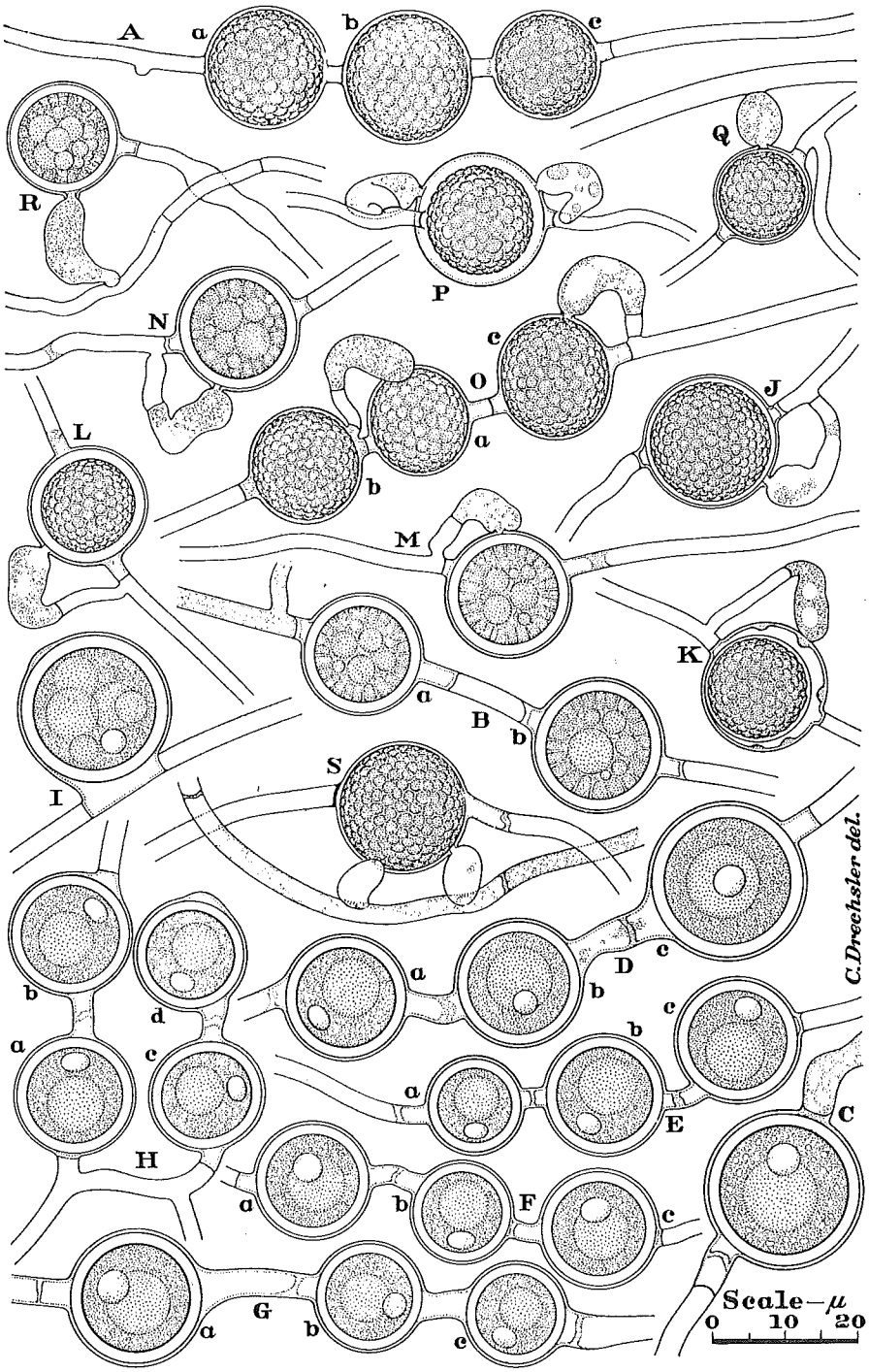

FIG. 14. Sexual reproductive apparatus of *Pythium salpingophorum* drawn from irrigated Lima-bean-agar (A, B, K–S) and from maizemeal-agar plate cultures (C–J) with the aid of a camera lucida; × 1000 throughout. A. Hypha bearing 3 young but full-grown oogonia, a–c, that are beginning parthenogenetic development. B. Hypha bearing 2 oogonia, a and b, each containing an immature parthenospore with thick wall. C. Intercalary oogonium of large size, with a mature parthenospore. D–G. Portions of hyphae, each bearing 3 mature parthenospores, a–c. H. Portion of mycelium bearing 4 mature parthenospores, a–d. I. Laterally intercalary oogonium with large parthenospore still

supplied with a male complement. Most frequently this complement consists of a single clavate crook-necked antheridium (Fig. 14, J–N; O, a, c) borne on a short branch arising from the oogonial hypha either in immediate proximity to the oogonium (Fig. 14, K–N; O, a, c) or occasionally at a distance of several microns from the oogonial boundary (Fig. 14, J). Now and then an androgynous branch antheridium is supplied from both the proximal and the distal side of the oogonium (Fig. 14, P). Occasionally, again, an antheridium (Fig. 14, Q, R) or 2 antheridia (Fig. 14, S) are contributed by a hypha having no close mycelial connection with the oogonium; male cells of such origin being more often sessile than those of androgynous origin, and usually lacking crook-necked curvature. As a rule the oogonial envelope becomes noticeably lipped about the short fertilization tube usually extended from the apex of the antheridium. In most instances a substantial portion, if not all, of the antheridial contents are delivered through the tube. Failure of fertilization may be inferred in other instances (Fig. 14, K, O, a), where no tube is intruded or where the antheridium retains its contents undiminished. Here and there an oogonium (Fig. 14, O, b) may be found to which is directly attached an antheridial branch supplying an adjacent older oogonium (Fig. 14, O, a); an appearance being thereby presented as if the antheridial branch were of oogonial origin. There is much reason to presume, however, that in all such cases the antheridial branch grew out from a portion of undifferentiated hyphal filament, and that it came into its anomalous positional relationship subsequently when the hyphal portion was distended to form the younger of the 2 contiguous oogonia. A similar relationship of parts is frequent in my *P. paroecandrum* (22, p. 208) and has been observed likewise in some cultures of the familiar *P. ultimum* that produced sexual apparatus very abundantly.

In irrigated preparations of *Pythium salpingophorum* scattered oogonia may be found enclosing oospores so much smaller (Fig. 14, K) that separation between oogonial envelope and oospore wall is no less distinct than in *P. debaryanum* or *P. ultimum*. However, in agar cultures such separation is usually evident only where the spherical contour of the oogonium merges with the cylindrical contour of the supporting filament; though here and there, especially in terminal (Fig. 14, H, d) or laterally intercalary (Fig. 14, I) oogonia, separation may be observable, besides, in blister-like irregularities of the oogonial envelope. For the most part, oospores of the species have little the aspect of endogenous reproductive bodies. On aging,

slightly immature with respect to distribution of reserve material among several globules. J. Oogonium fertilized by a branch antheridium arising nearby from same hypha; oospore in early stage of development. K. Oogonium apparently not fertilized though supplied with a branch antheridium arising nearby from the same hypha; the parthenospore here being unusual in lying loose within the oogonial chamber. L–N. Solitary oogonia, each supplied with an antheridium arising nearby from same hypha; the oospores here showing successively later stages in maturation. O. Three adjacent oogonia, one of them, a, supplied with an antheridium on a branch from its younger neighbor, b, while the third, c, is fertilized by an antheridium originating nearby from the parent hypha. P. Oogonium supplied with 2 antheridia, both borne sessile on the oogonial filament. Q, R. Oogonia, each supplied with an antheridium borne sessile on a neighboring hypha. S. Oogonium supplied with 2 antheridia sessile on a neighboring hypha.

after all antheridia have vanished from sight, they could readily be mistaken for chlamydospores, were it not for their internal organization. They show generally a fairly high degree of uniformity with respect to size. The metric data, given in the diagnosis, relative to oogonium and oospore were based on 200 measurements of specimens chosen at random in 14-day-old maizemeal-agar plate cultures containing very abundant sexual apparatus with virtually no degeneration. The 200 oogonia gave values for diameter, expressed in the nearest integral number of microns, distributable as follows: 11 μ, 1; 12 μ, 1; 13 μ, 10; 14 μ, 27; 15 μ, 42; 16 μ, 54; 17 μ, 41; 18 μ, 14; 19 μ, 6; 20 μ, 2; 21 μ, 1; 22 μ, 1; and the 200 oospores or parthenospores, all of correct internal structure, that were contained in them, gave values for diameter, expressed in the nearest integral number of microns, distributable thus: 10 μ, 1; 11 μ, 1; 12 μ, 13; 13 μ, 29; 14 μ, 40; 15 μ, 62; 16 μ, 33; 17 μ, 11; 18 μ, 6; 19 μ, 4.

Oospores from maizemeal-agar plate culture 250 days old were found to germinate freely when placed in a shallow layer of water kept at temperatures near 16° C. During the period of after-ripening the oospore wall becomes more distinctly differentiated into an outer colorless layer and a somewhat thicker yellowish inner layer. On immersion in water the differentiation is further accentuated through radial markings of the inner layer (Fig. 12, D). In some cases the single reserve globule now divides into several globules, and the refringent body also may undergo division (Fig. 12, E). Soon the inner layer of the oospore wall merges indistinguishably with the protoplast, which puts forth a protrusion that after pushing through both the outer layer of the oospore wall and the oogonial envelope continues growth externally as a germ tube (Fig. 12, F); the reserve globules and the 1 or 2 refringent bodies meanwhile being lost to view. Sometimes after the germ hypha has attained a length of 25 to 100 μ, all the protoplasmic contents of the oospore (Fig. 12, G–I: a) are utilized for the production of a terminal sporangium (Fig. 12, G–I: b), which may later put forth an evacuation tube (Fig. 12, H, t; I, t) much like a sporangium of mycelial origin. However, under favorable conditions the oospore (Fig. 12, J; K, a) functions directly as a sporangium; the germ tube extended by it (Fig. 12, J, t) forming on its expanded tip a cap of dehiscence which on yielding permits migration of the oospore contents into a terminal vesicle where they are fashioned into laterally biciliate zoospores, mostly 6 to 15 in number (Fig. 12, K, b). The empty evacuation tubes here are often rather strongly reflexed at the end (Fig. 12, L–P: t), though frequently, too, they are merely widened at the mouth without being folded backward (Fig. 12, Q–V: t), and occasionally they show no distal widening (Fig. 12, W, t). Owing very probably to lack of sufficient water the vesicle sometimes disintegrates prematurely, with the result that the young zoospores, not yet provided with flagella, encyst in irregular shapes and thus remain clustered near the mouth of the evacuation tube (Fig. 12, V, b; W, b). Where a portion of protoplasm fails to migrate from the chamber of the

oospore, it is nevertheless successfully fashioned into zoospores, which after a period of movement within their small enclosure round up to form spherical cysts (Fig. 12, W, a) indistinguishable from the cysts formed from normally liberated zoospores (Fig. 12, X, Y, Z). After being emptied of contents the persistent thin outer layer of the oospore wall sometimes remains appressed to the oogonial envelope (Fig. 12, G, H, K, N, O, T, U, V), but at other times shrinks away to become more clearly visible as a discrete membrane (Fig. 12, I, M, P, Q, R, S, W).

Like many congeneric forms *Pythium salpingophorum* is subject to severe attack when it is grown on maizemal agar in dual culture with *P. oligandrum*. Its mycelium is abruptly halted in its advance when it encounters growing mycelium of the echinulate species; the extension of the echinulate species, however, continuing without interruption. Everywhere in the zone of encounter young hyphae of *P. salpingophorum* (Fig. 15, A, a; B, a) become elaborately enveloped by branches of *P. oligandrum* (Fig. 15, A, b; B, b). Numerous threads of the smooth species (Fig. 15, C, a) are extensively invaded by assimilative filaments of the spiny one (Fig. 15, C, b), although plugs and irregular septa laid down in the former have some effect in restricting the field of each invasion. After the contents of the host thread (Fig. 15, D, a) have been appropriated, the assimilative filaments (Fig. 15, D, b) extend branches through the hyphal membrane to attack other threads. In dual culture with *P. acanthicum,* the mycelial advance of *P. salpingophorum* is likewise stopped abruptly in the zone of encounter; and its young hyphae (Fig. 15, E, a) here similarly become enveloped by branches of the opponent mycelium (Fig. 15, E, c). While large hyphae of the smooth form (Fig. 15, F, a) sometimes successfully resist invasion by the enveloping branches (Fig. 15, F, c), their protoplasmic contents nevertheless suffer thoroughgoing degeneration. Hyphae of *P. salpingophorum* having less indurated walls (Fig. 15, G, a; H, a) are readily invaded by *P. acanthicum* (Fig. 15, G, c; H, c) and when their contents have been appropriated (Fig. 15, I, a) the internal haustorial filaments (Fig. 15, I, c) erupt through the enveloping membrane to begin another attack. Similar injury is incurred by *P. salpingophorum* when it is grown in dual culture with *P. periplocum;* the advance of its mycelium being sharply arrested on meeting the opponent mycelium. Here, also, in the zone of encounter numerous hyphae of *P. salpingophorum* (Fig. 15, J–O: a) are penetrated by branches of the echinulate species (Fig. 15, J–O: d) and invaded lengthwise by assimilative filaments. Later these filaments often push branches (Fig. 15, P, d) through the membrane of the host (Fig. 15, P, a) to extend the destructive relationship to other hyphae of the smooth species.

In diseased pea roots from which it was originally described and from which it has since been isolated by Horsfall and Kertesz (29), *Pythium salpingophorum* is frequently found associated with the saprolegniaceous parasite *Aphanomyces euteiches* Drechsl. As it was isolated from spinach

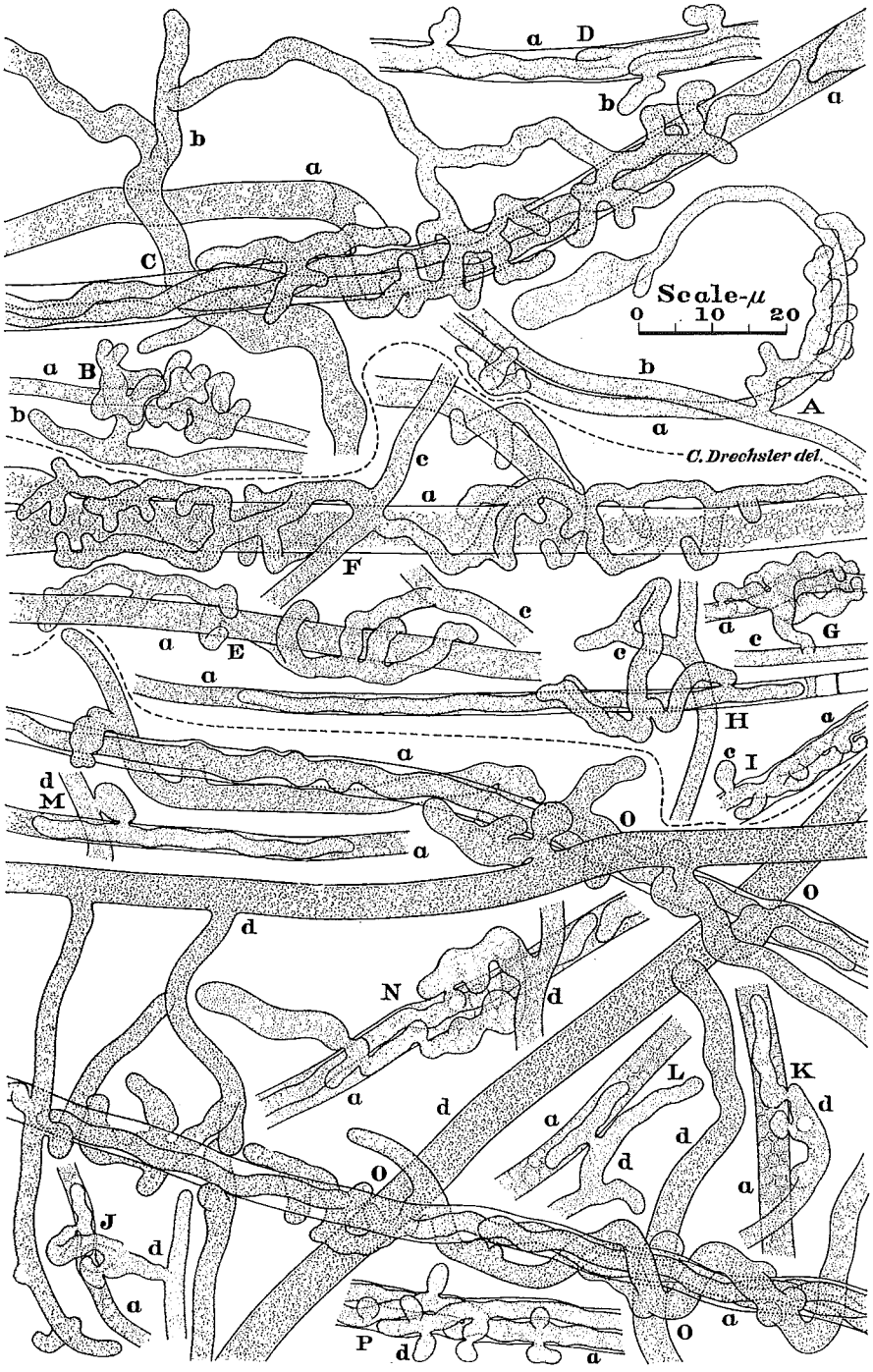

FIG. 15. Drawn from maizemeal-agar plate cultures with the aid of a camera lucida; ×1000 throughout. A–D. Hyphae of *Pythium salpingophorum*, a, attacked by *P. oligandrum*, b. E–I. Hyphae of *P. salpingophorum*, a, attacked by *P. acanthicum*, c. J–P. Hyphae of *P. salpingophorum*, a, attacked by *P. periplocum*, d.

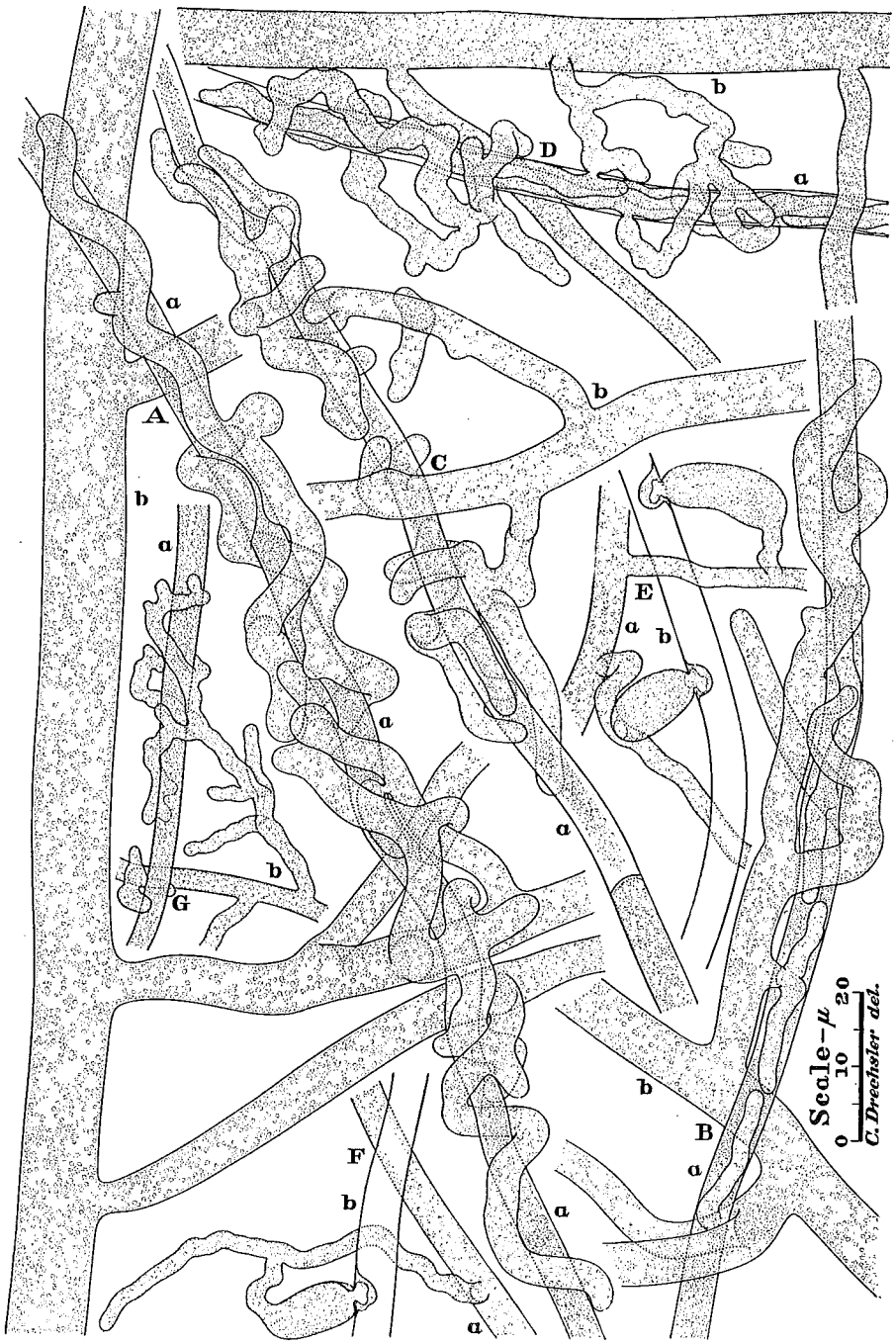

FIG. 16. Drawn from maizemeal-agar plate cultures with the aid of a camera lucida; ×1000 throughout. A, B. Hyphae of *Pythium salpingophorum*, a, attacked by *Aphanomyces cladogamus* (spinach strain), b. C. Hypha of *Pythium vexans*, a, attacked by *Plectospira myriandra*, b. D. Hypha of *Pythium vexans*, a, attacked by *Pythium periplocum*, b. E, F. *Pythium vexans*, a, attacking *Pythium periplocum*, b, by means of appressoria. G. *Pythium vexans*, a, attacked by *Pythium acanthicum*, b.

(*Spinacia oleracea* L.) roots collected near Norfolk, Va., late in November, 1932, and from tomato rootlets collected near Beltsville, Md., in September, 1942, *P. salpingophorum* is known to occur on at least 2 phanerogamic crop plants that have been recorded as hosts of another saprolegniaceous root-rotting species, *A. cladogamus* Drechsl. (15, 16, 19). When the fungus is grown in opposition to *A. cladogamus* on maizemeal agar, its mycelial advance is abruptly halted where it encounters the mycelium of the water mold. Many of its hyphae (Fig. 16, A, a; B, a) in the zone of encounter are soon copiously involved by branches extended from filaments of *A. cladogamus* (Fig. 16, A, b; B, b), their protoplasmic contents promptly showing degenerative changes by taking on a darkish opaque, lumpy appearance. Here and there the *Aphanomyces* branches narrowly penetrate into the *Pythium* filaments and intrude assimilative hyphae to appropriate the degenerating materials (Fig. 16, A, B).

THE MORPHOLOGY AND IDENTITY OF PYTHIUM VEXANS

In 1876 de Bary (2) briefly described under the binomial *Pythium vexans* a fungus which he first found in the month of July in dead exhausted cells of several potato tubers that had sprouted despite infection with *Phytophthora infestans;* the specific epithet being chosen because for two long years the fungus had given trouble with respect, more especially, to its separation from the late blight parasite. Five years later he (4) expanded his earlier account by adding a few more illustrations and by supplying further descriptive details, most of which provided contrast with *Pythium debaryanum.* Spherical monosporous oogonia, in most instances mature, were set forth as having been found attached laterally to delicate branched hyphae that could be traced into intercellular spaces of the spent tuber; the globose organ sometimes being borne on a very short stalk, sometimes being sessile on the parent filament, and sometimes, again, being broadly inserted upon the parent filament as a tangentially intercalary body. The single antheridium usually comprising the male complement—2 antheridia were present only rarely—was described as being mostly of curved clavate shape and as arising from the oogonial hypha in immediate proximity to the oogonium. Considerably smaller size of oogonium and oospore was represented as a feature separating the species from *P. debaryanum;* the diameter of the former structure having been determined from permanent mounts as 15 to 18 μ, that of the latter structure as 12 to 15 μ. The fungus was held distinguished from *P. debaryanum* also by the greater delicateness of its oogonial envelope, by greater size of its oospore in relation to the oogonium, by germination of its oospores after only a brief resting period, and by its inability in repeated trials to infect tissues of the potato plant. It gave further manifestations of saprophytism by growing luxuriantly on dead flies and on dead mites; within the mites it produced oospores, but within the flies it gave rise only to branching mycelial hyphae and to spherical conidia like those of *P. debaryanum.* In one of de Bary's figures (4: Taf. V, fig. 3)

wherein a portion of mycelium is shown, the main axial hypha would seem, from the magnification indicated in the legend, to vary in width from 3.5 to 4 μ, while a secondary branch would seem to have a width of about 1.5 μ. On this narrow branch is shown attached a unit of sexual apparatus that reveals in profile view broad application of an antheridium to a flattened portion of the oogonial wall; the portion of antheridial envelope not adnate to the oogonium presenting a semicircular contour. In a figure of another unit of sexual apparatus (4: Taf. V, fig. 4) the antheridium is shown advantageously in dorsal view as being extensively applied flatwise to the upper aspect of the oogonium; its attachment to a very short stalk arising ostensibly from the oogonial hypha in immediate proximity to the oogonium, and its divaricately bilobate shape, seeming especially worthy of attention.

Butler's account of *Pythium vexans* (10, p. 91–94) consists mainly of a first-hand description of a fungus which he found not uncommon in garden soil in Great Britain and France, and which he considered undoubtedly the same as de Bary's largely because of distinctive peculiarities he recognized in the frequently broad insertion of the oogonium and in the clavate or rounded shape of the relatively large antheridial cell. In *Abutilon*-root cultures the fungus gave rise extramatrically to oospores 20 to 22 μ in diameter within oogonia measuring 22 to 25 μ in diameter; its main measurements, therefore, not only considerably exceeding those originally ascribed to *P. vexans,* but also exceeding, even if only rather slightly, those ascribed to *P. debaryanum* (or to the synonymous *P. Equiseti* Sadebeck) both by de Bary (21 to 24 μ for oogonial diameter and 15 to 18 μ for diameter of oospore) and by Butler (20 to 25 μ for oogonial diameter and 14 to 18 μ for diameter of oospore). The individual oogonium was reported to be supplied with one antheridium, rarely with two; the antheridia usually arising from the oogonial stalk and sometimes being hypogynal. De Bary's account of *P. vexans* makes no mention of hypogynal antheridia, nor of an arrangement of sex organs which Butler found to be fairly common—an arrangement initiated through prolongation of the oogonial branch from below the female cell as a somewhat coiled, plurally diverticulate stalk which then cuts off terminally an antheridial cell that bends around to reach the apex of the oogonium and intrudes there a fertilization tube. Butler found the antheridial cell always closely applied to the oogonial wall so as to fuse with this wall over a large part of its circumference; the two conjugating organs together appearing commonly as a pear-shaped bilocular structure. In his figures of such structures (10: Plate V, fig. 8, a, b; 9) the male component is shown with a dome-like profile rather similar, it must be admitted, to the semicircular antheridial profile drawn by de Bary. While in some of his cultures Butler obtained zoosporangia in addition to conidia—both measuring 17 to 24 μ in diameter—other cultures yielded conidia but no zoosporangia; so that the fuller scope of asexual reproductive development in his fungus was readily reconcilable with de Bary's findings. Some departure from the conidial morphology implied in de Bary's account might not wholly

without reason be read into Butler's statements intimating that the conidia in his material were much more frequently of irregularly pyriform, ovoid, or subangular shape than of more symmetrical subspherical shape, and that their protoplasmic contents were of denser appearance than the contents of conidia in *P. debaryanum.* A vegetative character distinguishing his fungus from any other species known to him was recognized by Butler in the frequent prolongation of secondary and tertiary branches far beyond the primary hyphae, and their attenuation at the ends into very fine filaments. Lateral branches, according to his statement, were given off in a very irregular manner; and the mycelium, in general, was slender, finer than that of *P. debaryanum.*

In 1924 Braun (8) described as a new species, under the binominal *Pythium complectens,* a fungus he had isolated from blackened geranium (*Pelargonium sp.*) stems found in greenhouses at Washington, D. C., which in inoculation experiments caused decay in the stems of geranium cuttings and in *Coleus* cuttings, though it failed to attack either cucumber or watercress seedlings, and in radish (*Raphanus sativus* L.) seedlings caused only superficial black streaks on the stems. Without determining whether his fungus would attack the living potato plant—the only phanerogam de Bary tried out in the experimentation relevant here—Braun distinguished *P. complectens* from *P. vexans* partly on the score of its pathogenicity. He distinguished his fungus in part, again, on the ground that its hyphae, measuring 1.7 to 4.85 μ in width, were cylindrical with rounded tips and did not taper to fine points; although it would seem by no means certain that the very fine filaments of Butler's fungus need necessarily have been sharply pointed at the tip, or that the plant bearing them was actually referable to *P. vexans.* Owing to the uncertain identity of Butler's plant, the contrast that its rare, irregularly shaped sporangia offer to the subspherical or oval sporangia produced abundantly on various agar media by the geranium fungus appears of dubious relevance in separating this fungus from *P. vexans.* Braun assuredly erred in representing de Bary as having observed no sporangia or conidia in *P. vexans;* for, as has been mentioned, de Bary reported development of conidia when his species was grown on flies. Indeed, since the German mycologist likened his conidia to those of *P. debaryanum* they must have been rather similar to the sporangia Braun ascribed to *P. complectens,* which in shape and size—their diameter being stated to vary from 16.4 to 27.3 μ—show moderate resemblance to the conidia of *P. debaryanum* and of *P. ultimum.* According to Braun, the oogonia of the geranium parasite are borne each on a slender stalk, and are not inserted by a broad base into the mycelial tube after the manner of oogonia in *P. vexans.* The antheridium of his fungus, an organ described as "single, one-celled, arising from adjacent hypha or below oogonial stalk, persistent, varying from a trumpet shape flaring out at region of attachment, to a broad irregularly lobed mass clasping a large part of the oogonium and fused with it," Braun recognized as resembling in shape the broadly applied anther-

idium by which, in his view, de Bary characterized *P. vexans*. From his qualifying comment to the effect that a figure of de Bary's (4: Tab. V, fig. 3) shows, nevertheless, one clavate antheridium, it would seem that Braun regarded clavate antheridial shape in *P. vexans* as a feature rather exceptional in the morphology originally ascribed to the species by its author. In reality, however, de Bary set forth the antheridium of *P. vexans* as being usually of curved clavate shape and only seldom of other form. Somewhat curiously, therefore, de Bary's statement of antheridial morphology might have afforded a stronger argument in favor of separating the geranium pathogen from *P. vexans* than was derived from it by Braun.

After the publication of the paper presenting *Pythium complectens* as a new species, Braun kindly gave me a culture of his fungus. By comparison of material grown under like conditions, the fungus was readily seen to be identical with more than a dozen cultures sorted out, mainly because of resemblances in mycelial luster and antheridial morphology, from a numerous collection obtained in 1924 from softened pea roots and blackened sweet-potato rootlets—from the same collection in which, as has been noted, *P. oligandrum* was found so abundantly represented. The species has subsequently been recognized in cultures isolated from affected roots of pansies, tomatoes, peppers (*Capsicum annuum* L.), beans, giant ragweed plants, and pale touch-me-not plants collected in Arlington, Va., in Washington, D. C., and near Beltsville, Md. It has been recognized likewise in several cultures isolated from sugar-beet roots collected near East Lansing, Mich., and near Saginaw, Mich., in June, 1927, as well as in a few cultures among a much larger number isolated from celery (*Apium graveolens* L.) seedlings then collected near Kalamazoo, Mich. Later it was encountered also in a few cultures derived from discolored spinach rootlets gathered in fields near Norfolk, Va., in November, 1932.

In maizemeal-agar plate cultures the fungus produces a mycelium of markedly lustrous radiating appearance attributable here as in allied forms to a rather pronounced degree of parallelism in arrangement of the submerged and prostrate axial hyphae. This type of appearance, to which Braun aptly refers as a "combed silk effect," and which he illustrates rather satisfactorily (8: Plate 1, b), may justly be considered well worth mentioning in descriptive accounts, even though its presence in similar cultures of various congeneric species, including, for example, *Pythium complens* Fischer and *P. acanthicum*, somewhat abates its distinctiveness as a diagnostic character. Aerial mycelium is frequently absent on maizemeal agar in Petri-plate cultures, but usually is produced in moderate quantity on the same substratum in tube cultures. Somewhat more abundant aerial development ensues when a richer medium like Lima-bean agar is employed. In this medium the submerged mycelium reveals at times some cumulous variegation in density of hyphal elements.

After its vegetative growth in maizemeal-agar plate cultures has been concluded, the fungus begins to produce, as a rule, both asexual and sexual

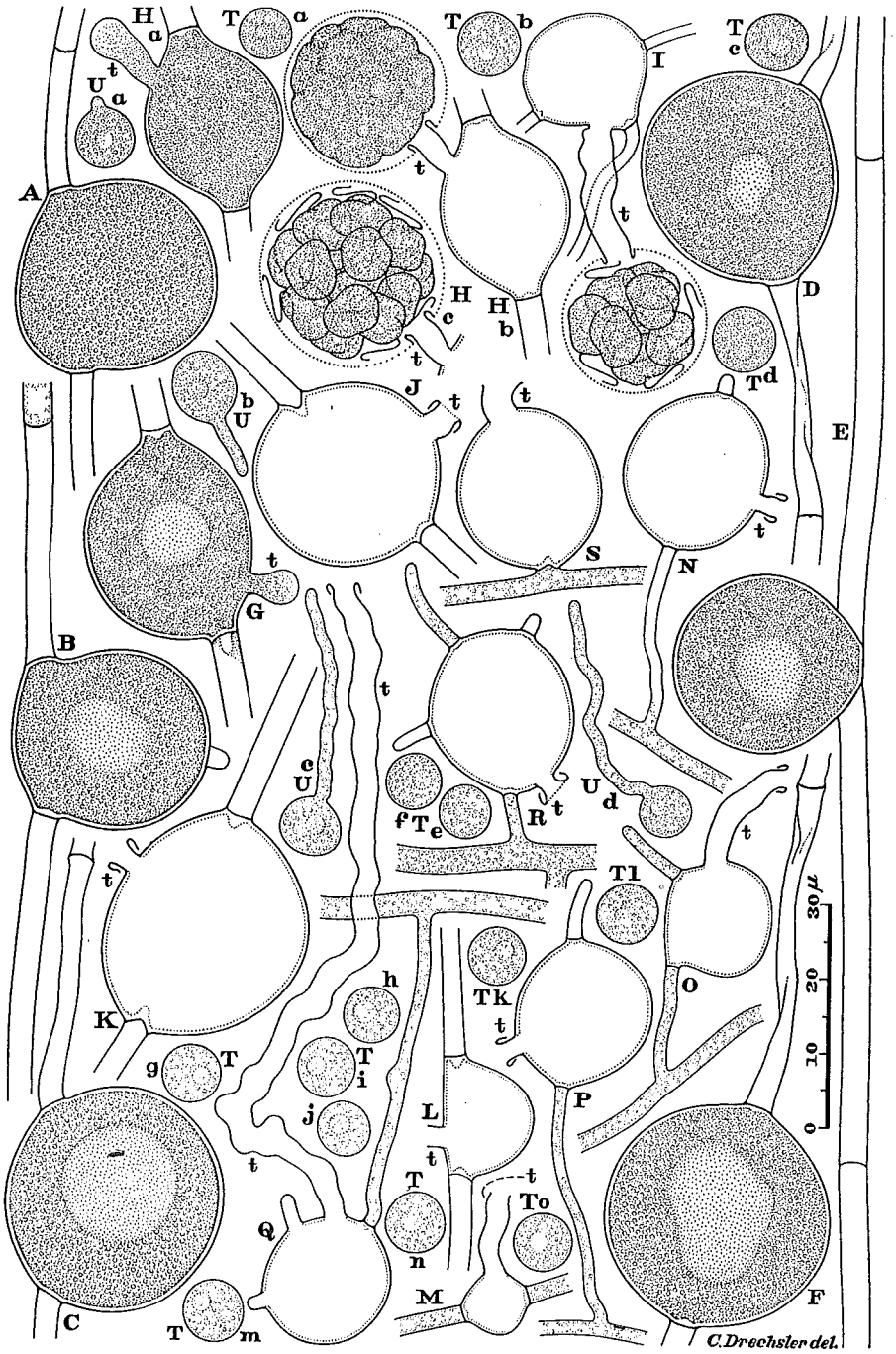

FIG. 17. Asexual reproductive apparatus of *Pythium vexans* drawn with the aid of a camera lucida from a maizemeal-agar plate culture (A–F), from irrigated maizemeal agar (G–K), and from irrigated Lima-bean agar (L–U); ×1000 throughout. A–F. Intercalary sporangia as found in rather soft maizemeal-agar plate cultures 51 days after inoculation. G. Sporangium that was formed in a maizemeal-agar culture, and that extended an evacuation tube promptly on addition of water. H. Sporangium, a, that was formed in a maizemeal-agar plate culture and that promptly extended an evacuation tube on addition of water; b, same sporangium after discharge of granular contents into a vesicle; c, empty, distally reflexed evacuation tube of same sporangium, together with the

reproductive apparatus. Since in such cultures zoospore formation cannot take place owing to lack of free liquid water, asexual reproduction is restricted to development of subspherical or ellipsoidal bodies mostly varying in diameter between 15 and 30 μ. In cultures 15 to 25 days old a substantial proportion of these bodies often measure from 25 to 30 μ in diameter. For the most part specimens of such large size are found in laterally intercalary (Fig. 17, A–E) or mesially intercalary (Fig. 17, F) positions in the stouter main mycelial filaments; thus offering, with respect to size and hyphal relationships, the similarity to conidia of *Pythium debaryanum* and *P. ultimum* that is to be inferred from de Bary's account of *P. vexans*. When the globose bodies are sparingly irrigated with distilled water they usually germinate rather promptly. Sometimes germination takes place almost exclusively by emission of germ tubes that grow directly into branched mycelia; whereas at other times by far the greater number of globose bodies give rise to zoospores. The frequent predominance of the one or the other of the alternative modes of development affords some measure of reconciliation between the findings of de Bary and of Braun relative to asexual reproduction.

Butler's statement that in *Pythium vexans* zoospores are given only when sporangia are sown immediately in fresh water would seem to imply that de Bary's failure to obtain zoospore development could well have been due to the age of the conidia in his material—an implication not necessarily devoid of merit because of the questionable identity of Butler's fungus. Braun reported that in *P. complectens* the asexual reproductive bodies gave rise to zoospores for 10 days after their formation; the proportion of bodies that produced zoospores when placed under circumstances favorable for germination thereafter diminishing with increasing age. My cultures of the species have shown at times even more enduring capacity for zoosporangial development. Thus, although the reproductive bodies shown in figure 17, A–G, which were drawn from a Petri-plate culture prepared with rather soft, slightly moist agar 51 days after planting, mostly showed clear evidence of aging in the presence of a central vacuole (Fig. 17, B–G), all of them and the generality of their very numerous fellows germinated as sporangia on addition of a small quantity of distilled water. In this material, moreover, germination began very promptly. Many of the globose bodies were observed individually putting forth an evacuation tube (Fig. 17, G, t; H a, t) within 10 minutes after the water had been added; exten-

vesicle shortly before the motile zoospores escaped. I. A smaller intercalary sporangium which was formed in a maizemeal-agar plate culture, and which on addition of water promptly extended an evacuation tube and discharged its contents into a vesicle for transformation into 8 zoospores. J, K. Empty envelopes left after irrigation of large intercalary sporangia from a maizemeal-agar plate culture; each envelope bearing a short reflexed evacuation tube. L–S. Empty envelopes of sporangia that were formed and discharged in an irrigated Lima-bean-agar preparation; illustrating intercalary (L, M), subterminal (N, O, P, R), terminal (Q), and lateral (S) positional relationships to supporting hyphae; and showing plain-rimmed (L, M, S) and reflexed (N–R) conditions in empty evacuation tubes, as well as pronounced variation in length of these tubes. T. Encysted zoospores, a–o, showing variations in size and shape. U. Zoospores, a–d, each germinating by production of a germ tube. (t, evacuation tube.)

sion of the tube to its definitive length in the course of the ensuing 15 minutes being followed by abrupt yielding of its hyaline expanded tip and by migration of the sporangial contents into a terminal vesicle (Fig. 17, H b, t) for conversion into motile zoospores (Fig. 17, H c; I). Within 45 minutes after addition of the distilled water zoospores were observed swimming about in easily noticeable numbers; in 3 hours they were swarming abundantly throughout the irrigated preparation. Of the numerous sporangia a large proportion were now represented only by empty envelopes (Fig. 17, J, K), each provided with an empty evacuation tube (Fig. 17, J, t; K, t).

Zoospore development can readily be induced in the species, as in most congeneric forms, by excising from maizemeal-agar or Lima-bean-agar plate cultures thin slabs well permeated with vigorous mycelium and transferring them to a thin layer of water. In such preparations sporangia and motile zoospores make their appearance in moderate numbers after about 24 hours, and with occasional renewal of water will ordinarily continue to be formed in some quantity for several days. Since the conditions necessary for zoospore development are here constantly present, the sporangia fail to attain generally as large a size as in agar cultures devoid of free water; their transverse diameter varying usually from 8 to 23 μ (Fig. 17, L–S). While many are found in intercalary positions (Fig. 17, L, M), others are produced subterminally on relatively slender branches, so that a terminal portion of the branch is borne somewhat like a distal appendage (Fig. 17, N, O, P). Sometimes the distal portion of the supporting branch does not become delimited by a septum, and then will appear as a diverticulum of a terminal sporangium (Fig. 17, Q). In other instances not only the distal portion of the supporting branch but also 1 or 2 short lateral secondary branches are each cut off by a septum, and thus likewise come to be borne on the subterminal sporangium as appendages (Fig. 17, R). Occasionally a sporangium is borne laterally on an axial filament (Fig. 17, S).

In sparingly irrigated material, where the sporangia are not deeply submerged and yet are adequately bathed in a thin layer of water so that the positional relationships to water and air are nearly everywhere favorable for zoospore development, the evacuation tube usually is not extended beyond a length of 15 μ, and sometimes not beyond a length of 10 μ. However, where local conditions are less favorable, it pushes out farther (Fig. 17, I, t; Q, t), occasionally attaining a length of 100 μ (Fig. 17, Q). For the most part it varies in width from 2.5 to 5 μ. It is rather markedly expanded at the tip (Fig. 17, G, t; H, t) without, however, sharing the pronounced apical modification characteristic of the evacuation tube in *Pythium salpingophorum*. After discharge of the sporangium its empty membrane in some instances widens noticeably near the orifice (Fig. 17, S, t). Much more often the membrane becomes reflexed at the tip (Fig. 17, H b, t; I–K: t; N–R: t), though such eversion is absent here and there (Fig. 17, L, t; M, t; S, t).

The vesicle attached to the frequently reversed rim of the evacuation

tube is often, especially in its proximal portion, only faintly discernible. In any case the film yields soon after the swarm of active zoospores formed within it begin their battering, whereas in most congeneric species the clearly visible bladder commonly resists the collective impact of the fully fledged swarm for a period of 5 to 8 minutes. Owing in large part to their less prolonged impoundage the zoospores of *Pythium vexans* are often liberated in 13 to 15 minutes after discharge of the sporangium, rather than after the more usual period of approximately 20 minutes. They swim about for some time, then come to rest and round up into subspherical cysts commonly 6.8 to 9 μ in diameter (Fig. 17, T, a–o). They germinate, as a rule, by putting forth a germ tube approximately 1.5 μ wide (Fig. 17, U, a–d).

Besides producing sporangia and zoospores, tracts of young mycelium in slabs excised from maizemeal or Lima-bean-agar plate cultures conveniently give rise, on irrigation, to sexual reproductive apparatus in moderate quantity; the softened substratum allowing patently normal development and still retaining enough firmness to hold all imbedded apparatus securely in place for close microscopical examination. As Braun pointed out, pairing of the sex elements takes place at a very early stage. Indeed, even when the young oogonium consists only of a terminal (Fig. 18, A, a) or subterminal (Fig. 18, B, a) enlargement no more than 6 or 7 μ in width, which by itself would not yet be clearly distinguishable from miscellaneous enlargements of vegetative character, it is often found rather extensively in contact with, or extensively enwrapped by a young male complement constituted of a swollen hyphal termination (Fig. 18, B, b) or of such a termination together with a similarly swollen lateral branch (Fig. 18, A, b). Frequently the mycelial connection between oogonial stalk (Fig. 18, A–I: a) and antheridial branch (Fig. 18, A–I: b) is too remote to be traced with certainty amid the confusion of ramifying hyphae. With about equal frequency, however, a connection between the paired elements is plainly evident (Fig. 18, J–O). Sometimes the oogonial stalk (Fig. 18, J, a; K, a) arises from the same hypha as the antheridial branch (Fig. 18, J, b; K, b); sometimes it (Fig. 18, L, a) originates as a secondary ramification from the hypha directly bearing the antheridial stalk (Fig. 18, L, b); or, again, it (Fig. 18, M, a; N, a) provides the very familiar androgynous arrangement of parts in giving rise at a variable distance from the growing oogonium to an antheridial branch (Fig. 18, M, b; N, b) which to reach the place of union follows an arcuate course often considerably rangier (Fig. 18, N, b) than the course of the antheridial branch in monoclinous sexual apparatus in *Pythium debaryanum*. Remoteness with respect to mycelial connection and proximity with respect to position are combined ingeniously in instances where an axial hypha gives off, on the same side, 2 branches (Fig. 18, O, a, b) of which one (Fig. 18, O, a) bears plural oogonia (Fig. 18, O, w, x) that are supplied with antheridia borne on ramifications (Fig. 18, O, y, z) arising from the other (Fig. 18, O, b). In units of sexual apparatus developed directly in maizemeal-agar plate cultures prepared with a medium softer

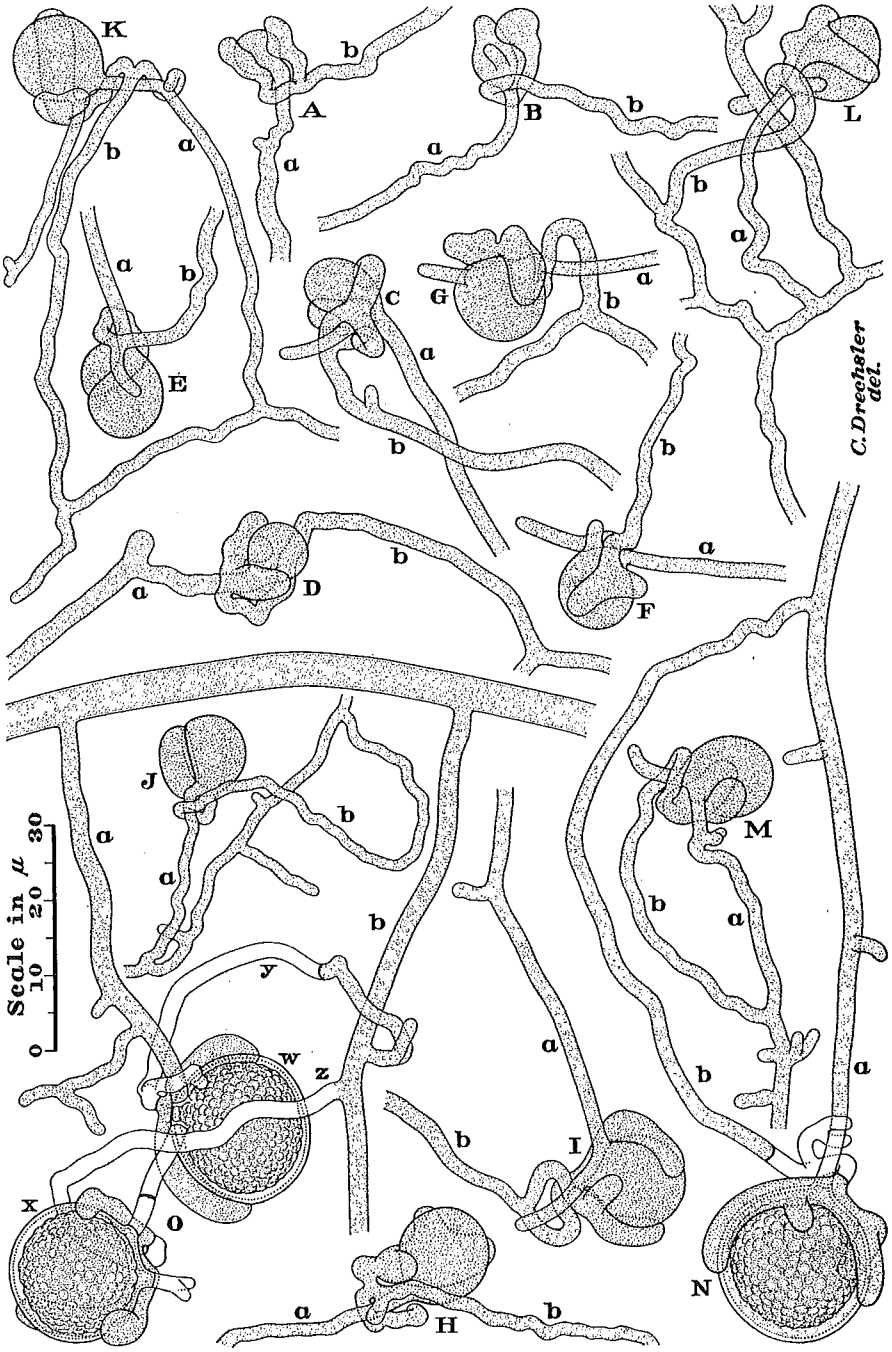

FIG. 18. Immature units of sexual reproductive apparatus of *Pythium vexans* as found produced in soft, irrigated maizemeal agar (A–M) and in irrigated Lima-bean agar (N, O); drawn to a uniform magnification with the aid of a camera lucida; ×1000 throughout. A–I. Young units without evident mycelial connection between the paired organs. J–M. Young units each showing moderately close mycelial connection between the apposed organs. N, O. Units about ready for fertilization, each showing a somewhat remote mycelial connection between oogonium and antheridium. (a, hypha supporting oogonium; b, hypha supporting antheridium.)

than the media commonly employed, the mycelial relationships of the conjugating parts differ little from the relationships manifest in irrigated agar slabs. Where a mycelial connection can be made out, the 2 filamentous elements supporting the paired organs here likewise are often contributed by the same hypha (Fig. 19, A, a; Fig. 20, A); or the antheridial branch may arise from the oogonial stalk (Fig. 19, A, b; B–D; Fig. 20, B, C); or secondary and possible tertiary branching may be present in one if not in both of the supporting filamentous elements (Fig. 19, E; Fig. 20, D, E). And, naturally, in soft agar substratum much as in irrigated agar slabs, numerous units of sexual apparatus show no demonstrable mycelial connection (Fig. 19, F–I; Fig. 20, F, G).

As in related species the oogonium develops into a subspherical body. It is often found attached more or less mesially to the end of a hyphal stalk which may be somewhat narrow not only during the earlier formative stages (Fig. 18, A, a; E, a; H, a; J, a) but also during later stages (Fig. 18, N, a; Fig. 19, C, D, F, H; Fig. 20, D), or, again, may be moderately stout (Fig. 18, D, a; Fig. 19, B; Fig. 20, A). A more distinctive hyphal relationship frequently results when the oogonium grows out laterally a short though somewhat variable distance below the tip of the supporting filament (Fig. 18, B, a; C, a; F, a; G, a; I, a; K, a; L, a; M, a), so that a terminal portion of filament, usually about 10 μ long but occasionally measuring less than 5 μ (Fig. 18, B, a; F, a; L, a) or more than 25 μ (Fig. 18, K, a) in length, is borne on the young oogonium after the manner of an appendage. Owing to the circumstance that in soft, yielding substratum the lateral growth of the oogonium often pushes the distal element out of its earlier alignment into a position approximately at a right angle with the supporting element, the origin of the appendage as a termination of the supporting stalk is frequently obscured. When the female organ later comes to be delimited, the distal element is commonly cut off by a cross-wall (Fig. 18, O, x; Fig. 19, A, a, b; I). However, where the distal element is very short, it often remains as a spur-like diverticulum (Fig. 20, D) continuous with the oogonium; this organ thereby being left in a terminal position with a hyphal attachment similar to that of some oogonia having a subspherical shape devoid of marked modification (Fig. 20, G). Sometimes an oogonium develops in laterally intercalary position some distance from the tip of its supporting filament (Fig. 18, O, w). Frequently too, where the oogonium grows out laterally from a base so narrow that the spherical contour does not encroach on the supporting hypha, it becomes delimited as a sessile structure, or is borne terminally on a very short lateral branch (Fig. 19, E, G; Fig. 20, B, C, E, F).

The oogonia of the fungus thus show not only the several relationships to the mycelium that were expressly ascribed by de Bary to the oogonia of *Pythium vexans,* but also the one relationship—attachment to the tip of a slender hypha—which Braun set forth as prevailing in *P. complectens.* Indeed the latter relationship is not wholly unrecorded in de Bary's treat-

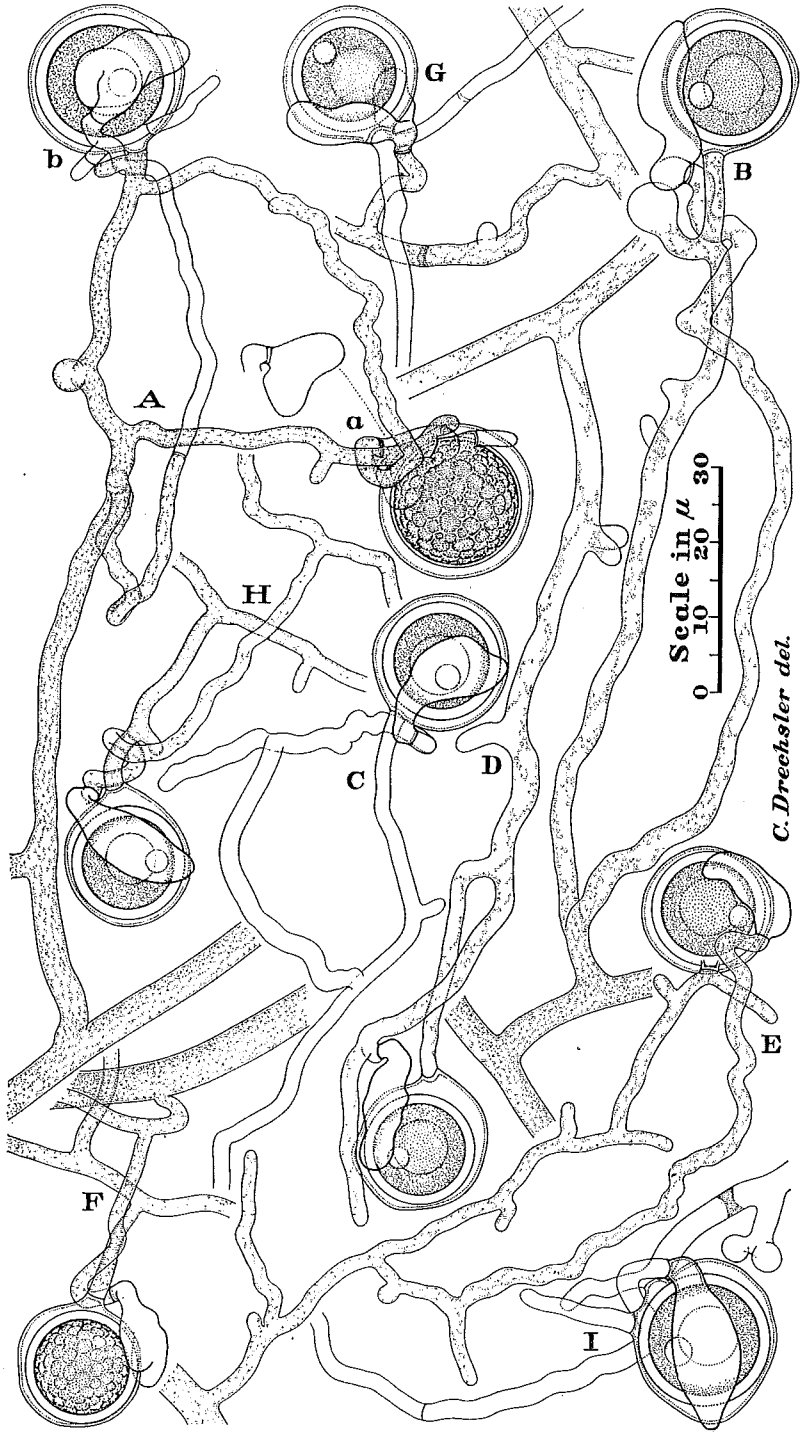

FIG. 19. Sexual reproductive apparatus of *Pythium vexans* drawn with the aid of the camera lucida from Petri-plate cultures prepared from maizemeal agar of only moderate firmness; ×1000 throughout. A. Two reproductive units—one (a) with immature oospore, the other (b) with mature oospore—whereof the apposed sex organs are supplied from a single parent filament. B–E. Units of mature apparatus varying with respect to aggregate length of hyphal parts connecting oogonium with antheridium. F.

ment of *P. vexans;* for though not mentioned in his descriptive text, terminal attachment would seem to be represented in one of his figures (4: Taf. V, fig. 4). In my material the supporting hypha has shown no noteworthy tendency to widen markedly below the attachment of a terminally borne oogonium. Such widening appears to have been considered by Braun a feature that de Bary held characteristic of *P. vexans.* Marked hyphal widening immediately below the unipolar attachment of an oogonium borne mesially in alignment with its supporting filament, was figured by Butler as illustrative of the broad insertion of oogonium he deemed especially distinctive of the species. Although de Bary's words describing the oogonium as "theils selbst mit breiter Ansatzstelle eingeschaltet in die Continuität des Schlauches, also, mit andern Worten, intercalar aber einseitig blasig vorgewölbt," unquestionably make reference to broad attachment, the breadth here in question relates to the basal dimension of a laterally intercalary oogonium, and consequently is to be measured lengthwise along, not transversely across, the supporting hypha.

The enwrapment of the young oogonium by the young antheridium, which, as has been mentioned, is observable in irrigated agar preparations at a very early stage, is shared in varying measure by the distal portions of the hyphae bearing the developing sex organs. Often the antheridial branch (Fig. 18, A, b; B, b; E, b; F, b; G, b; L, b) winds half way around the oogonial stalk before extending its widened termination along or about the young oogonium. In the case of subterminal, laterally intercalary oogonia, the distal prolongation rather than the proximal supporting element is often enwrapped by, or interlocked with, the antheridial branch (Fig. 18, C, b; F, b; M, b). Sometimes, again, the antheridial branch passes partly around the young oogonium before it engages with the oogonial stalk and extends its expanded termination over other regions of the globose body (Fig. 18, D, b). The close contact of oogonium and antheridium is maintained as both organs continue growing. While the oogonium merely rounds out into a more nearly spherical shape as it increases in size, the antheridium usually elongates considerably and at the same time often ramifies more or less (Fig. 18, C, b; D, b; F, b; G, b; H, b; I, b; K, b; L, b; M, b). Eventually when the oogonium has attained its full growth, and its readiness for fertilization is made manifest by shrinkage of its lumpy contents from the enveloping wall (Fig. 18, N; O, w, x), it may be found embraced by an antheridium consisting of 2 (Fig. 18, O, w), 3 (Fig. 18, O, x), or 4 (Fig. 18, N) curving finger-like branches measuring individually 5 to 20 μ in length and 2 to 4 μ in width.

Branching of the antheridium is both less frequent and less elaborate in Petri-plate cultures prepared from maizemeal agar somewhat firmer than irrigated agar slabs yet not so firm as most gelose media employed in laboratories. Usually in such cultures antheridial branching is represented only

Somewhat immature unit of sexual apparatus without visible mycelial connection between apposed organs. G–I. Mature units of sexual apparatus, each contributed by a pair of hyphal elements without evident mycelial connection.

in simple dichotomy of the ypsiliform (Fig. 19, A, a), bilobate (Fig. 19, F), and biramous (Fig. 19, G) male organs that are observable in moderate numbers. Rather commonly the unbranched male cells here are of elongate saccate shape, perceptibly widened at the middle (Fig. 19, C, D, E, H, I; Fig. 20, B–F). They mostly vary in length from 15 to 30 μ and in width from 5 to 8 μ; their thickness often being substantially less than their width. Many are applied their entire length to the oogonium; some of the longer ones thus enfolding more than one-third of the circumference of the globose body (Fig. 19, H, I; Fig. 20, B). Contrasting with the numerous antheridia that by their longitudinal application from base to apex recall the homologous organs of my *Pythium helicoides* (20, p. 412–414) and others which because of their application only along their distal half (Fig. 19, D, F; Fig. 20, F) invite comparison rather with the anteriorly applied antheridia occasionally to be observed in my *P. palingenes* (23, p. 491, Fig. 8, C). Somewhat as in irrigated preparations, the antheridial branch in softish agar cultures is often found wound about the oogonial stalk to the extent, as was noted earlier (18, p. 444), of a half turn or whole turn (Fig. 19, A, a, b; B; C; F; H; Fig. 20, B). Even where such involvement is absent, contact of the paired organs or, more especially, contact of their proximal parts, is often accompanied by some more haphazard sort of engagement between the supporting hyphal elements (Fig. 19, G, I; Fig. 20, C, D, E, G); although in other instances no interlocking of the supporting hyphae is evident (Fig. 19, D, E; Fig. 20, A, F).

In irrigated preparations the extensive enwrapment of the oogonium by the antheridium has apparently very little direct effect in modifying the outward form of either organ. Nor is conspicuous modification of shape evident in most units of sexual apparatus produced in rather soft maizemeal agar; though here an occasional oogonium may usually be found of which the envelope is broadly indented or flattened in the region of contact with the antheridium (Fig. 19, B; Fig. 20, C), so that it is brought snugly against the oospore wall in the region underlying the antheridium and usually also in the antipodal region. In Petri-plate cultures prepared with maizemeal agar of customary firmness, such flattening of the oogonium appears as a virtually constant character, and is commonly associated with malformation of the antheridium often so pronounced that this organ offers an appearance unknown among congeneric species. The scope of deformity displayed in cultures prepared with hard agar is by no means exaggerated in the assortment of misshapen male cells illustrated in Braun's drawings (8: Plate 5, B–D). While conveniently helpful in identifying the species, the bizarre conformation of such antheridia would seem perhaps more nearly a teratological feature than a character pertaining to normal morphology. Owing to the very early apposition of the male and female elements the antheridium in a somewhat unyielding ambient is necessarily subjected throughout the period of its growth to persistent pressure arising especially from the simultaneous expansion of the more massive oogonium; and thus

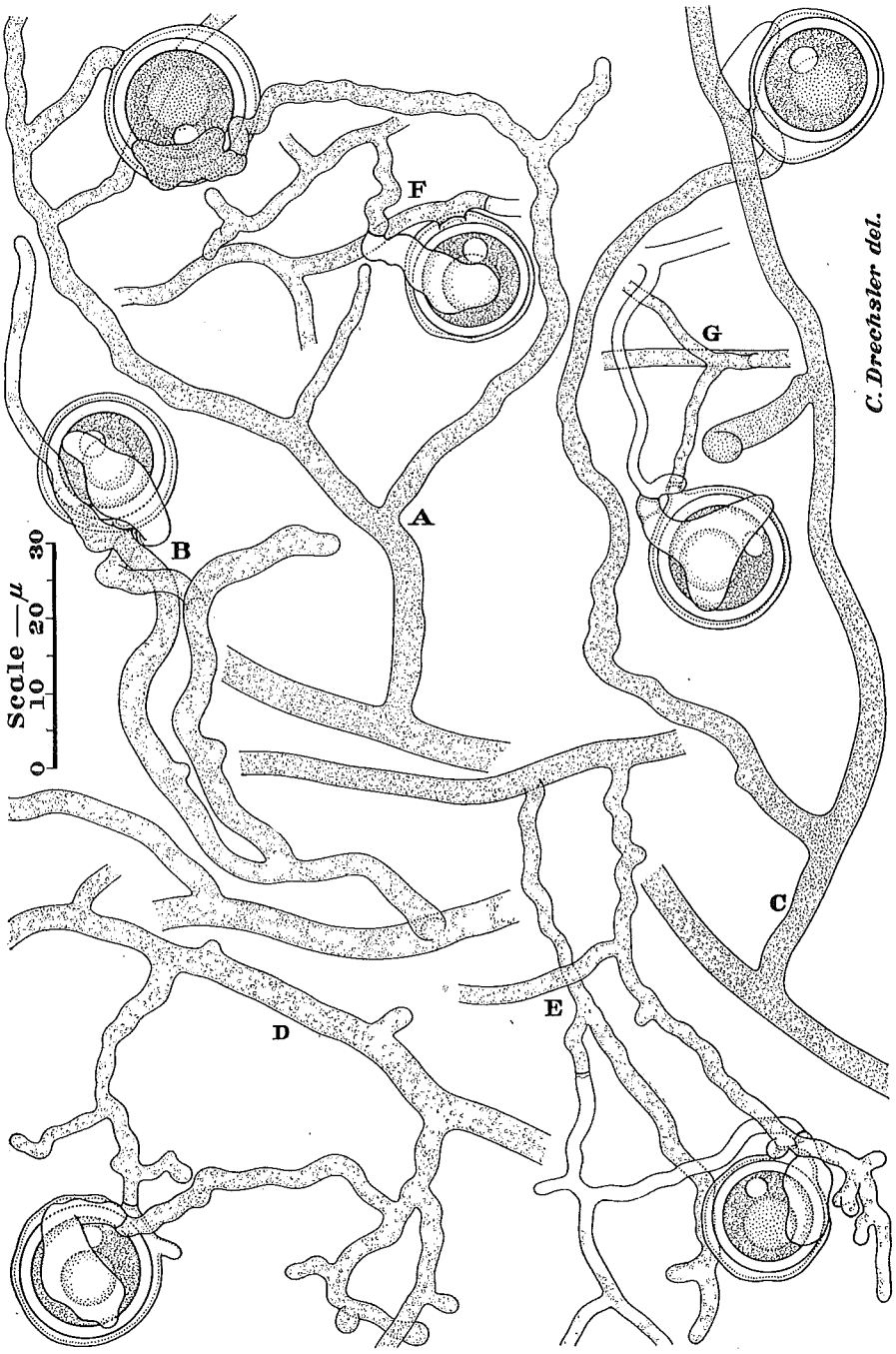

FIG. 20. Mature sexual reproductive apparatus of *Pythium vexans* drawn with the aid of a camera lucida from Petri-plate cultures prepared from maizemeal agar of only moderate firmness; ×1000 throughout. A–E. Units of apparatus with obvious mycelial connection between oogonium and antheridium; the antheridium in A, of deep yellow coloration, being applied distally to the oogonium; the antheridium in B–E being empty and colorless and in a deceptive manner having its basal attachment spatially near the base of the oogonium. F, G. Units of sexual apparatus without evident mycelial connection; the empty antheridium in either unit has its base some little distance from the base of the oogonium, but in G a short spur of the supporting branch helps to give somewhat the appearance of close monoclinous relationship.

is squeezed or constrained to grow into a flattened shape fitting the flattened or indented shape of the oogonium. In expanding jointly against the physical resistance of the ambient, the general tendency is for the 2 apposed organs to assume shapes such that the combined unit of sexual apparatus will have a more or less subspherical compact form. As might be expected, the proximal portion of the antheridium is in many instances less affected by this tendency than the median and distal portions.

Although the bilobate ypsiliform antheridium figured by de Bary was mentioned as illustrating a male cell of unusual outward form, it yet provides the most important clue whereby Braun's geranium pathogen can be referred to *Pythium vexans*. Among the members of the genus whose oogonia and oospores approach at all closely the measurements for diameter given by de Bary, I have found such bilobate antheridia only in the particular species here under consideration. Bilobate branching has been noted occasionally in *P. helicoides* and in *P. palingenes* (23, p. 491, Fig. 8, F; p. 492) where the elongated antheridium is similarly applied lengthwise in its extensive enwrapment of the oogonium; but these 2 species, as also the allied *P. oedochilum* Drechsl. (23, p. 478–486), have oogonia and oospores conspicuously larger, not smaller, than those of *P. debaryanum* and *P. ultimum*. The antheridium of semicircular profile shown in one of de Bary's figures (4: Taf. V, Fig. 3) is aptly illustrative of the usual appearance presented by the elongate antheridium of Braun's fungus when it is applied in an equatorial region of the oogonium with its long axis oriented vertically or nearly vertically, that is, in a direction nearly parallel to the line of vision. The very close androgynous relationship figured by de Bary in 2 instances (4: Taf. V, Fig. 3, lower right; Fig. 4), wherein the antheridium is borne on a very short stalk arising from the oogonial hypha in immediate proximity to the oogonium, has not been recognized with certainty in my material. Quite frequently in mature reproductive apparatus the antheridium, because of the position of its basal septum, offered much the appearance of arising in such close monoclinous relationship; but in these instances wherever on careful scrutiny the hyphal connections could be accurately ascertained the male cell was found borne on a separate branch (Fig. 19, A, a, b; B; C; F; H; I; Fig. 20, B–E). Neither has an immediate androgynous relationship been revealed unmistakably in young sexual apparatus (Fig. 18, A–M) where all parts are filled with living protoplasm and thus are most favorable for observation. Later, when the supporting hyphae have been evacuated, their thin, highly transparent membranous envelopes often become so faintly visible as to tax the capabilities of a good modern microscope with good illumination. Since, further, the tubular membranes near the base of the oogonium are often more or less intertwined or interlocked, optical difficulties intrude that would seem well beyond the capacities of the microscopes in use 65 years ago. In fine, regardless of whether the very close monoclinous relationship figured by de Bary is absent in Braun's fungus, or whether it is perhaps occasionally present there, the

very frequent and persuasive simulation of such relationship in my cultures appears under the circumstances to provide sufficient resemblance for identifying the fungus with *P. vexans.*

The passageway through which the antheridial contents migrate into the oogonium is generally even more difficult to see than the hyphae supporting the sex organs. In my material it has been most clearly discernible when observed in profile view in ripened units of sexual apparatus wherein at least locally the oogonial envelope lay in contact with the oospore; the fertilization canal then appearing merely as an aperture, 1.5 to 1.9 μ wide, in the oogonial envelope (Fig. 21, A, B). At maturity, the oospore, commonly 12.5 to 16 μ in diameter and somewhat loosely contained in an oogonial envelope 16 to 21.5 μ in diameter, reveals the unitary organization frequent among members of the genus; its wall, mostly 1 to 1.5 μ thick, surrounding a finely granular layer of protoplasm which encloses a single reserve globule, usually 6 to 9 μ wide, as well as a single globose or slightly flattened refringent body ordinarily 2.6 to 4 μ in diameter (Fig. 19, A, b; B–E; G–I; Fig. 20, A–G). In maizemeal-agar cultures 110 days old fully 9 out of 10 oospores showed no change with respect to internal structure, though some few specimens now revealed 2 refringent bodies. Despite their inert behavior in a stale ambient, the oospores, when transferred to a shallow layer of distilled water, germinated readily by production of zoospores. De Bary recognized the capacity of newly ripened oospores to germinate by the production of swarmers as a characteristic attribute of *Pythium vexans;* and in my material likewise no extended resting period has been required for such development. Like the zoosporangia of mycelial origin among which they developed, oospores taken from maizemeal-agar cultures 40 days old produced swarm spores freely. Fairly abundant development of zoospores ensued also after irrigating oospores removed when the cultures were 110 days old, and again when they were 150 days old.

During the earlier stages of germinative development in an oospore (Fig. 21, A, C–E) the reserve globule changes from a spherical to a somewhat irregular shape, while at the same time the refringent body undergoes division into 4, 5, or 6 bodies appreciably smaller than their parent. Gradually the plural refringent bodies become less clearly recognizable, and before long are lost to view in their granular matrix. The reserve globule also loses some of its distinctness without, however, vanishing from sight. The inner layer of the oospore wall, which as a rule equals or slightly exceeds the outer layer in thickness, takes on more and more the appearance of the protoplasm bordering it, and finally merges indistinguishably with the granular mass. The persistent outer layer then dissolves in a circular area 2.5 to 5 μ wide, permitting the protoplast to protrude against the oogonial envelope (Fig. 21, F). This envelope likewise gives way, and the protrusion emerges externally as a germ tube, except that in occasional instances an overlying antheridium interposes an additional membranous barrier (Fig. 21, G). Often before it has attained a length of 25 μ, the germ tube forms a cap of dehis-

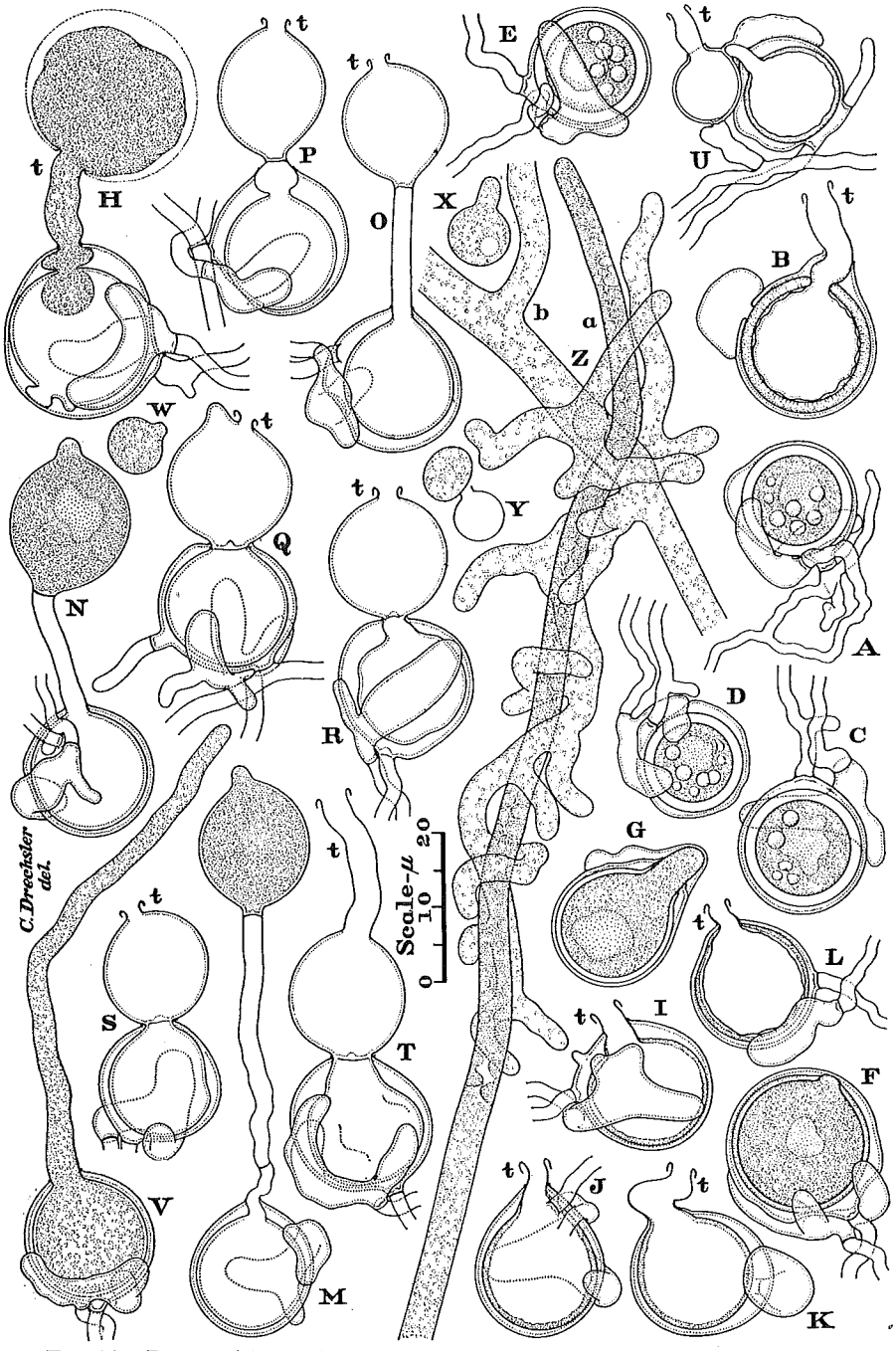

FIG. 21. Drawn with the aid of a camera lucida; ×1000 throughout. A–Y. Germination of oospores of *Pythium vexans* from maizemeal-agar plate cultures 5 months old. A. Oospore showing modification in internal organization preliminary to germination; fertilization passage visible in profile view about 2 μ to left of septum delimiting the antheridium. B. Oospore that germinated without reducing the thickness of its wall; a structurally distinct sporangium within the oospore chamber has left its empty envelope continuous with the evacuation tube; showing also the fertilization passage in profile near the middle of the region covered by the antheridium. C–E. Oospores revealing onset of germinative development in irregular outline of reserve globule and in presence of several refringent bodies. F. Oospore whose protoplast has assimilated a thick inner

cence, which yields to permit migration of the protoplasmic materials (Fig. 21, H) into a terminal vesicle where they are fashioned into zoospores. After the zoospores have been liberated the rim of the empty evacuation tube is usually found reflexed (Fig. 21, B, t; I–L: t) much as in sporangia of mycelial origin. Usually when the evacuation tube is followed backward it is found to be continuous with the persistent outer layer of the oospore wall (Fig. 21, I–K). Now and then, however, it appears to be continuous instead with a separate sporangial envelope either nested within the frequently somewhat irregular contour of the yellowish residual layer (Fig. 21, L) or, in the occasional instances where the inner layer has not been digested, nested within the undiminished oospore wall (Fig. 21, B).

Apart from the type of oospore germination wherein the rather broad germ hypha functions directly as an evacuation tube—a type already ascribed to *Pythium vexans* by de Bary—germination in my irrigated preparations has often taken place by the emission from the individual oospore of a somewhat narrower germ hypha that fulfills its function by bearing at its tip a sporangium into which the entire protoplasmic contents are received (Fig. 21, M, N). This sporangium not uncommonly is of citriform shape, being often provided at the apex with a short protuberance or beak rather suggestive of the prominent papilla familiar especially in certain species of *Phytophthora,* as, for example, *P. cactorum* (Lebert & Cohn) Schroeter. The beak represents an incipient evacuation tube, which frequently, after some slight elongation, yields at the apex to permit the granular contents to migrate into a terminal vesicle for transformation into zoospores; the rim of the empty tubular membrane becoming reflexed (Fig. 21, O, t) in the manner usual for the species. The length of the sporangiferous germ hypha varies usually from 10 to 50 μ (Fig. 21, M–O), yet sometimes it exceeds 100 μ. In many instances, however, similar germinative development takes place without any germ hypha being extended at all; the sporangium (Fig. 21, P–U) here being formed sessile on the oogonium in such wise that after evacuation its membrane is found directly continuous with the residual outer layer of the oospore wall nested within the oogonial envelope, though its basal septum, which usually is found in approximate alignment with the spherical oogonial contour, separates its empty chamber

layer of the wall and has pushed a protrusion through an opening in the outer layer against the oogonial envelope. G. Oospore whose germ tube has broken through the oogonial envelope and is pushing against the farther wall of the overlying antheridium. H. Granular contents of oospore migrating into a vesicle through an evacuation tube conspicuously widened in the space between the oospore wall and the oogonial envelope. I–L. Empty membranous envelopes left behind after escape of swarm spores brought into being following direct conversion of oospore into a zoosporangium. M, N. Oospores, each of which has germinated by producing a sporangium at the tip of a germ hypha. O. Oospore that produced a sporangium on a germ hypha; the sporangium later becoming evacuated in giving rise to zoospores. P–T. Oospores, each of which produced a sporangium sessile on the oogonial envelope; the sporangium then becoming evacuated in giving rise to zoospores. U. Oospore that gave rise within the empty overlying antheridium to a sporangium which subsequently became evacuated in producing swarm spores. V. Oospore with germ hypha apparently of vegetative character. W–Y. Zoospores illustrating stages in the emergence of a secondary motile swarmer. Z. Hypha of *P. vexans,* a, attacked by branches of *Aphanomyces cladogamus* (spinach strain), b. (t, evacuation tube.)

from the equally empty chamber of the oospore. When fully grown such sessile sporangia, like those borne on germ hyphae, are often provided individually with a distal beak, which, again, usually on meager elongation, functions in conveying the protoplasmic contents into a terminal vesicle, and thereafter appears as a short membranous tubulure with reflexed rim (Fig. 21, P, t; R, t; S, t). Sometimes an empty reflexed evacuation tube arising from the distal end of a sporangial envelope measures more than 20 μ in length (Fig. 21, T, t); wherefore it is evident that the apical beak may elongate rather considerably before serving in discharge of the sporangium. On the other hand, the beak occasionally undergoes no elongation and takes no part in dehiscence; discharge then being effected by means of an evacuation tube having a separate origin (Fig. 21, Q, t). Now and then the germ hypha, after having forced its way through the oogonial envelope or grown through the fertilization canal, enters the empty chamber of the antheridium to produce there a terminal sporangium which consequently has to thrust its evacuation tube (Fig. 21, U, t) through the antheridial membrane to form a vesicle outside. Extension of a germ tube beyond a length of 50 μ or 75 μ (Fig. 21, V) often betokens the beginning of mycelial growth and incapacity for immediate development of zoospores. The swarm spores produced in the germination of oospores agree morphologically with those produced from sporangia of mycelial origin; and it seems wholly fortuitous that protrusion of a papilla by encysted zoospores (Fig. 21, W, X) preliminary to emergence of the protoplast (Fig. 21, Y) in the repetitional development of a second swimming generation, has so far come under my observation only in some irrigated preparations containing swarm spores that originated exclusively from oospores.

The frequency of an apiculate shape among sporangia produced from oospores would seem related to their habitually terminal development either on the tip of a germ hypha or directly on the oogonial envelope. Sporangia of similar conformation are found borne terminally also in maizemeal-agar cultures and irrigated preparations but there invite little attention, being often greatly outnumbered by sporangia or conidia of generally subspherical shape that occur in intercalary or subterminal positional relationships. The apiculate sporangia of the fungus contribute to the parallelism with *Pythium helicoides* and *P. palingenes* shown more especially in its frequently elongated clasping antheridia. This parallelism is sustained further in the tendency of the sexual apparatus to take on a peculiar deep yellow coloration distinguishable from the yellowish coloration widely prevalent among species of *Pythium,* not only by its greater intensity but also by its different distribution. For while in *P. vexans,* as in nearly all congeneric forms, the ordinary yellowish coloration is concentrated mainly in the oospore wall, the more unusual coloration most often pervades the interior of the antheridium, giving this organ an appearance as if it were filled with yellow, translucent, homogeneous or faintly granular material (Fig. 20, A), though the presence of a mature oospore of correct internal organization

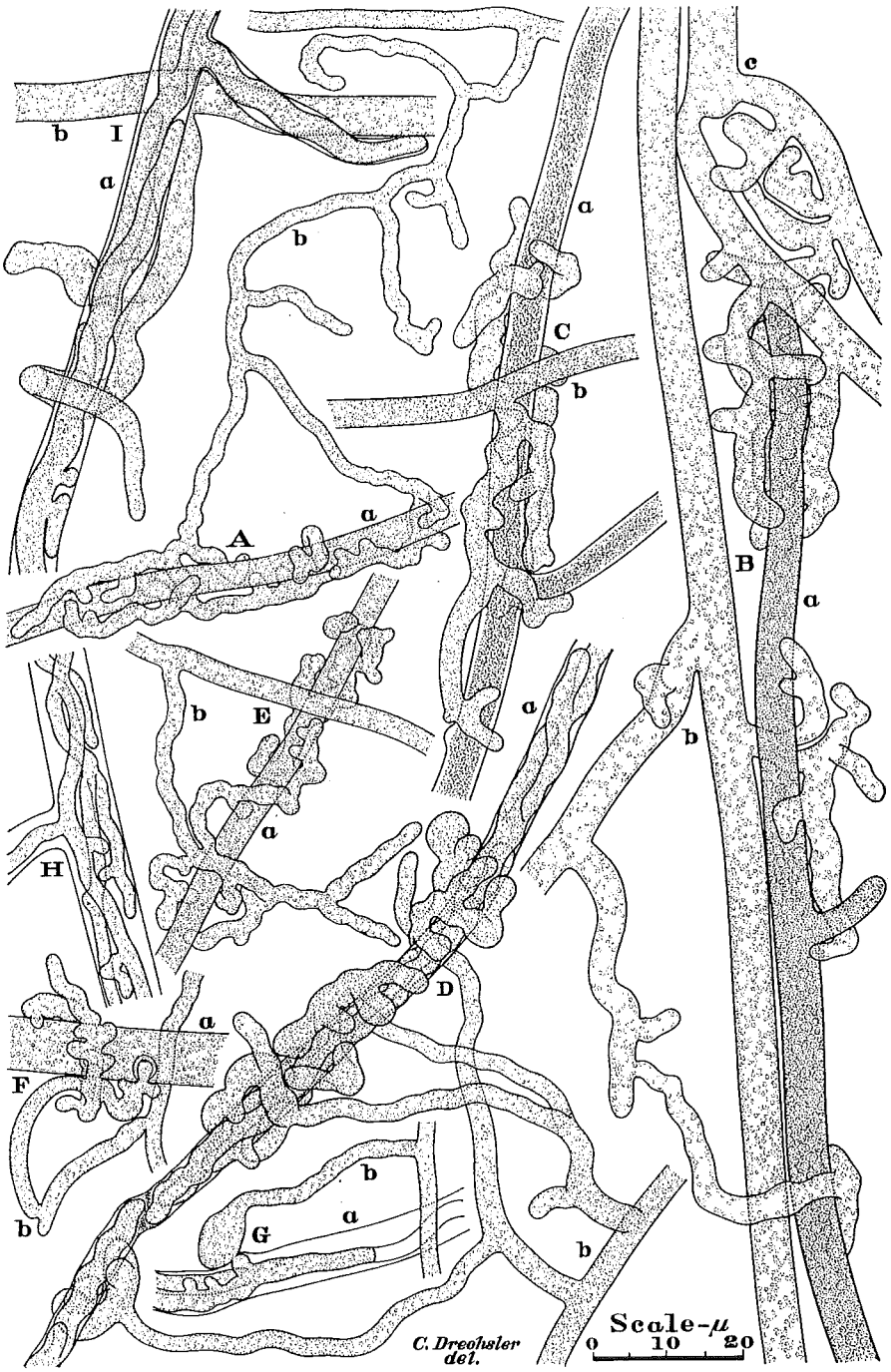

FIG. 22. Antagonistic and parasitic relationships; drawn with the aid of a camera lucida; ×1000 throughout. A. Hypha of *Pythium vexans,* a, attacked by ramifications of *Pythium acanthicum,* b. B. Hypha of *Pythium undulatum* Petersen *sensu* Dissmann, a, attacked by ramifications from filaments of *Plectospira myriandra,* b and c. C, D. Hyphae of *Pythium undulatum* Petersen *sensu* Dissmann, a, attacked by ramifications of *Pythium oligandrum,* b. E–H. Hyphae of *Pythium undulatum* Petersen *sensu* Dissmann, a, attacked by *Pythium acanthicum,* b. I. Hypha of *Pythium undulatum* Petersen *sensu* Dissmann, a, attacked by *Pythium periplocum,* b.

may give ample proof of effective fertilization. Often the same coloration likewise permeates thoroughly the space between the oogonial envelope and the oospore. In *P. vexans,* as in *P. helicoides* and *P. palingenes,* such coloration, if of moderate intensity, is not usually concomitant with perceptible abnormality of structure; yet when widespread degeneration of sexual apparatus occurs in these 3 species, it is often accompanied by intense coloration. Whatever the nature of the coloration may be, its development in *P. vexans,* together with resemblances in antheridia and sporangia, suggests that the fungus may perhaps be somewhat more closely related to the *helicoides* series than most of the numerous congeneric forms similarly having nonproliferous sporangia and oospores of unitary internal organization.

The species, as was noted, has been isolated from discolored tomato rootlets, and thus is known to occur on the same host as the saprolegniaceous form I have described as *Plectospira myriandra* (15). When it is grown in maizemeal-agar plate cultures in opposition to that water mold its advance is halted abruptly at the line of encounter, its individual hyphae (Fig. 16, C, a) being made to degenerate internally soon after they have become elaborately invested by short branches extended from the main filaments of the opponent mycelium (Fig. 16, C, b). Likewise when it is grown in opposition to the saprolegniaceous root-rot fungus *Aphanomyces cladogamus,* with which it shares common host relationships through its known occurrence in roots of tomatoes, spinach, and pansies, its mycelial advance is abruptly halted at the line of encounter, and its hyphae at the forefront (Fig. 21, Z, a) suffer visible degeneration promptly after they have been enwrapped by elaborately ramifying branches from filaments of the water mold (Fig. 21, Z, b). Growing in the presence of *Pythium periplocum* the species often shows markedly varied behavior in different portions of the same Petri-plate culture. In some regions many of its hyphae (Fig. 16, D, a) may become extensively if somewhat loosely invested by irregular branches arising from the filaments (Fig. 16, D, b) of the spiny form, and then suffer invasion by assimilative elements intruded into them. In other regions its hyphae not only remain wholly unharmed but are found bearing rather massive appressoria (Fig. 16, E, a; F, a), each of them affixed apically to a *periplocum* filament (Fig. 16, E, b; F, b); a short, frequently lobate protrusion which extends from the tip of the appressorium into a thick deposit of golden yellow substance within the filament indicating that invasion was stopped by secretion of a defensive barrier. When the species is grown in Petri-plate cultures in opposition to *Pythium acanthicum,* many of its hyphae (Fig. 16, G, a; Fig. 22, A, a) along the zone of encounter become enveloped by intricately ramifying branches of the echinulate fungus (Fig. 16, G, b; Fig. 22, A, b). The injury sustained appears usually not very serious, for although some of the invested filaments suffer internal degeneration while others in addition are invaded by assimilative elements, envelopment in numerous instances seems not to result in any abnormal changes.

PYTHIUM ANANDRUM

Since the descriptive account (20, p. 415–420) supplementary to the original diagnosis (17, p. 410–411) of *Pythium anandrum* was written, the fungus has been isolated by Hickman (28) from strawberry (*Fragaria* sp.) roots received from Scotland, and, besides, has been made known by Middleton (34) as occurring in the United States on cucumber fruits, bean roots, and spinach roots. In view of the wider host range and more extensive geographical distribution thus disclosed, it is of moment that the main difficulty hitherto experienced in trustworthy identification of the fungus—the difficulty of obtaining the papillate zoosporangia distinctive of the species through irrigation of young mycelium—can be circumvented advantageously, if the occasion is not too pressing, by using, instead of young mycelium, the parthenospores always abundantly formed in maizemeal-agar cultures. Structurally separate zoosporangia of the sort most helpful in making determinations are commonly formed in ample quantity by germinating parthenospores even though many parthenospores dispense with the development of such bodies in giving rise to swarmers. The behavior of the fungus in the laboratory suggests that under natural conditions *P. anandrum* may very probably produce its zoospores in larger measure in the germination of its parthenospores than from its sporangia of mycelial origin.

On transfer to a shallow layer of water, parthenospores of *Pythium anandrum* taken from a maizemeal-agar plate culture 11 days after planting, that is, only a few days after they had achieved the unitary internal organization of maturity, showed germination in scattered instances. When the cultures were 90 days old, nearly all of the parthenospores germinated on similar treatment, with few exceptions giving rise to swarm spores. In the earliest recognizable stage of germinative development, 2 (Fig. 23, A) to 4 (Fig. 24, A) refringent bodies, evidently derived by division of the single refringent body present earlier, may be seen in the finely granular parietal layer of protoplasm. The reserve globule, which during the resting period has an accurately spherical boundary, now shows a noticeably irregular contour; and the inner layer of the oospore wall, embracing about two-thirds of the thickness of this envelope, reveals closely arranged radial linear markings (Fig. 23, A). Through further change the inner layer of the wall gradually becomes indistinguishable from the granular layer in contact with it (Fig. 23, B), and soon its substance amalgamates with the protoplast, which thus becomes expanded to reach the persistent outer layer of the wall. This outer layer dissolves in a round area 2.5 to 8 μ wide, permitting the protoplast to protrude against the oogonial envelope. When the oogonial envelope likewise gives way locally, the protrusion pushes out as a germ hypha (Fig. 23, C, t; Fig. 24, B, t; C, t). Germ tubes destined to function directly as evacuation tubes grow out, often with abrupt changes in direction, to a length of 10 to 65 μ and at a width varying commonly from 4.5 to 6.5 μ (Fig. 23, D–G: t; Fig. 24, D, t; E, t), though here and there some may expand locally to a width of approximately 10 μ (Fig. 23, G, t; Fig.

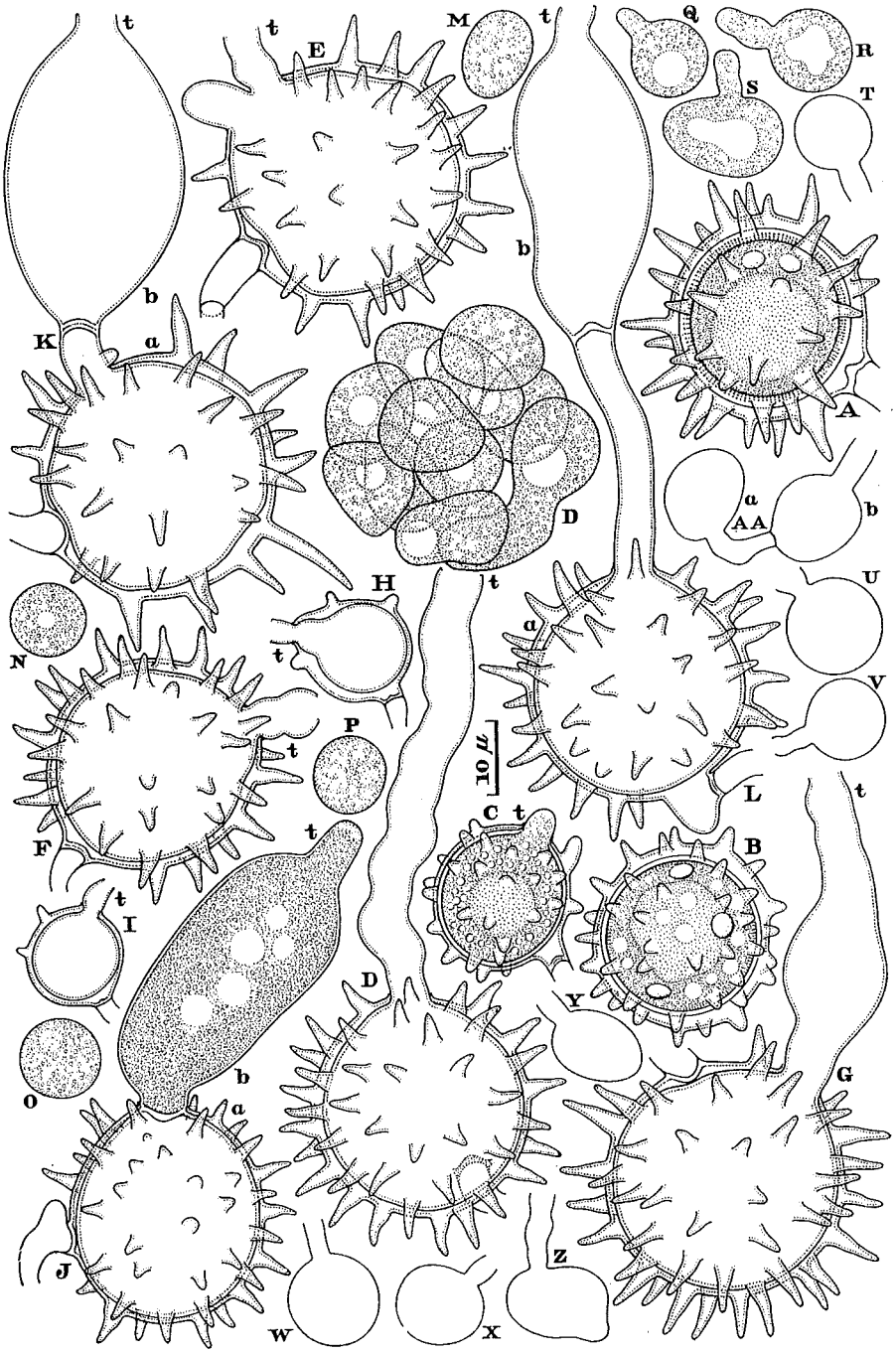

FIG. 23. Germination of parthenospores of *Pythium anandrum* from maizemeal-agar cultures 3 months old; drawn with the aid of a camera lucida; ×1000. A, B. Parthenospores showing onset of germinative development in assimilation by protoplast of inner layer of wall, in irregular outline of reserve globule, and in presence of plural refringent bodies. C. Parthenospore that has resorbed inner layer of wall and extended a germ tube through oogonial envelope. D. Parthenospore that has discharged its contents by way of a long evacuation tube into a vesicle which has disappeared; the resulting zoospores having encysted in place. E–I. Membranous envelopes left behind after escape of the zoospores formed through conversion of parthenospores directly into sporangia. J. Parthenospore, a, that has produced a sporangium, b, sessile on the oogonial envelope. K, L.

24, E, t). A very small parthenospore measuring approximately 12 μ in diameter may produce an evacuation tube scarcely 5 μ long and 3 μ wide (Fig. 23, H, t; I, t). In the vesicle formed apically when the protoplasmic contents of so small an oospore flow through the minute evacuation tube only 2 zoospores are fashioned. The granular materials from an oospore 27 μ in diameter (Fig. 23, D) are sufficient for about 12 swarmers. As many as 15 or 16 zoospores have been produced in the largest parthenospores, which measure 29 μ or 30 μ in mean diameter (Fig. 23, G; Fig. 24, E). Individualization of the motile spores always takes place within a vesicle after the manner usual in the genus. If from lack of water the vesicle disintegrates somewhat prematurely the developing zoospores often encyst in irregular shapes to form a cluster near the open end of the evacuation tube (Fig. 23, D).

The parthenospores of *Pythium anandrum,* like the homologous reproductive bodies of *P. salpingophorum* and *P. vexans,* give rise to swarmers not only by becoming directly transformed into zoosporangia, but also, as has been intimated, by producing sporangia structurally distinct from themselves. In instances of the latter type of germinative development the germ hypha may attain a length exceeding 200 μ before its tip begins to expand in initiating the formation of a terminal apically papillate elongated-ellipsoidal sporangium which receives all or very nearly all the protoplasmic content of the parthenospore before it is delimited by a basal septum. Where the layer of water is kept shallow, so that deep immersion is avoided, the sporangiferous germ hyphae only occasionally will exceed 100 μ in length (Fig. 24, F), and most often will measure less than 75 μ in this dimension (Fig. 24, G). They commonly vary in width from 3 to 5.5 μ (Fig. 23, K, L; Fig. 24, F, G) and thus are appreciably narrower, besides being less irregular in course, than germ hyphae destined to operate as evacuation tubes. In meagerly irrigated preparations a special sporangiferous hypha is frequently dispensed with altogether, as the germ tube here often widens immediately after pushing through the oogonial envelope (Fig. 23, J, a) and forms a sessile sporangium (Fig. 23, J, b) delimited at the base by a septum flush with the parthenospore membrane. A sporangiferous hypha may not be formed even where the germ tube elongates as a stout filament for some distance outside of the oogonial envelope, since the basal septum delimiting the sporangium is sometimes laid down as a broad convex partition within the chamber of the parthenospore (Fig. 24, H). At times a germ tube that elongated externally as a filament only for a few microns may nevertheless furnish a recognizable, if short, supporting stalk (Fig.

Parthenospores, a, of which each produced a sporangium, b, at the end of a germ hypha; the sporangia later becoming evacuated in giving rise to swarm spores. M–P. Encysted zoospores. Q–S. Encysted zoospores, showing different stages in production of an evacuation tube for emission of a secondary motile swarmspore. T–Z. Empty cyst envelopes left behind after escape of a secondary motile zoospore from each. AA. Membranous envelopes evidencing production by encysted zoospore, a, of a minute zoosporangium, b, at the tip of a germ tube; the sporangium then having produced a secondary motile swarm spore. (t, evacuation tube.)

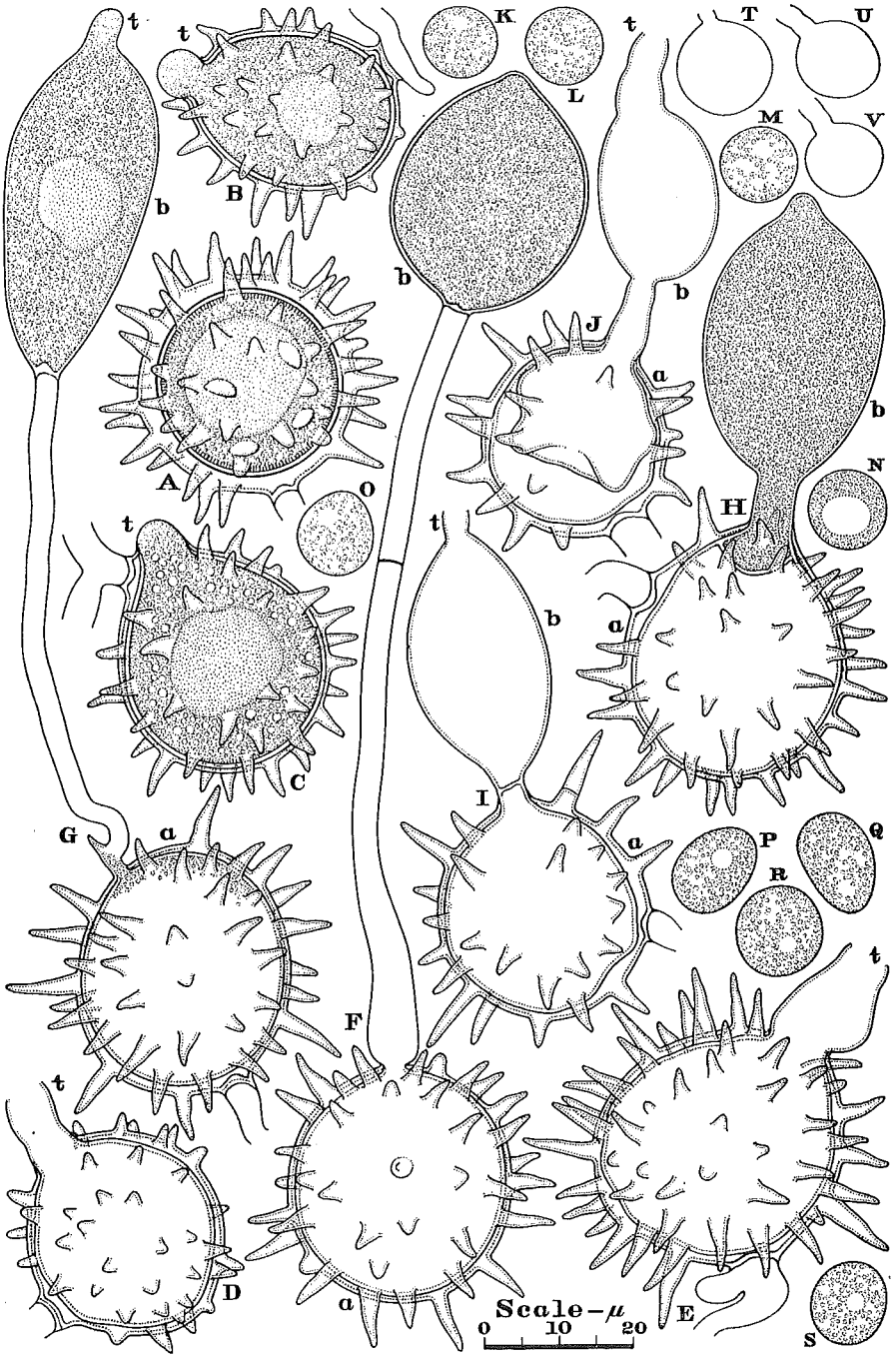

FIG. 24. Germination of parthenospores of *Pythium anandrum* from maizemeal-agar plate cultures 3 months old; drawn with the aid of a camera lucida; ×1000. A. Parthenospore showing onset of germinative development in assimilation by the protoplast of inner layer of wall, in irregular outline of reserve globule, and in presence of plural refringent bodies. B, C. Parthenospores that have resorbed an inner layer of wall and extended a germ tube through oogonial envelope. D, E. Membranous envelopes left behind after escape of zoospores formed through conversion of parthenospores directly into sporangia. F, G. Parthenospores, a, each of which has produced a sporangium, b, at the end of a germ hypha; the germ hypha in G emerging from one of the oogonial spines. H. Partheno-

24, I); whereas at other times a germ tube that elongated externally as a filament for fully 10 μ before widening may come to constitute the constricted median portion of a dumbbell-shaped sporangium one of whose expanded parts is deeply nested within the chamber of the parthenospore (Fig. 24, J, a), while the other occupies a position corresponding to the usual position of a stalked sporangium (Fig. 24, J, b). In instances of such partly endogenous origin of the germ sporangium, the obvious separateness of the sporangial wall from the parthenospore membrane suggests that where the empty evacuation tube in *P. vexans* is found continuous with a separate membranous envelope deeply inserted into the chamber of the oospore (Fig. 21, B, L), formation of swarm spores came about by development of a wholly endogenous sporangium rather than through direct conversion of the oospore into a sporangium.

Germ sporangia, when borne terminally on germ hyphae (Fig. 24, F, b; G, b) or sessile on oogonia (Fig. 23, J, b), correspond well in their generally ellipsoidal and distally papillate shape to the sporangia of mycelial origin that were described earlier. While the apical papilla here likewise often forms a cap of dehiscence directly, so that the vesicle into which the protoplasmic materials migrate is often sessile on the sporangium, numerous instances came to light in which the papilla elongated materially before discharge took place (Fig. 23, J, t; Fig. 24, G, t). Consequently the empty sporangial envelope was often found extended distally into an evacuation tube varying mostly from 1 to 10 μ in length (Fig. 23, K, t; L, t; Fig. 24, I, t; J, t). Sometimes, though less frequently than in *Pythium vexans,* the evacuation tube was found in a position apart from the apical papilla. Proliferous development of sporangia, such as takes place rather sparingly when young mycelium in agar slabs is irrigated, has never been observed in the germination of parthenospores. Lack of renewed sporangial growth here has sufficient explanation in the limited volume of the parthenospore; for even when a relatively large specimen contributes its entire contents, the resulting sporangium is yet substantially smaller than the average sporangium of mycelial origin. Nor have parthenospores ever been observed giving rise to plural sporangia on separate germ hyphae.

It is not evident that the zoospores brought into being through germination of parthenospores differ from those produced after appropriate irrigation of young mycelium. In a random assortment of encysted individuals (Fig. 23, M–P; Fig. 24, K–S) some may usually be found to measure only 10 μ in diameter (Fig. 23, N, P; Fig. 24, K). Smaller size of cysts is sometimes partly attributable to prevalence of repetitional development. Most frequently such development is initiated by the cyst through extension of an

spore, a, that has produced a sporangium, b, partly inserted into the chamber of the parthenospore envelope. I. Parthenospore, a, that has produced a sporangium, b, on a very short stalk; the sporangium later having become evacuated in giving rise to swarm spores. J. Parthenospore, a, that produced a sporangium, b, with a basal part deeply nested within the parthenospore envelope; the whole sporangium having become evacuated in giving rise to zoospores. K–S. Encysted zoospores. T–V. Empty cyst envelopes left behind after escape of a motile secondary zoospore from each. (t, evacuation tube.)

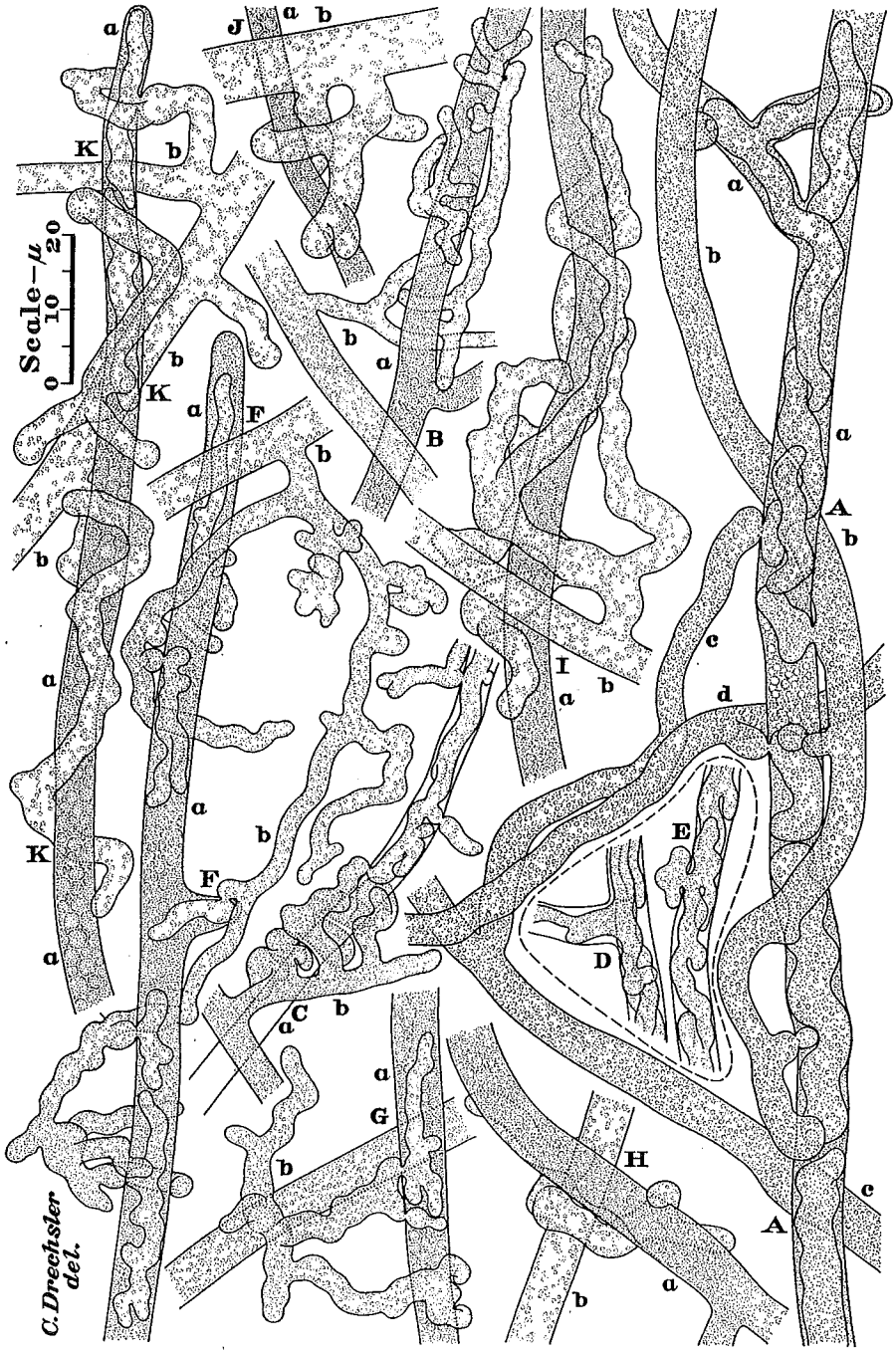

FIG. 25. *Pythium anandrum* attacked by other oomycetes; drawn with the aid of a camera lucida; ×1000 throughout. A. *Pythium anandrum*, a, attacked by *Pythium oligandrum*, b, c, d. B, C. *Pythium anandrum*, a, attacked by *Pythium acanthicum*, b. D, E. Hyphae of *Pythium anandrum* invaded by *Pythium acanthicum*. F, G. *Pythium anandrum*, a, attacked by *Pythium periplocum*, b. H, I. *Pythium anandrum*, a, attacked by *Plectospira myriandra*, b. J, K. *Pythium anandrum*, a, attacked by *Aphanomyces cladogamus* (pansy strain), b.

evacuation tube (Fig. 23, Q–S) 1.8 to 10 μ long and 2 to 4 μ wide. The empty cyst envelope left after the secondary zoospore escapes from the small terminal vesicle shows considerable variation with respect to the length, width, and shape of the open membranous tube (Fig. 23, T–Z; Fig. 24, T–V). Empty membranous envelopes (Fig. 23, AA) were occasionally observed that supplied evidence of less direct repetitional development; the primary zoospore (Fig. 23, AA, a) in each instance having manifestly given rise on the tip of a germ tube to a small sporangium (Fig. 23, AA, b) which then extended an evacuation tube to permit escape of the contents into a terminal vesicle for transformation into a secondary swarmer.

When *Pythium anandrum* is grown in maizemeal-agar plate cultures in opposition to *P. oligandrum,* its mycelial advance is halted at the zone of encounter, and along this zone many of its hyphae become loosely enwrapped with hyphae and branches of the more delicate spiny form. Such enwrapment is usually soon followed by visible degeneration of protoplasmic contents. Some of the invested filaments (Fig. 25, A, a) further undergo invasion lengthwise by numerous assimilative elements intruded here and there by branches and diverticula arising from *oligandrum* filaments (Fig. 25, A, b–d). When the fungus is grown in opposition to *P. acanthicum* its mycelium is likewise halted at the zone of encounter; its hyphae at the forefront (Fig. 25, B, a; C, a) again becoming abundantly enveloped by intricately ramifying branches of the less robust echinulate species (Fig. 25, B, b; C, b). Many of the enveloped hyphae rupture near the tip, releasing considerable quantities of protoplasmic material. In any case the hyphal contents soon take on the somewhat opaque appearance associated with degeneration (Fig. 25, B, a). Often, besides, the enveloped hyphae are invaded by irregularly ramifying assimilative elements (Fig. 25, C, a; D; E) which after appropriating the granular materials will frequently push out through the confining membrane to extend the infection, if possible, to neighboring hyphae. Similar injury is sustained by *P. anandrum* when it is grown in opposition to *P. periplocum.* Along the zone of encounter its hyphae (Fig. 25, F, a; G, a) are promptly arrested in their growth as they are beset by intricate ramifications extended from the *periplocum* filaments. The hyphae attacked soon take on a darkish appearance; their degenerating protoplasm in many instances being appropriated by assimilative branches invading them longitudinally (Fig. 25, F, G). When *P. anandrum* is grown in opposition to *Plectospira myriandra,* its mycelial advance, again, is abruptly halted at the zone of encounter. Its hyphae (Fig. 25, H, a; I, a) at the forefront become enveloped by ramifications put forth from filaments of the saprolegniaceous fungus (Fig. 25, H, b; I, b). Such envelopment is regularly followed by internal degeneration, and often also by invasion with assimilative branches. Under similar conditions the related *Aphanomyces cladogamus* likewise arrests the mycelial advance of *P. anandrum* at the zone of encounter; many of the *Pythium* hyphae (Fig. 25, J, a; K, a) soon becoming enwrapped by branches extending from *Aphanomyces* fila-

ments (Fig. 25, J, b; K, b). The enwrapped portions of hyphae degenerate internally, and frequently, moreover, undergo invasion by assimilative branches of the saprolegniaceous species.

PYTHIUM UNDULATUM PETERSEN SENSU DISSMANN

In the well-known account of Danish fresh-water phycomycetes published by Petersen in 1909 (37) and again in 1910 (38) this author describes as a new species under the binomial *Pythium undulatum* a fungus he had found living especially on the leaves and petioles of both the white waterlily, *Nymphaea alba* L., and the European yellow pondlily, *Nuphar luteum* (L.) Sibth. and Smith, though occasionally occurring also on the buds of these aquatic phanerogams and on old fruits of iris as well as on branches of trees. Its extramatrical mycelium was set forth as consisting of unbranched, more or less undulating hyphae, often several millimeters long and 3 to 6 μ wide. To the species were ascribed terminal (rarely lateral) ellipsoidal sporangia, about 130 μ long and 50 μ wide, which sometimes were provided with a small apical papilla. The sporangia were stated to open at the apex, sometimes with a papilla; no explanation being given, however, as to whether the papilla was always operative in dehiscence when it was present, or by what means dehiscence was accomplished when it was absent. Laterally biciliate zoospores similar to those of *Pythium,* it was asserted, issued forth, measuring 15 to 20 μ in length presumably while in their motile condition rather than after their encystment. Despite the phrase "in vesica ut in Pythio," the sequence in which the descriptive details are given leaves uncertainty, as Blackwell, Waterhouse, and Thompson (7, p. 154) have justly intimated, whether the zoospores are really fashioned within a *Pythium* vesicle, or whether, as sometimes happens among species of *Phytophthora,* they are surrounded for a very short time in a highly evanescent vesicle after being discharged from the sporangium in a full-fledged state. After escape of the zoospores new sporangia are sometimes formed within the old ones, while at other times the supporting hypha produces a sporangium after growing lengthwise through the empty envelope. The protoplasm was characterized as refractive, and the membranes of the hyphae as more or less brownish. Of the several sporangia shown in Petersen's 4 relevant drawings, 2 are filled with contents and show an apical protuberance that might represent either a very prominent sessile papilla or a short evacuation tube (37: Fig. VIII, a (right); d); one of these two being borne at the tip of a hypha in a group with 2 empty sporangial envelopes (37: Fig. VIII, a, top, left) which reveal distally a recognizably narrowed prolongation of the membrane as if a short evacuation tube, not a sessile papilla, had been operative in discharge. However, no similar narrowed prolongation is evident in the illustrations of 2 other sporangial envelopes (37: Fig. IX), neither of which assuredly could at any stage have been continuous with an evacuation tube. From the scale of magnification indicated for them the 2 distally unmodified empty envelopes would seem to have dimen-

sions most extraordinary for sporangia of such ellipsoidal type; the larger one appearing to measure about 240 μ in length, 70 μ in greatest transverse diameter, and 28 μ in width of apical opening.

Even if these extraordinary dimensional values are disregarded, so that only the measurements given in the diagnosis are left for consideration, the large size of the ellipsoidal sporangia appears to be the most distinctive character presented in the original account of *Pythium undulatum*. Despite wide variability in size resulting from differences in environal conditions, ellipsoidal sporangia 130 μ long and 50 μ wide are not often encountered among species of *Pythium*. In most representatives of the genus—certainly in most of those commonly found in a terrestrial habitat—the tendency toward moderation in volume of the sporangium appears fairly pronounced; somewhat extensive swollen branching systems in luxuriant irrigated material of *P. Butleri*, for example, often becoming divided by a dozen cross-walls to no other end, apparently, than to lessen the size of the individual reproductive units. It is true, Apinis (1) ascribed elongated oval sporangia, 50 to 167 μ long and 20 to 50 μ wide, to a submerse fungus that he held referable to Petersen's species; but he transferred this species to *Pythiomorpha* presumably because in his material the zoospores were formed directly within the sporangia. Matthews (32, p. 69–71) somewhat doubtfully recognized *Pythium undulatum* in a fungus isolated from soil in North Carolina which when grown on hemp seeds in distilled water produces proliferous cylindrical sporangia, 25 to 55 μ long and 15 to 18 μ wide, that evidently discharge their contents into a vesicle for transformation into zoospores. Narrowly ovoid proliferous sporangia, likewise functional in asexual reproduction typical of *Pythium*, but of even smaller size than those of Matthews —their length usually varying from 40 to 45 μ, and their width from 12 to 15 μ—were produced by an aquatic fungus that Sparrow (40, p. 299–300) discussed at first hand under the binomial contributed by Petersen. Working in Denmark a quarter of a century after Petersen, Lund (31, p. 48–51) applied the binomials *Pythiomorpha undulata* and *Pythium undulatum* to things he regarded as separate: Apinis' species being recognized in a fungus which he isolated from various natural substrata—plant remains, *Sphagnum*, soil, sand, twigs (including *Picea* twigs), roots of *Salix repens* L.—and which formed zoospores directly within terminal sporangia usually oval in shape, 45 to 117 μ long, 35 to 43 μ wide, frequently 70 μ long and 40 μ wide; while Petersen's species was doubtfully recognized once in a sporangium of similar morphology that bore at its mouth a vesicle in which zoospores were lying. Despite the meagerness of the material that provided Lund's momentary experience ostensibly with *Pythium undulatum*, his view that members of 2 different genera have come to share the specific epithet *undulatum* given by Petersen seems amply justified. This view is reflected in Sparrow's (41, p. 707) recent treatment of *Pythiomorpha undulata* as a phycomycete distinct from *Pythium undulatum;* the widely divergent usage with respect to the epithet having somewhat earlier been reviewed by Blackwell, Waterhouse,

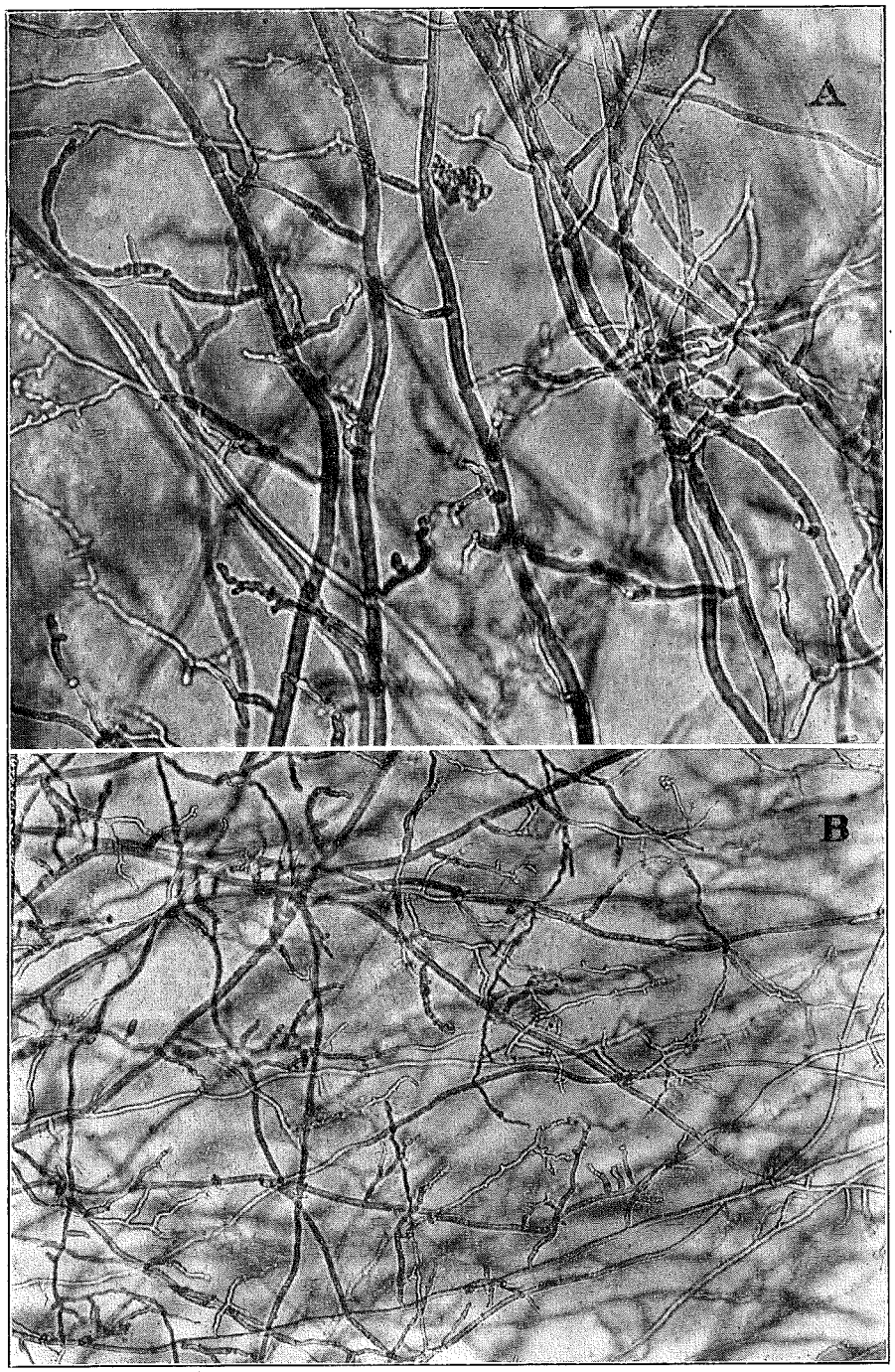

FIG. 26. Mycelium of *Pythium undulatum* Petersen *sensu* Dissmann as found in a 2-day-old maizemeal-agar plate culture; photomicropraphs; magnification in A about × 240, in B about × 120.

and Thompson (7) in their elucidation of the evident synonymy of *Pythiomorpha* (including *Pythiomorpha undulata* as understood by Apinis and Lund) with *Phytophthora.*

If ellipsoidal proliferous sporangia approximately of the extraordinary dimensions given in the original diagnosis of *Pythium undulatum* were to be found usual and characteristic for some one particular species—preferably for a species in which undifferentiated protoplasmic contents are delivered into a vesicle and then are fashioned into motile zoospores—no serious misgivings could be entertained with respect to the correct application of the binomial. Although the relevant literature does not offer any completely satisfactory metric agreement, a rather likely application of the binomial is given in Dissmann's (13) account of 2 *Pythium* species he isolated from prematurely discolored, yellowing leaves of waterlily (*Nymphaea candida* Presl.) plants that grew abundantly in an artificial pond near Hirschberg in Bohemia; both species, therefore, being similar to Petersen's in regard to their host relationship and their aquatic habitat. In peptone-saccharose solution the fungus Dissmann identified as *Pythium undulatum* produced a mycelium with hyphae up to 6 or 8 μ in width; in pea decoction its mycelium branched more irregularly and grew more delicately by extending hyphae often only 3 to 4 μ wide. The sporangia obtained by transferring mycelium from a liquid culture to water varied greatly in size with changes in the concentration of the nutrient solutions employed: a solution containing from 0.5 to 1.0 per cent of peptone yielded sporangia 30 to 40 μ long, whereas a 1.0 per cent solution yielded sporangia 40 to 76 μ in length. Use of a 0.6 per cent haemoglobin solution resulted in production of sporangia up to 60 μ long. When the fungus was transferred to unmodified pond water after being grown in pond water containing 5 per cent of maltose it produced sporangia up to 80 μ long. On transfer to unmodified pond water, mycelium that was grown in pond water fortified with water-lily-leaf decoction gave rise to very large sporangia, with an occasional individual measuring up to 120 μ or 140 μ in length—dimensional values close to those found in material developed under natural conditions. In a figure (13: Fig. 1) showing zoosporangia drawn as they were found occurring naturally in a mixture on waterlily leaves, those ascribed to *Pythium undulatum* (13: Fig. 1, a)—all represented by empty envelopes borne on a single ramified trunk—appear from the scale of magnification to range in length from 60 to 150 μ. None of these envelopes shows any narrow prolongation at the open distal end. Their general appearance suggests that discharge must have been accomplished by means of a sessile cap of dehiscence rather than by means of an evacuation tube. In the multiple nesting of empty sporangia illustrated in the figure some of the inner envelopes terminate so far within the outer envelope that formation of a globose vesicle would have been obstructed. Among proliferous species of *Pythium* such obstruction does not ordinarily occur, since here as a rule the inner sporangium is extended either broadly or by an evacuation tube until its tip is flush with,

or protrudes beyond, the mouth of the older membranous mantle. Instances of recessed nesting are, however, not infrequent among proliferous species of *Phytophthora*, where no vesicle needs to be formed, and where the full-fledged zoospores released from an inner sporangium are capable of making their way if necessary through a series of apical openings. The generous width of the apical openings, which here and there would seem to exceed 15 μ, is likewise especially suggestive of *Phytophthora,* in which the non-papillate sporangia of various proliferous species, as, for example, *P. cryptogea* Pethyb. & Laff. and *P. cambivora* (Petri) Buisman, are given to dehiscence by a relatively broad distal aperture. In contrast, both of the correctly evacuated zoosporangial envelopes (13: Fig. 8, left; Fig. 9) drawn by Dissmann from material derived from the pure culture that he treated as *Pythium undulatum* show a recognizable evacuation tube at the apex; the wider of the 2 tubes measuring about 9 μ in diameter. There is good reason to suspect that the strongly proliferous sporangia drawn from waterlily material originating in nature were alien to the fungus represented in the pure culture—that they belonged more probably to a species of *Phytophthora,* which, owing perhaps to the slower mycelial extension usual in members of this genus, may have been consistently outgrown in isolation cultures by the accompanying species of *Pythium* and thus kept from being recognized.

Apart from sporangia, Dissmann's pure culture of *Pythium undulatum* readily gave rise on various agar substrata to terminal or intercalary subspherical reproductive bodies he termed chlamydospores; the protoplasm for their growth being obtained through a progressive evacuation of adjacent portions of hypha entailing deposition of successive boundary walls. The size of the chlamydospores was found to increase with the richness of the agar medium employed. In maizemeal-agar cultures prepared with media whose nutrient concentration varied in a 1–5–10 ratio, the most frequent values for diameter of the globose spores were about 36 μ, 59 μ, and 69 μ, respectively; a total range extending from 6 μ to 92 μ being indicated for the dimension. Rather early in the development of the chlamydospore its wall could be recognized as composed of 2 layers, and with advancing maturity the two-layered construction became more distinctly visible: the very thin outer layer consisted of the original hyphal envelope, while the inner layer, of more variable thickness, represented a special membrane secreted by the protoplast. In chlamydospores older than those present in maizemeal-agar cultures 4 weeks after inoculation, the outer layer was stated to persist as flakes or lumps externally adnate to the inner layer. With respect to the internal organization of the chlamydospore, aging was found accompanied by conspicuous accumulation of fat (Fett); this material first appearing in small droplets, and later, following union of the small droplets, in larger globules. Attempts at germinating the chlamydospores were successful only with very young specimens in which no accumulation of fat, nor any thickening of the wall, had yet taken place. In such young

specimens the thin envelope ruptured, or a short evacuation tube was put forth; the protoplasmic contents, in either event, being then emptied into a vesicle for transformation into zoospores. Failure of the older chlamydospores to germinate, it was pointed out, might derive from conditions similar to those present in oospores, which likewise constitute a thick-walled resting stage with abundant accumulation of fat, and which likewise, again, have only rarely and under conditions little understood been induced to germinate. Dissmann reported that his persistent effort to find sex organs of the fungus on host tissue failed, and that no oogonia came to light in numerous culture media he tried out during nearly 2 years.

A *Pythium* whose specific identity with the one Dissmann grew in pure culture as *P. undulatum* seems beyond question, developed in 9 among 42 tubes of maizemeal agar that were planted late in July, 1936, with separate pieces of discolored leaf tissue taken at random from waterlily (*Nymphaea sp.*) plants growing in a drainage ditch in a cranberry bog near East Wareham, Massachusetts. After the isolation cultures were freed of bacteria and other contaminating microorganisms the fungus displayed a robust mycelial habit recalling such coarse congeneric forms as *P. ultimum, P. debaryanum,* and *P. anandrum.* Growing in maizemeal agar its main hyphae often attain a width of 8 μ within 100 μ or 150 μ of the tip, though if the filaments are followed backward hardly any further widening is to be noted. Its lateral branches, which usually develop in moderate quantity, are of lesser thickness, and show more or less irregular secondary ramification. Despite a certain degree of coarseness, the vegetative mycelium (Fig. 26, A, B) offers the generally flexuous appearance familiar among species of *Pythium* rather than the stiffly branching aspect common in species of *Phytophthora.*

In July and August, 1944, occasion was taken to try out the fungus on young unblemished waterlily (*Nymphaea sp.*) leaves supplied from an artificial pond in Arlington, Va. Soon after their removal from the pond, the leaves were placed in large glass damp-chambers, planted with slabs excised from a maizemeal-agar plate culture of the phycomycete, and stored at a temperature of 18° C. In 2 or 3 days the leaf areas under the slabs took on a dark brown discoloration. This discoloration continued to spread steadily, with the result that in 10 days it had come to extend over irregularly circular patches 40 to 60 mm. in width, though the leaves that had not been planted with the fungus still retained then their fresh green color throughout. When pieces of discolored tissue near the periphery of the brown waterlogged patches were removed to a shallow layer of distilled water in a Petri dish, and then stored at a temperature near 18° C., extramatrical hyphae 3 to 7 μ wide grew out into the liquid to produce terminally a moderate number of prolate ellipsoidal sporangia mostly 30 to 90 μ long and 20 to 40 μ wide. These sporangia on attaining definitive size were often found provided with an apical papilla, which sometimes, after renewal of the water, would form a virtually sessile cap of dehiscence. More often,

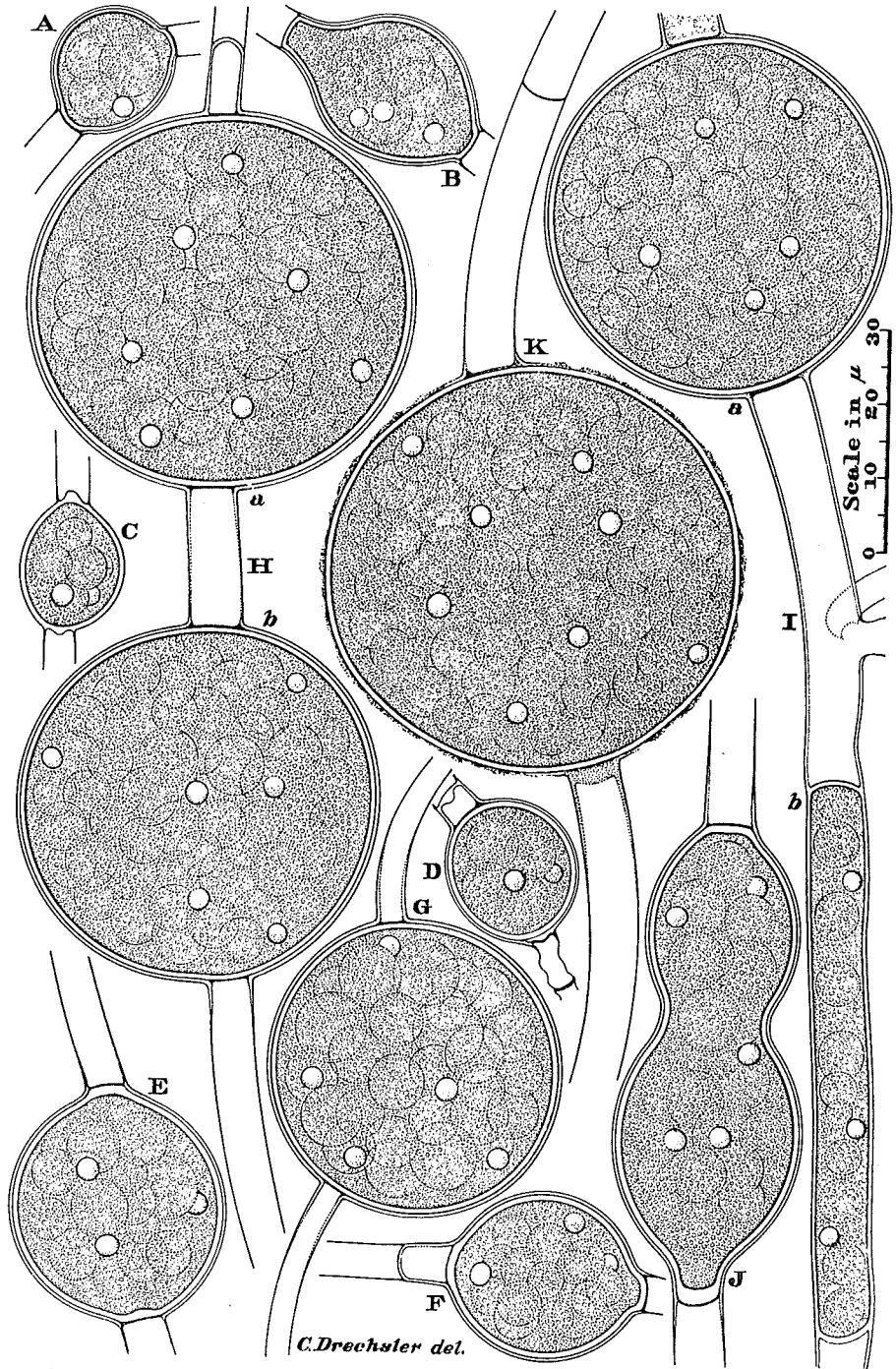

FIG. 27. Resting spores of *Pythium undulatum* Petersen *sensu* Dissmann as found in a 25-day-old maizemeal-agar plate culture; drawn with the aid of a camera lucida; ×1000. A–F. Small smooth-walled specimens. G; H, a, b. Smooth-walled specimens of moderate size. I. Portion of hypha bearing a fairly well-developed smooth-walled resting spore, a, and a cylindrical resting spore, b. J. Smooth-walled resting spore of aberrant hour-glass shape. K. Rather large rough-walled resting spore.

however, the papilla grew out into a short evacuation tube before the hyaline cap was formed; so that after the undifferentiated protoplasmic contents had migrated into the inflated vesicle, and had been converted into zoospores, the empty sporangial envelope was usually found bearing distally a recognizable tubular prolongation much like the similar envelopes drawn by Dissmann from material referable to his pure culture. That the extramatrical hyphae and the sporangia really derived from the material planted on the leaves, rather than from some adventitious parasite, could hardly be doubted in view of their close resemblance to the extramatrical hyphae and sporangia produced in moderate quantity following irrigation of slabs excised from young maizemeal-agar plate cultures permeated exclusively with vigorous mycelium of the Massachusetts fungus.

More distinctive than the sporangia formed in irrigated preparations are the large globose reproductive structures or resting spores that first become noticeable in maizemeal-agar cultures about 3 or 4 or 5 days after inoculation. These structures continue development for about 20 days to present eventually a display scarcely less impressive with respect to the number of individual units than with respect to their collective bulk. In maizemeal agar of moderate nutrient content, such as I have employed, they have usually ranged in diameter from 15 to 75 μ. Occasional departures from their usual subspherical shape (Fig. 27, A–G; H, a, b; I, a; Fig. 28, A, B) are recognizable in cylindrical (Fig. 27, I, b) and in transversely constricted (Fig. 27, J) specimens. Their identity with the chlamydospores described by Dissmann becomes clearly manifest at maturity, when they are found crowded internally with an abundance of globules varying commonly from 4 to 10 μ in diameter. The smaller resting spores often contain only 4 or 5 of these globules (Fig. 27, A–D; Fig. 28, A, B), but the largest specimens (Fig. 27, I, a; K) probably contain more than 200. Their size and their distribution in a matrix of granular protoplasm provide a striking parallelism with the plural reserve globules found in the oospores of *Pythium helicoides* and its allies. This parallelism gains in suggestiveness from the presence of orbicular bodies, mostly 2.5 to 3 μ wide, that are scattered presumably throughout the protoplast, even if, as a rule, they are discernible only in the upper aspect of the massive spore. For the most part these bodies appear less brilliant than the refringent bodies present singly in oospores of unitary organization, and, perhaps, somewhat less brilliant even than the plural refringent bodies in oospores of multiplicate organization; though their lack of luster could well be attributable to the feebler illumination associated with the unusual thickness of the spore.

The mature resting spores of the Massachusetts fungus, much like the chlamydospores described by Dissmann, are surrounded individually by a wall composed of 2 layers. While in some of the smallest specimens (Fig. 27, C) the two layers can be made out only with some difficulty, in specimens of moderate size they are readily seen to be distinct from one another. The outer layer is colorless and continuous with the membrane of the parent

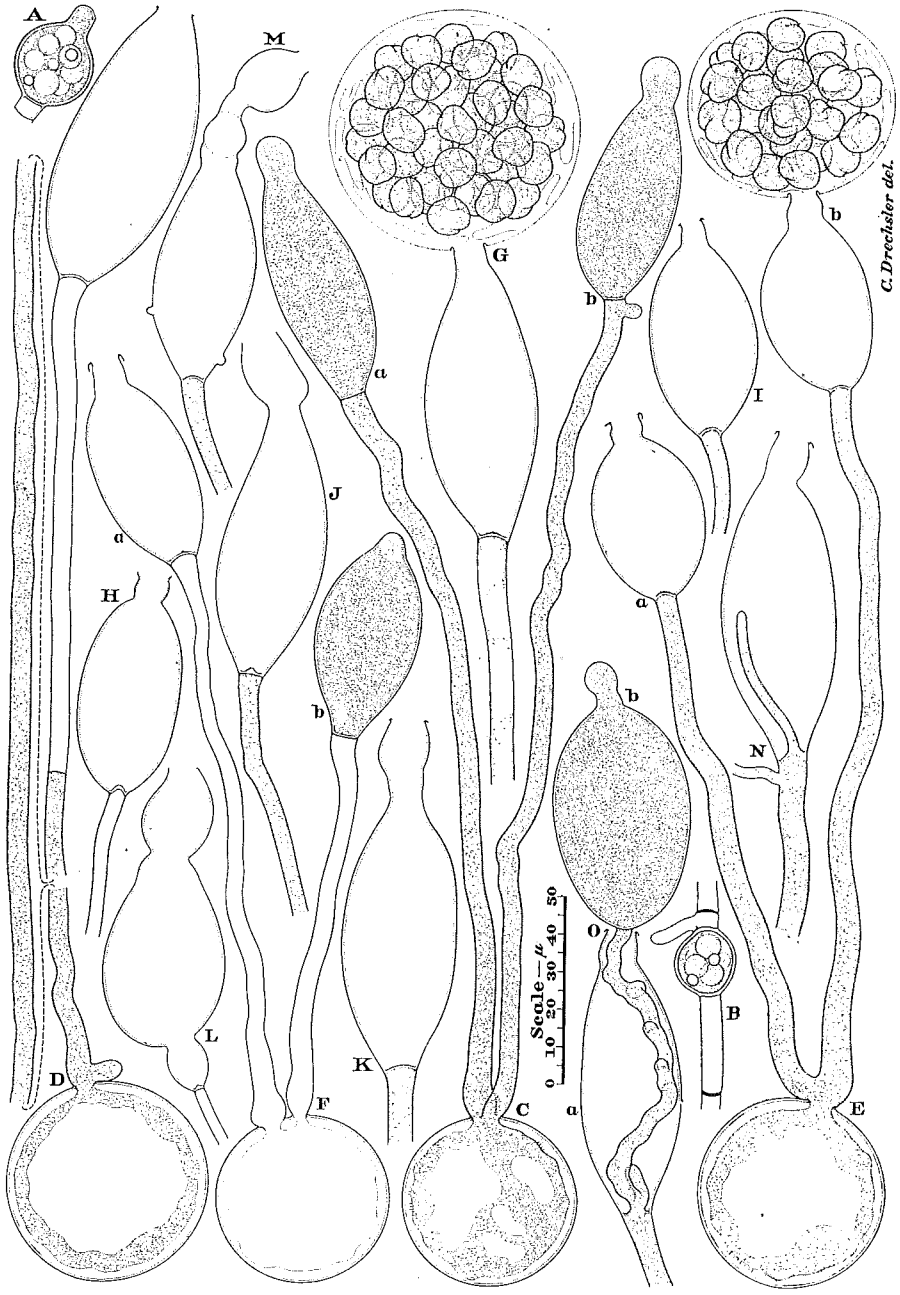

FIG. 28. *Pythium undulatum* Petersen *sensu* Dissmann; drawn with the aid of a camera lucida; ×500 throughout. A, B. Very small resting spores, showing the internal organization of maturity. C–F. Resting spores from a 6-months-old maizemeal-agar plate culture, which on being transferred to water germinated by producing zoosporangia on germ hyphae: the 2 sporangia, a and b, produced by C are each extending an evacuation tube preparatory to discharge, and the resting spore retains enough protoplasmic material for the development of 1 or 2 additional sporangia; D also retains much granular material after producing a large sporangium that has given rise to zoospores; E apparently retains enough protoplasm for development of 1 or 2 more sporangia, after having produced the 2 sporangia, a and b, of which the former is represented only by its empty envelope, while the latter supports a vesicle with zoospores about ready to escape; F has contributed all its contents in producing 2 sporangia, a and b, of which the former has given rise to zoospores while the latter is still filled with granular contents. G. Large sporangium borne

hypha; the inner one, secreted by the massive protoplast, offers noticeable contrast in its yellowish coloration. In spores measuring 30 to 50 μ in diameter (Fig. 27, E; G; H, a, b; I, a) the wall commonly appears to have a total thickness of about 1.3 μ; the outer layer usually contributing about 0.5 μ and the inner layer about 0.8 μ to the composite measurement. Many of the larger spores, including mainly specimens more than 45 μ in diameter, show a markedly irregular outer contour, and present an appearance as if they were covered with an uneven darkish incrustation (Fig. 27, K). In comparison with smooth-walled resting spores, those with a rough wall look more or less misshapen, as their subspherical form is usually found modified perceptibly by a number of broadly curved bulges. As these bulges sometimes occur in regions where the external incrustation is either very thin or wholly absent, the impression is gained that in the later stages of spore enlargement the outer membranous layer yields locally here and there or is ruptured outright in several places, permitting the somewhat elastic inner layer to push outward in the weakened regions.

Attempts to germinate newly mature resting spores of the Massachusetts fungus have always been wholly unsuccessful. When spores from a 65-day-old maizemeal-agar plate culture were transferred to a shallow layer of water in a Petri dish and stored at 18° C. a substantial proportion of them—most often between 10 and 25 per cent—germinated in the course of 7 days. On repeating the trials 100 days after the plate cultures had been planted, fully half of the spores germinated within 2 days. Virtually all spores transferred to water from cultures 180 days old germinated within 24 hours, and in strongly predominant measure germinated by the development of zoosporangia. When the resting spores contained in 0.1 to 0.2 cc. of agar from a 6-months-old culture were distributed over the floor of a Petri dish and sparingly watered, a much livelier display of motile zoospores often resulted than was obtained by irrigating ten times as much maizemeal agar or waterlily-leaf tissue permeated with young mycelium.

Preparatory to germination the resting spore takes on a somewhat opaque appearance as the reserve globules lose their clear boundaries, and together with the refringent bodies become gradually obliterated in the augmented volume of densely granular protoplasm. The inner layer of the wall dissolves away in a circular region, allowing the protoplast to protrude against the outer layer. After the outer layer has also given way the protrusion emerges to elongate externally as a germ hypha (Fig. 29, A). Apparently this germ hypha never functions directly as an evacuation tube, but like the hypha extended from the germinating oospore of *Pythium ostracodes* (25, p. 276–286) commonly forms a terminal sporangium (Fig. 29, B) from protoplasmic materials made available through increasing vacuolization

on a germ hypha and supporting a vesicle that contains approximately 45 zoospores nearly ready for escape. H–M. Empty sporangial envelopes produced in germination of resting spores. N. Empty sporangial envelope whose supporting hypha, produced in the germination of a resting spore, shows both uniaxial elongation and subsporangial branching. O. Development of 2 successive sporangia, a and b, through uniaxial elongation of the supporting hypha, which was produced in the germination of a resting spore.

within the parent spore (Fig. 28, C–E; Fig. 29, B). Where a substantial quantity of protoplasm remains, as is usually the case with large resting spores, the hypha may continue growth by putting forth a branch immediately below (Fig. 28, C, b; Fig. 29, C; E, a) or some little distance below (Fig. 29, D, a) the base of the first sporangium; the branch subsequently giving rise at its tip to a second sporangium (Fig. 29, D, b; E, b). Often 2 germ hyphae, each bearing a terminal sporangium, may be extended from well-separated positions on the resting spore (Fig. 29, F, a, b), or from positions rather close together (Fig. 28, F, a, b). Frequently, again, 2 sporangium-bearing hyphae may arise through basal branching of a single germ tube (Fig. 28, C, E; Fig. 29, G, a, b; H, a, b). Under environal conditions that favor immediate development of zoospores (Fig. 28, E, b; G) and thus permit prompt evacuation of sporangia (Fig. 28, H–M) while germination is still proceeding, the supporting filament in many instances elongates straightforwardly (Fig. 28, N) to produce a second sporangium within or beyond (Fig. 28, O, b; Fig. 29, H, c) the empty envelope of the first (Fig. 28, O, a; Fig. 29, H, a). Since in judiciously watered preparations many of the larger resting spores afford uniaxial production of 2 successive sporangia, proliferous development takes place in connection with germination on about the same modest scale as in the asexual reproduction obtained by irrigating young mycelium. Obviously no proliferous development is possible where relatively small resting spores—specimens less than 30 or 35 μ in diameter—are concerned, as these usually give rise only to a single sporangium (Fig. 29, I–L); the supporting hypha in such instances sometimes measuring less than 50 μ in length, and occasionally even less than 10 μ (Fig. 29, J). The sporangium-bearing hyphae extended from the more robust spores commonly measure 3 to 8 μ in width and 100 to 500 μ in length (Fig. 28, C–F; Fig. 29, G, H), though a considerable proportion of them may measure 0.5 to 1 mm. in length (Fig. 29, E, F) and some as much as 2.5 mm. or 3 mm.

The sporangia resulting from germinative development closely resemble those of mycelial origin in all particulars including size, since resting spores less than 30 μ in diameter are ordinarily too few to contribute any large proportion of noticeably undersized progeny. While awaiting conditions favorable for zoospore formation they are nearly always found provided with an apical papilla (Fig. 28, F, b; Fig. 29, B; C; D, a, b; E, a, b; F, a, b; G, a, b; H, b; I, J); yet now and then (Fig. 29, H, c; K), especially in the intercalary specimens (Fig. 29, L) to be seen occasionally, no distal modification is evident. The papilla sometimes is converted directly into a sessile cap of dehiscence, so that the empty sporangial envelope, after evacuation of the protoplasmic contents, will terminate abruptly in an aperture commonly 8 to 10 μ wide (Fig. 28, D), without displaying any sign of a tubular prolongation. More often, however, the papilla becomes extended into a rather short evacuation tube (Fig. 28, C, a, b; O, b) which eventually leaves its empty membrane superadded to the empty sporangial envelope (Fig.

28, E, a, b; F, a; G–N; O, a; Fig. 29, H, a). Usually the empty tube either terminates abruptly with a plain rim (Fig. 28, H, J, L, M; Fig. 29, H, a) or is minutely lipped at the orifice (Fig. 28, E, b; G; I; K; O, a), but in scattered examples it is found reflexed (Fig. 28, E, a; F, a; N) in a manner reminiscent of *Pythium vexans*. The vesicular membrane is always clearly visible. On disintegrating it releases commonly from 25 to 50 broadly reniform, laterally biciliate zoospores, which after swimming about for some time come to rest and round up into spherical cysts 9.5 to 13.5 μ in diameter (Fig. 29, M, a–z). The cysts occasionally give rise to secondary swarmers through repetitional development entailing the production of an evacuation tube usually 2 to 10 μ long and 2.5 to 4 μ wide (Fig. 29, N, a–f). More often, of course, they germinate vegetatively by putting forth 1 or 2 germ hyphae 2 to 3 μ wide (Fig. 29, O–Z).

Germination is accompanied by a marked change in the appearance of the wall surrounding the resting spore. The inner layer of the wall, which in spores of moderate size seemed earlier to measure about 0.8 μ in thickness, will usually show a thickness of 2 or 3 μ after a substantial portion of the protoplasmic contents has been contributed toward the development of germ hyphae and zoosporangia (Fig. 28, C–E). Later when the spherical chamber of the spore has been completely emptied of granular materials, the generally increased thickness of the inner layer is revealed as being varied locally by the presence of scattered pits which here and there seem to extend clear through to the outer layer. In some preparations the substance of the inner layer offers a nearly homogeneous or cartilaginous appearance (Fig. 28, F) while in others it exhibits numerous radial striations (Fig. 29, D–H) suggestive of the striations familiarly observed during germination in oospores of many congeneric species. Thus the empty two-layered envelope shows rather good correspondence with the two membranous envelopes, considered jointly, that are left from the germination of oospores in allied species; the correspondence being perhaps most obvious if comparison is made with such forms as *Pythium salpingophorum* where usually the oogonial membrane and the oospore wall are for the most part intimately fused.

The similarities shown by its membranous vestments after germination, taken together with similarities in structure of its protoplast during the long period of dormancy, provide persuasive grounds for interpreting the resting spore as a parthenospore homologous more particularly with the oospores of multiplicate internal organization that are found in *Pythium helicoides* and related species. One might be inclined to dismiss the morphological parallelism as being perhaps of fortuitous character if it were not so strongly corroborated by the physiological similarity manifest in the prolonged dormancy of the reproductive bodies under discussion; such dormancy being familiar among oospores and parthenospores, but wholly unknown among the subspherical conidia and chlamydospores formed by numerous species of *Pythium*, including, for example, *P. debaryanum* and *P. ultimum*. Kinship in the *helicoides* series would seem indicated further in the germinative

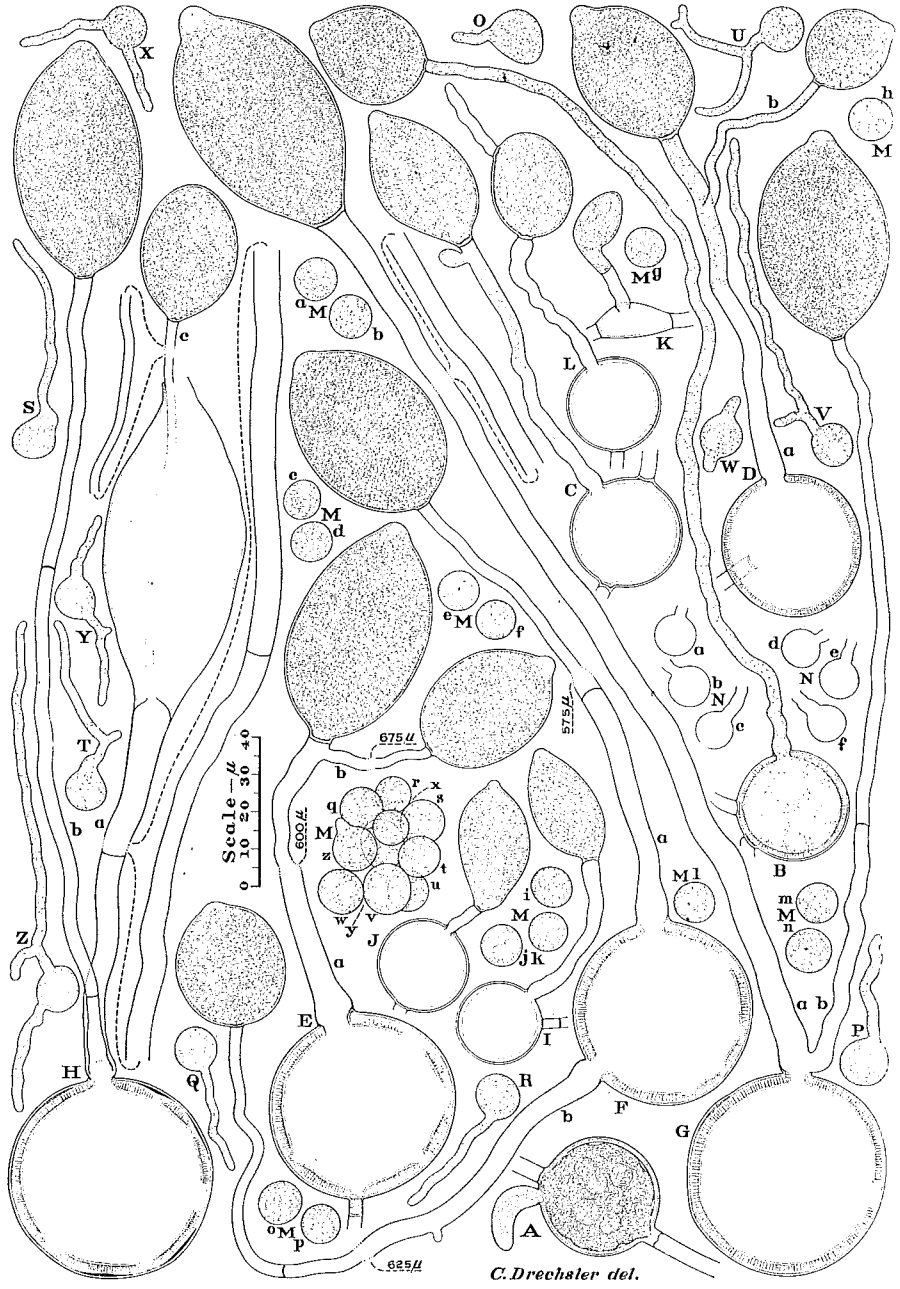

FIG. 29. Germination of resting spores of *Pythium undulatum* Petersen *sensu* Dissmann from a maizemeal-agar plate culture 6 months old; drawn with the aid of a camera lucida; ×500 throughout. A. Small resting spore from which a germ hypha is being extended. B. Rather small resting spore that has produced a small sporangium on a germ hypha, and still retains a substantial quantity of protoplasm. C. Rather small resting spore that has produced a sporangium at the end of a germ hypha; the germ hypha thereupon branching out below the delimiting septum although containing only a small quantity of residual protoplasm. D. Resting spore whose germ hypha, a, has formed a terminal sporangium and given off a branch, b, which also supports a sporangium. E. Resting spore whose germ hypha has produced a terminal sporangium, and then put forth distally a branch, b, to bear a second sporangium; from lack of space intercalary portions of hypha measuring 600 μ and 675 μ in length, respectively, are omitted at places indicated.

behavior of the fungus; for, as has been noted, the resting spores, much like oospores of my *P. ostracodes* give rise to zoospores exclusively through the production of structurally distinct zoosporangia, never by the more direct course of development wherein the protoplasmic contents are conveyed to a vesicle by way of an evacuation tube originally extended as a germ hypha—a course of development frequent in the unitary oospores of *P. anandrum.* The zoosporangia, whether borne on germ hyphae or on mycelial hyphae, conform satisfactorily in their typically terminal position, prolate ellipsoidal shape, distal papillate modification, and occasionally successive uniaxial development, with the type of sporangium prevalent in the *helicoides* series; though this type of sporangium, it must be admitted, is characteristic also of *P. anandrum,* and besides has been recognized for many decades as distinctive of *P. proliferum* de Bary (3, p. 18, 19; 4, p. 558–562), a species which from its smooth oogonia and from the unitary internal organization ascribed to its mature oospores (3, p. 61, lines 21–25) would not seem intimately related either to *P. helicoides* or to *P. anandrum.*

Indeed, the type of sporangium here concerned—the occasionally proliferous, obliquely oriented, bursiform sporangium of my *Pythium marsipium* (23, p. 492–506) and the very meagerly proliferous, frequently subterminal sporangium of *P. salpingophorum,* obviously differ from it in substantial measure—occurs too widely even among terrestrial members of the genus to provide alone a really trustworthy indication of either the identity or the intimate kinship of a species. The extraordinarily large sporangial measurements given by Petersen, which might be adequate for determining the application of his binomial if they were found usual for some fungus properly referable to the genus, have assuredly not been found usual in the Massachusetts waterlily fungus, whether it was grown on artificial media or on its natural substratum, though its sporangia have regularly been of generous dimensions. However, my fungus agrees well with the one that Dissmann isolated and referred to Petersen's species; the agreement being satisfactory with respect both to the zoosporangia and to the very distinctive resting spores. Dissmann's report of zoospore formation by direct discharge of contents from very young chlamydospores is not incon-

F. Resting spore that likewise became completely evacuated in giving rise to 2 sporangia, which here, however, are borne on 2 separate germ hyphae, a and b; from lack of space portions of hypha measuring 575 μ and 625 μ in length, respectively, are omitted at places indicated. G. Resting spore that has become evacuated in producing 2 sporangia on 2 separate germ hyphae, a and b, arising from a single trunk by basal branching; from lack of space, germ hypha a is shown in parts whose proper connection is indicated by broken lines. H. Large resting spore that has become evacuated in producing 3 sporangia of which 2 were formed terminally on separate germ hyphae, a and b, arising through basal branching from a single trunk, whereas the other was formed terminally on the uniaxial prolongation c of the germ hypha a; from lack of space a and c are shown in portions whose proper continuity is indicated by broken lines. I–K. Small resting spores, each of which became evacuated in producing a sporangium on a short germ hypha. L. Small resting spore that has put forth a germ hypha with an intercalary sporangium. M. Encysted zoospores, a–z, derived through germination of resting spores, and showing variations in size and shape. N. Empty cyst envelopes, a–f, each with an evacuation tube that served in the emergence of a secondary motile zoospore. O–V. Encysted zoospores, each germinating with 1 germ tube. W–Z. Encysted zoospores, each germinating with 2 germ tubes.

sistent with my statement of germinative behavior in properly after-ripened resting spores; for, as Dissmann pointed out, the juvenile reproductive bodies he found active resembled greatly the zoosporangia formed in water, not having yet undergone, either in their protoplasm or in their envelopes, any modification tending toward the mature condition. Since he makes no mention of using material several months old in his germination trials, there is good reason to suspect that his failure with mature chlamydospores was attributable to inadequate aging.

Although the soil fungus discussed under Petersen's binomial by Matthews produced "thick-walled chlamydospores" which she held similar to Dissmann's, their small size—a range in diameter from 14 to 24 μ being given for them—would seem to make identity with my waterlily parasite quite improbable. Greater likelihood of such identity is offered by the aquatic fungus that Sparrow discussed as *Pythium undulatum,* since it gave rise on maizemeal agar to "dark brown, rough-walled chlamydospores" 10 to 50 μ in diameter; though serious misgivings are aroused here by the small dimensions of the sporangia. The possibility is not to be ignored that reproductive bodies frequently rough-walled like those described by Dissmann may be formed by several members of the genus, and more particularly, perhaps, by aquatic members intimately akin to the waterlily parasite.

When the waterlily parasite is grown on maizemeal-agar plate cultures in opposition to *Plectospira myriandra,* its mycelial advance is halted along the zone of encounter as its individual hyphae (Fig. 22, B, a) become enveloped by short branches extended from filaments of the saprolegniaceous form (Fig. 22, B, b, c); envelopment in all instances being followed by darkish degeneration of the protoplasm within the *Pythium* hyphae. Similar injury is sustained by the fungus when it is grown in opposition to *Pythium oligandrum.* Its hyphae (Fig. 22, C, a; D, a) on being invested with ramifying branches put forth from filaments of the spiny form (Fig. 22, C, b; D, b) soon suffer evident degeneration of their protoplasmic contents (Fig. 22, C, a), and, besides, are often invaded lengthwise by assimilative elements (Fig. 22, D). Likewise when the fungus encounters mycelium of *Pythium acanthicum* its hyphae (Fig. 22, E, a) at the forefront of advance are halted and promptly enveloped by irregular ramifications of the delicate echinulate species (Fig. 22, E, b). Often small diverticulations intruded into a newly enveloped *undulatum* hypha (Fig. 22, F, a) from an *acanthicum* branch (Fig. 22, F, b) are found surrounded by a rather thick deposit of yellow material that gives the appearance of having been secreted as a barrier against invasion. Although invasion is frequently delayed for some time, many *undulatum* hyphae (Fig. 22, G, a; H) ultimately come to be permeated by *acanthicum* filaments (Fig. 22, G, b; H). Again, when the waterlily parasite encounters a mycelium of *Pythium periplocum* its hyphae (Fig. 22, I, a) are rather extensively though not very elaborately enveloped by ramifications from filaments of the echinulate species (Fig. 22, I, b); whereupon they soon degenerate internally, and often, in addition, are invaded longitudinally by assimilative branches.

SUMMARY

Pythium oligandrum has been found frequently in damped-off seedlings as well as in decaying stems and roots of older phanerogamic plants originating from widely separated localities in the eastern United States. Its usual occurrence in association with congeneric species familiar as agents causing damping-off and root rot, together with its ready parasitism on these species, suggests that it probably operates more commonly as a secondary than as a primary invader. Once, however, it was found, unaccompanied by any other likely pathogen, in a cucumber fruit affected with watery decay in the field; and on inoculation by incision was found capable of causing decay both in nearly full-grown cucumber fruits and in watermelon fruits. Its zoosporangia resemble those of *P. acanthicum,* but appear somewhat more often to become relatively large in volume, and to include plural globose parts. The oogonium, typically subspherical and spiny, is usually delimited proximally by a massive plug and distally by a cross-wall; it often includes a cylindrical prolongation at one or at both ends, and occasionally may be wholly cylindrical. Parthenogenetic development is generally very common; its frequency varies between different strains, and, besides, is influenced by environmental conditions. Where a male complement of 1 or 2 antheridia is present, it is usually supplied from a single branch. In most instances the mycelial connection between the male and female organs is too remote to be traced. Where such connection can be traced, it often has a total length of 250 to 600 μ, occasionally a length of only 125 μ. The type of antheridium consisting of a hyphal segment adjacent to the oogonium —the type that presumably prevails in *P. artotrogus* to the exclusion of other types—has not been recognized in any material held referable to *P. oligandrum.* The oospore when mature, shows very distinctive internal organization, as it contains usually 4 to 15 refringent bodies imbedded in the granular parietal layer surrounding the single reserve globule. After a resting period of 6 months it germinates readily on shallow irrigation, often giving rise to zoospores by discharging its undifferentiated contents directly into a vesicle through an evacuation tube 10 to 50 μ long.

Oospores of *Pythium periplocum* likewise germinate readily in pure water after a resting period of 6 months. Preliminary to germination, much as in *P. oligandrum,* an inner layer of the oospore wall amounting to about two-thirds of the thickness of the envelope, is assimilated by the protoplast. The germ hypha after attaining a length of 50 to 200 μ frequently functions as an evacuation tube in conducting the granular contents into a vesicle where they are fashioned into zoospores.

Pythium salpingophorum requires a lower temperature for zoospore production than most congeneric species. In irrigated preparations its globose sporangia are more often formed in subterminal than in terminal or intercalary positions, and are only in rather small measure given to successive development through either uniaxial elongation or subsporangial branching of the supporting hypha. The species is distinguished by pronounced distal

widening of the evacuation tube; the empty membrane of this tube becoming reflexed at the orifice somewhat in the manner of a trumpet. The rather small, smooth, subspherical oogonia frequently develop parthenogenetically, but many are supplied with 1 or 2 antheridia which may be borne on a short branch arising from the oogonial filament in close proximity to the oogonium, or, again, may be sessile either on the oogonial hypha or on a neighboring hypha. Except in the proximal and distal regions the oogonial envelope is usually adnate to the oospore wall, which at maturity encloses a protoplast of unitary organization—a single refringent body being imbedded in the granular layer surrounding the single reserve globule. After a resting period of 8 months the oospore germinates freely in pure water, often through the production of swarmers. The protoplast generally assimilates a thick inner layer of the oospore wall before putting forth a germ hypha that sometimes functions directly as an evacuation tube and at other times bears terminally a sporangium similar to sporangia of mycelial origin.

In *Pythium vexans* (= *P. complectens*), after discharge of the sporangium, the empty membrane of the evacuation tube is often though not always reflexed at the open end. As the oogonium and antheridium in this species are brought together at a very early stage, they necessarily expand in intimate contact with one another. Where a hard agar culture medium offers considerable resistance to their expansion, their outward shapes are noticeably modified; the oogonium becoming flattened or broadly indented in the region of contact, the antheridium at the same time being squeezed to fit snugly until in extreme instances it appears as an irregularly lobed mass. However, in water or soft agar the subspherical shape of the oogonium undergoes little modification, while the antheridium develops rather often into a ramified body consisting of 2 to 4 elongate digitate or more broadly lobate parts that clasp the oogonium extensively. The antheridium is regularly borne terminally on a branch arising either from a neighboring hypha or from the oogonial hypha at some distance from the oogonium. Since its base is often very close to the oogonial attachment, it frequently has much the appearance of being sessile on the oogonial filament close to the oogonium. Oospores of *P. vexans* soon germinate freely in pure water, often giving rise to swarmers. The protoplast, after assimilating a thickish inner layer of the oospore wall, sometimes extends a germ hypha that functions directly as an evacuation tube, and at other times produces a structurally distinct zoosporangium which may be sessile on the oogonial envelope or may be terminal on a germ hypha of variable length.

On shallow irrigation, oospores of *Pythium anandrum* from cultures 3 months old germinate readily, usually giving rise to swarm spores. After absorbing a thick inner layer of the oospore wall the protoplast often puts forth a stout germ hypha that subsequently functions directly as an evacuation tube. In other instances a zoosporangium structurally distinct from the oospore is produced terminally on a germ hypha up to 200 μ in length. Such a sporangium may also be formed sessile on the oogonial envelope, or

may even be deeply inserted into the chamber of the oospore. Repetitional development of zoospores is accomplished in *P. anandrum* usually, as in *P. oligandrum, P. vexans,* and *P. undulatum,* by direct production of an evacuation tube, but also takes place occasionally through production of a miniature sporangium.

A fungus isolated from waterlily leaves in Massachusetts is referred to *Pythium undulatum* Petersen *sensu* Dissmann by reason of its prolate ellipsoidal, distally papillate sporangia and its large rough-walled resting spores. In their mature condition the resting spores show an internal organization similar to that in oospores of *P. helicoides,* and are surrounded individually by a wall consisting of a thin (0.5 μ) colorless layer and a somewhat thicker (0.8 μ) yellowish inner layer. Resting spores from cultures 6 months old germinate readily on shallow irrigation by producing 1 to 4 sporangia on germ hyphae 10 μ to 3 mm. long. After germination the inner layer of the spore wall appears much thicker (2 to 3 μ) than before. It seems probable that the resting spore represents a parthenospore homologous with the oospore of *P. helicoides.*

When *Pythium salpingophorum, P. vexans, P. undulatum,* and *P. anandrum* are grown on an agar substratum in opposition to *P. oligandrum* or *P. periplocum* or *P. acanthicum,* their mycelial advance is halted as their hyphae in varying measure become enveloped by branches extended from the filaments of the opponent fungus; the enveloped hyphae usually soon showing degeneration of their contents, and often in addition undergoing invasion by assimilative elements. Similar injury is sustained by them when they are grown in opposition to *Aphanomyces cladogamus* or *Plectospira myriandra. P. vexans* appears occasionally to retaliate upon *P. periplocum* by applying appressoria to hyphae of the echinulate form.

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