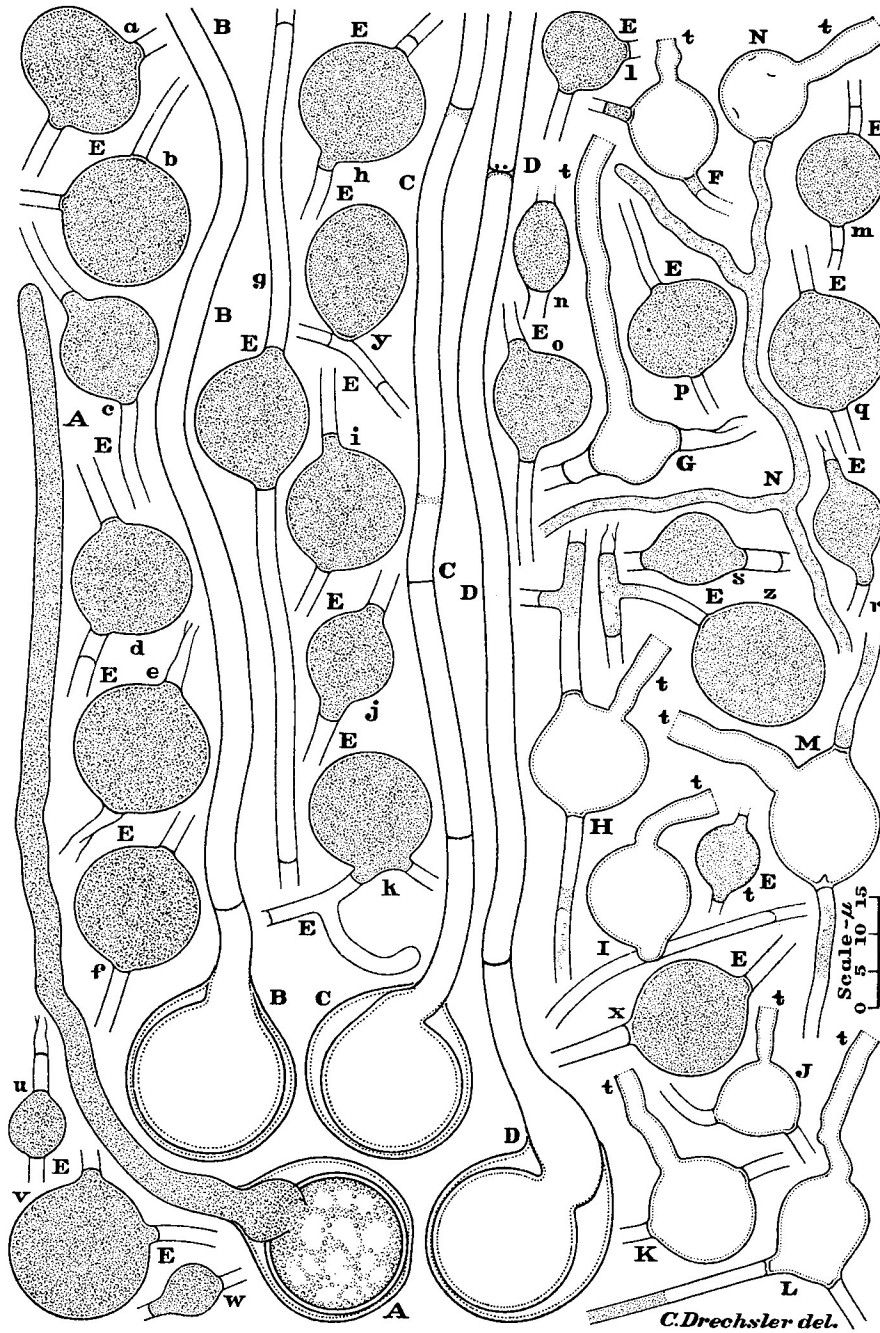


PRODUCTION OF ZOOSPORES FROM GERMINATING
OOSPORES OF *PYTHIUM ULTIMUM* AND
*PYTHIUM DEBARYANUM*¹CHARLES DRECHSLER²

Seventy years ago de Bary (1881b, p. 525) described oospores of *Pythium debaryanum* Hesse as germinating, after a resting period of 4 to 5 months, by production of a germ hypha, which in pure water devoid of nutrients would grow out and ramify rather considerably before it eventually died off in forming a resting conidium. If the germ hypha reached a favorable substratum it formed an extensive mycelium capable of normal reproduction. It was not seen giving rise to zoosporangia. De Bary's (1881 a, p. 12-14, Taf. I, figs. 9a, 10-13, 14b, 16-19; 1881 b, fig. 1) detailed account of the developmental morphology of the antheridium in *P. debaryanum*—the manner of origin of this organ, its shape, and its most usual positional and postural relationships to the oogonium—indicates that his studies on the formative and mature stages of sexual reproduction very probably were carried out for the most part on material of the exceedingly widespread damping-off parasite which on grounds set forth earlier (Drechsler 1927, 1935) seems best referable to *P. ultimum* Trow. Yet as the damping-off fungus best conforming to the figures of sporangia and sexual reproductive apparatus that are given in the original account of *P. debaryanum* (Hesse 1874, figs. 5-9, 11, 13, 14) is likewise ubiquitous and abundant in moist temperate lands, often, indeed, occurring together with *P. ultimum*, it could presumably have also been present in addition to the 6 other much less frequent congeneric species de Bary found growing out of affected garden cress (*Lepidium sativum* L.) seedlings. Assuredly the zoospore development which de Bary ascribed to *P. debaryanum* and which puzzled him by its pronounced inconstancy—some cultures producing rather many functional zoosporangia while others produced none—could more readily have come from an admixture of the species named in his honor than from the parasite whose sexual development he so accurately described. It seems therefore not altogether impossible that the single germinating oospore he figured without an attached empty antheridium (de Bary 1881b, fig. 2) may really have belonged to Hesse's species, though, again, it may with at least equal probability have belonged to *P. ultimum*. When Trow (1901) twenty years later described *P. ultimum* as a new species he set forth its oospores as germinating

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always by the production of one or more germ tubes, either soon after reaching maturity or after they had rested for a variable period extending up to 7 months.

The commonplace vegetative germination ascribed by de Bary to the oospores of *Pythium debaryanum* and by Trow to those of *P. ultimum* can be achieved readily and abundantly in these 2 species at the temperatures ordinarily prevailing in most American laboratories. Maizemeal-agar plate cultures of either species containing in suspension some finely divided maize-meal will usually show rapid mycelial growth, copious sexual reproduction, and subsequently gradual after-ripening of oospores, not only in summer when indoor temperatures fluctuate commonly between 25° and 33° C., but also in winter when rooms are kept heated to temperatures ranging from 22° to 28° C. To insure this sequence of development it is necessary only to keep the cultures from drying out—a matter easily accomplished by placing them in stacks under bell-jars or inverted battery jars. If after such storage for a period of 2 to 4 months, portions of agar are removed to a shallow layer of pure water, many of the oospores will germinate promptly at ordinary laboratory temperature by putting forth a germ hypha (fig. 1, A-D) capable of extensive mycelial growth.

As has been made known in a preliminary report (Drechsler 1946a) more distinctive germination by production of motile zoospores ensues when after-ripened oospores of *Pythium debaryanum* or of *P. ultimum* are transferred to pure water at lower temperatures. This type of germinative development is of moment especially in the case of *P. ultimum*, since this species generally displays very little inclination, even under chilly conditions, to produce zoospores from its ordinary asexual conidia. All pure cultures so far tried out, which from the character of their mycelium and the morphology of their sexual reproductive apparatus could be referred to *P. ultimum*, have proved reluctant to form zoospores from their conidia of mycelial origin, while showing much readiness and consistency in producing them from their after-ripened oospores. The figures herein given to illustrate the formative and germinative phases of oospore development in Trow's species were prepared mainly from 2 cultures obtained as manifest causal agents in separate instances of plant disease recorded in earlier papers. Of these 2 cultures one (fig. 2, A-M; fig. 3, A-J) was isolated in 1924 from a cabbage (*Brassica oleracea* L. var. *capitata* L.) head affected with stump rot

FIG. 1. *Pythium ultimum*, drawn to a uniform magnification with the aid of a camera lucida; $\times 1000$ throughout. A, after-ripened oospore germinating by production of a germ tube. B-D, Oospore envelopes, each with empty proximal portion of a long vegetative germ hypha. E, Small conidia or zoosporangia, a-z, formed on germ hyphae. F-N, Empty envelopes of such small zoosporangia that have discharged their contents through the evacuation tube, t, for conversion into zoospores. (A-D, M, N, drawn from sweet-potato strain; E (a-z), F-L, drawn from cabbage strain.)

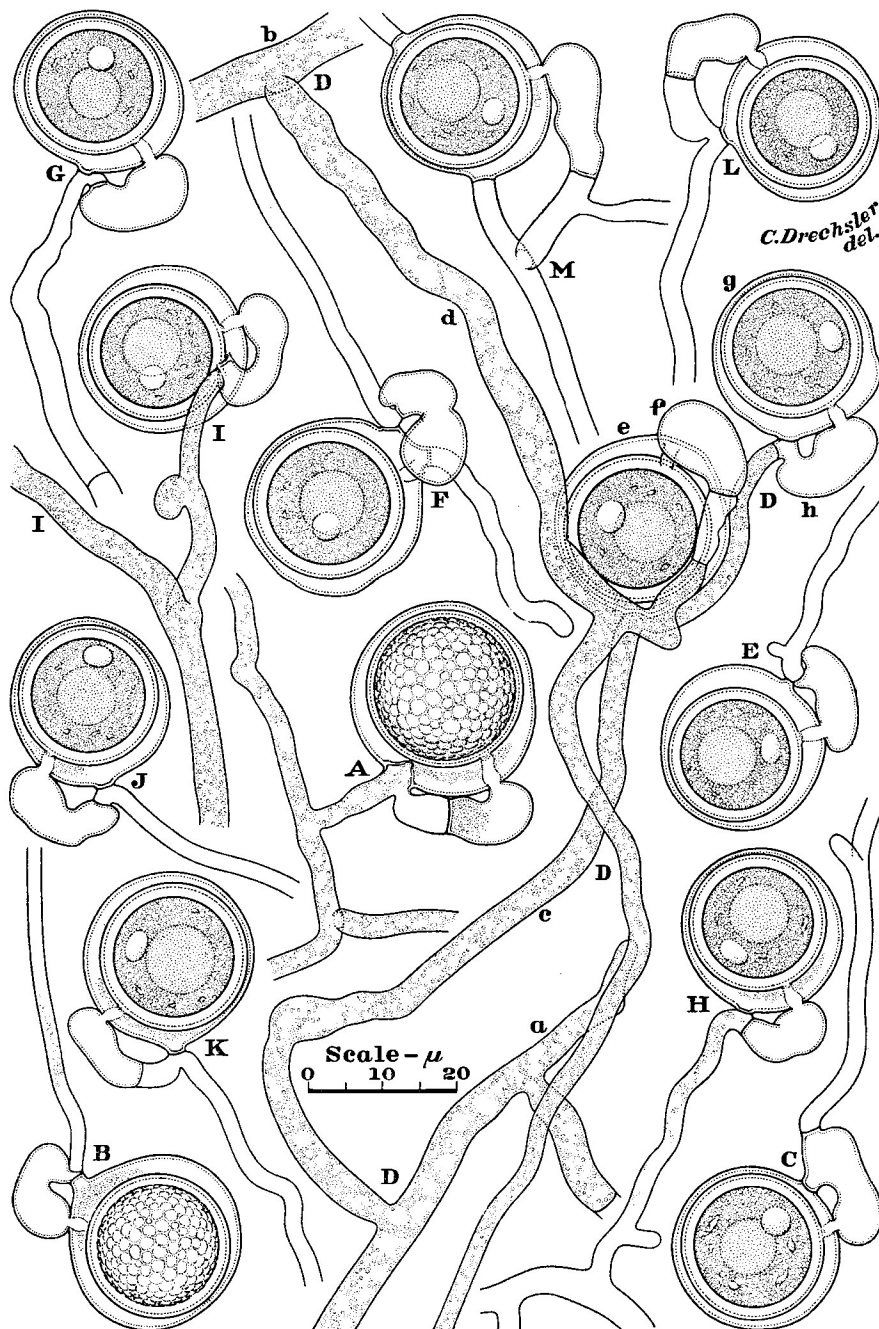
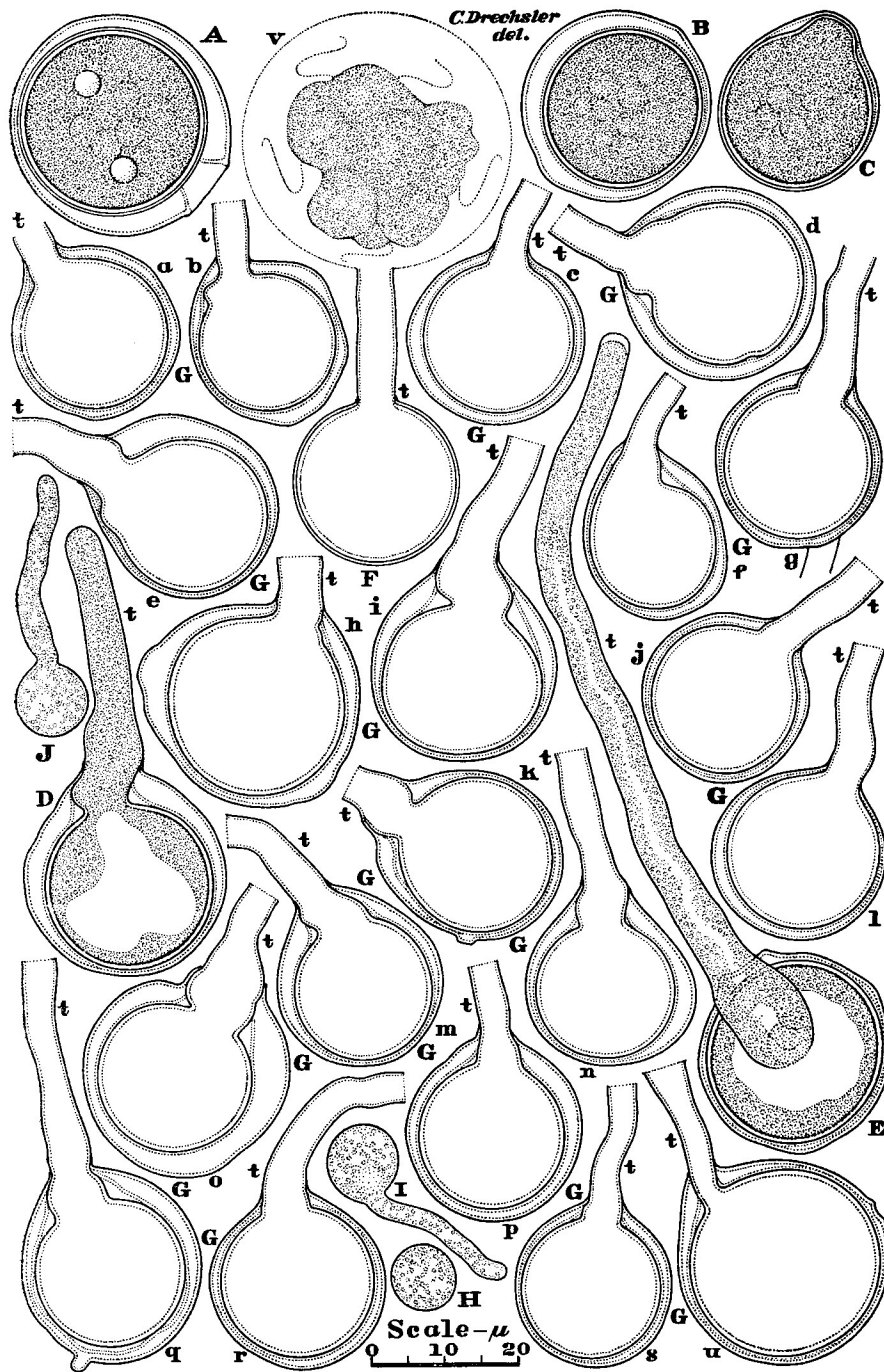


FIG. 2. Sexual reproductive apparatus of *Pythium ultimum* (cabbage strain) drawn with the aid of a camera lucida, at a uniform magnification, from the underside of maize meal-agar plate cultures; $\times 1000$ throughout. Explanation of all parts given in text.

(Drechsler 1925), while the other (fig. 4, A-O; fig. 5, A-Z) was received the same year from L. L. Harter, who had isolated it from a darkened rootlet of a sweetpotato (*Ipomoea batatas* (L.) Lam. var. Red Brazil) slip in the spring of 1924 (Harter 1925). Occasion is taken to include also a few supplementary drawings of conidia and sexual reproductive apparatus that were prepared from 2 cultures labelled "G 5" (fig. 6, A-G) and "G 3" (fig. 6, H-M), respectively, which Harry Braun kindly gave me in February, 1925, as being representative of forms he had isolated from decaying geranium (*Pelargonium* sp.) cuttings and had treated separately under the names *P. debaryanum* and *P. debaryanum* var. *pelargonii* in a paper that was published some months later (Braun 1925). Both of Braun's cultures were clearly referable to *P. ultimum* as that species is understood here and were wholly alien to Hesse's species. Several units of sexual apparatus (fig. 6, N-R) drawn from a culture isolated from a partly decayed geranium cutting in a greenhouse near Cedarville, N. J., on May 29, 1924, likewise reveal the make-up characteristic of *P. ultimum*, as do also 3 additional units (fig. 6, S-U) drawn from 3 separate cultures isolated originally from separate collections of softened pea (*Pisum sativum* L.) roots. Of the 3 pea-root collections one was made near Onalaska, Wis., in the spring of 1922 (fig. 6, S), another near Salt Lake City, Utah, on August 20, 1923 (fig. 6, T), and the third in Arlington, Va., on June 9, 1921 (fig. 6, U). A culture originally isolated from a softened pea root taken from a field near Rochelle, Ill., on June 12, 1924, was used in preparing the figures relating to sexual reproduction (fig. 7, A-T) and oospore germination (fig. 8, A-V) in *P. debaryanum*.

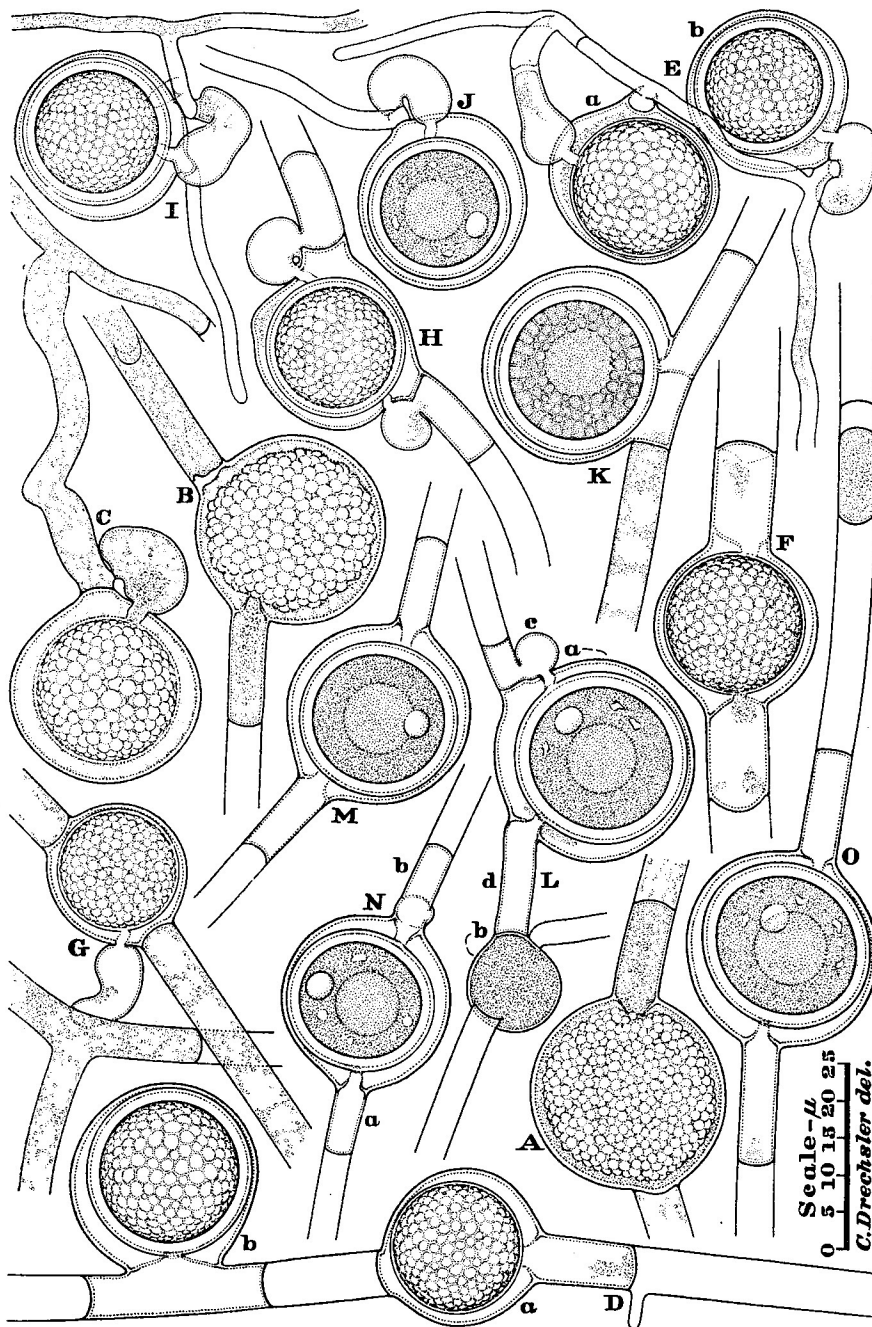
The oogonia of *Pythium ultimum* are borne either terminally (fig. 6, A, I-K, N-P, R, S) or intercalarily (fig. 6, B-D, H, Q, T, U). Their position in considerable degree seems to be governed by the width of their supporting hyphae. On the under side of agar plate cultures, where mycelial filaments are relatively narrow, the female cell is formed terminally (fig. 2, A-C; D, g; E; G-L) more often than in subterminal (fig. 2, F) or intercalary (fig. 2, M) positions. Units of sexual apparatus produced by the wider hyphae abundant on the upper side of a maize meal-agar plate culture commonly show the oogonium in an intercalary position (fig. 4, A; B; D, a; F-H; K; L, a; M-O), though here and there a stout hypha may bear an oogonium terminally (fig. 4, C) or laterally (4, D, b). Slender hyphae on the upper surface of an agar plate culture will, as elsewhere, most often give rise to oogonia terminally (fig. 4, E, b; J) or subterminally (fig. 4, I). Now and then an oogonium that was formed terminally (fig. 2, D, e; fig. 4, E, b) comes to occupy an approximately lateral position when the supporting hypha grows out below the basal septum, often to produce a second oogonium (fig. 2, D, g; fig. 4, E, a) some distance forward.



The width of the supporting filament affects also the position and shape of antheridia in monoclinal units of sexual reproductive apparatus. Where a wide filament bears an intercalary oogonium, fertilization is accomplished often by a single antheridium that consists merely of an adjacent cylindrical hyphal segment (fig. 4, A; B; D, a; K. fig. 6, Q). Frequently, again, a male cell of unmodified cylindrical shape is delimited at each pole of the oogonium (fig. 4, F, M, O; fig. 6, D, a, b), though often when 2 adjacent antheridia are present one of them may be of plain cylindrical shape (fig. 4, L, d; N, a. fig. 6, T, a), while the other is modified by a protrusion close to the oogonium (fig. 4, L, c; N, b. fig. 6, T, b), or each of the two may have a bulbous protrusion (fig. 4, H). Adjacent antheridia of unmodified cylindrical shape always extend their fertilization tube through the cross-wall delimiting them from the oogonium, and this is true also of some antheridia which have only a rather small lateral protrusion (fig. 4, N, b). Where the protrusion is fairly large the fertilization tube is usually pushed through the subspherical envelope of the oogonium in a position not far from the cross-wall (fig. 4, H; L, c. fig. 6, T, b). In monoclinal units of sexual apparatus that are borne terminally on rather slender hyphae an adjacent antheridium often consists of a small hyphal part together with a lateral protuberant part many times larger (fig. 2, B; C; D, h; I. fig. 6, A, I, K, O, P, S). Very often, too, in such terminal monoclinal units the antheridium includes no hyphal part at all, but is derived entirely from the lateral outgrowth. The whole outgrowth may be included in the antheridium, so that this organ appears as a plump arched body sessile on the supporting hypha (fig. 2, E, F; fig. 4, C, J; fig. 6, H, N); or a proximal portion of the outgrowth, sometimes very short (fig. 2, G, H, J. fig. 4, E, b; I) but at other times longer (fig. 2, A, K, L), may serve as a stalk terminally supporting the curved male cell.

Development of an antheridium largely or wholly from a lateral outgrowth extended from a position immediately below the basal septum of a terminal oogonium often brings the oogonium markedly out of alignment with its supporting hypha. In water preparations, as de Bary (1881a, p. 14) noted, and similarly in very soft gel substrata, the antheridium, though originating laterally below the terminal oogonium, will tend so strongly to

FIG. 3. Germination of oospores from 90-day-old maize-meal-agar plate cultures of *Pythium ultimum* (cabbage strain); all parts drawn to a uniform magnification with the aid of a camera lucida; $\times 1000$ throughout. A, B, After-ripened oospores. C, After-ripened oospore beginning to germinate. D, Oospore with elongating germ tube, t. E, Oospore with unusually long germ tube, t, surmounted by a cap of dehiscence. F, Oospore that has discharged its contents through the evacuation tube, t, into the vesicle, v, where they are being fashioned into zoospores. G, Empty membranous envelopes (a-s, u) of oogonium, oospore, and evacuation tube, t, remaining after escape of zoospores. H, Encysted zoospore. I, J, Encysted zoospores, each putting forth a germ tube.

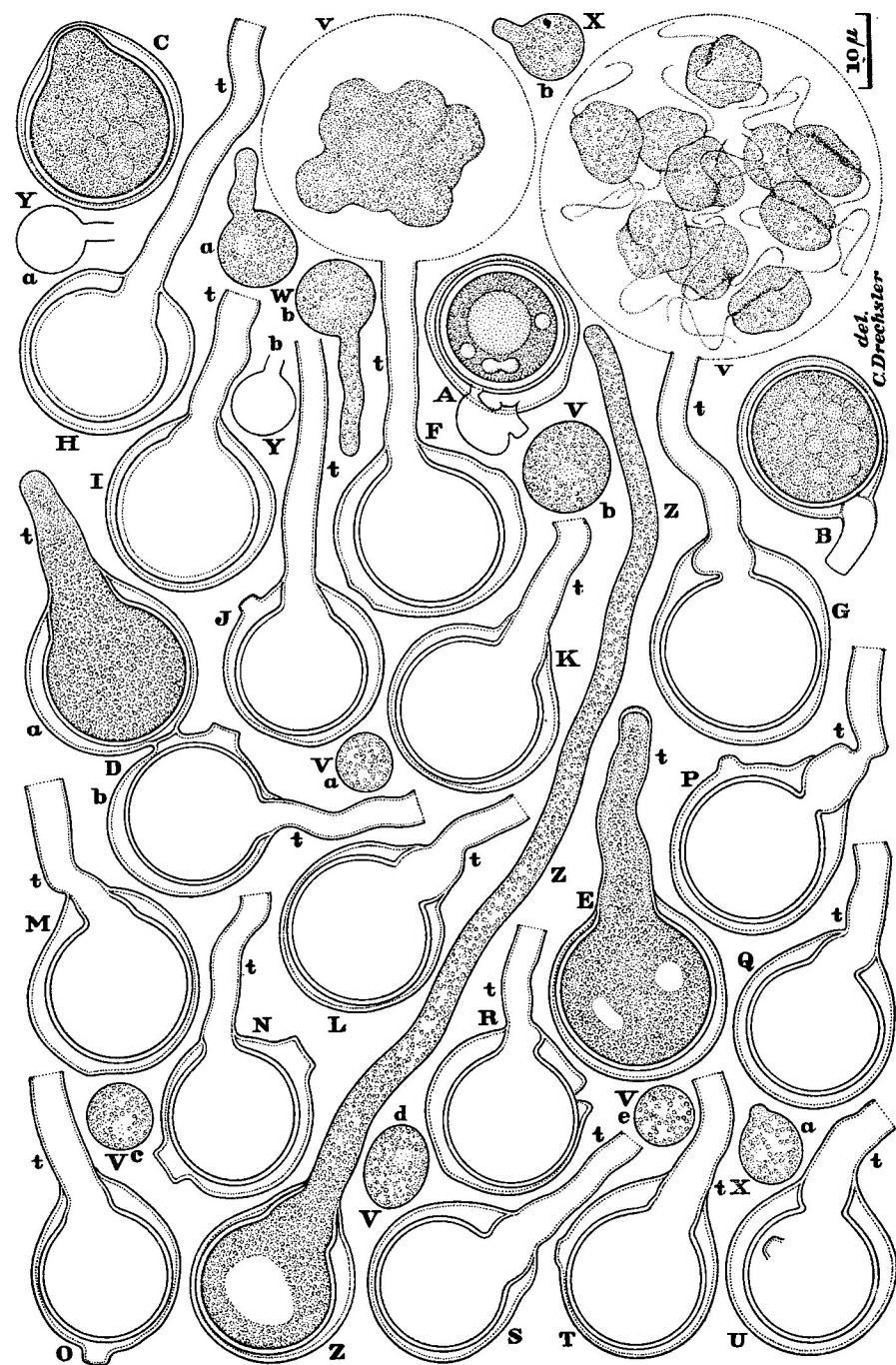


grow straightforward that the oogonium is pushed aside and may come to lie at a right angle with the supporting hypha. In moderately firm agar cultures, where massive parts cannot move freely in response to growth pressures, displacement of the oogonium is impeded, though some approximation to the angular relationships observable in irrigated material may come about here through rather abrupt bending of the distal portion of the supporting hypha (fig. 2, B; D, g, h. fig. 6, I, P, S).

Although the sexual reproductive apparatus of *Pythium ultimum* is predominantly monoclinal, units of diclinal origin are not infrequent in the species. Sometimes two mycelial filaments (fig. 2, D, a, b) rather far apart give off long branches (fig. 2, D, c, d) on which the oogonium (fig. 2, D, e) and the antheridium (fig. 2, D, f) fertilizing it are borne terminally, the male cell then usually being of curved clavate shape. In instances where the two mycelial filaments supplying the apposed organs are closer together, the antheridium may be formed on a short stalk (fig. 6, R) or may be borne laterally and sessile as a straight or somewhat curved, elongated utriform structure (fig. 4, G). Most often when the male hypha lies close to the oogonium, the antheridium consists of an intercalary segment of the hypha together with a protuberant part (fig. 6, B; D, c; T, c) in somewhat broad contact with the oogonium—a make-up more or less suggestive of the antheridia in *P. butleri* Subr. and *P. aphanidermatum* (Eds.) Fitzp.² Sometimes an intercalary antheridium of diclinal origin will have 2 protuberant parts, each of them provided with a fertilization tube (fig. 6, C).

² These 2 closely related species, which were adjudged to be identical by Carpenter (1921) and Fitzpatrick (1923) though apparently neither author had studied material referable to either species, are distinguished here on grounds set forth briefly in an abstract (Drechsler 1934). My examination of Dr. Edson's excellent microscope mounts, together with the adverse comments he made when on various occasions during the 5 years following 1922 he kindly examined maize-meal-agar plate cultures and irrigated preparations of the larger, strongly lobulated species, gave good reason for holding that his description (Edson 1915) was based on the smaller, often rather meagerly lobulated species which soon afterwards came to light in 3 cultures that R. D. Rands isolated from sugarcane roots in Louisiana. Since these cultures were found unmistakably conspecific with 2 Sumatra cultures that represented the same stem-burn parasite—the "*Pythium debaryanum*" of Joehms (1927)—on which Meurs (1934) based his description of *P. deliense*, it would seem that *P. aphanidermatum* has a synonym in *P. deliense* rather than in *P. butleri*.

FIG. 4. Sexual reproductive apparatus of *Pythium ultimum* (sweet-potato strain) drawn with the aid of a camera lucida, at a uniform magnification, from the upper side of maize-meal-agar plate cultures; $\times 1000$ throughout. A, B, Oogonia being fertilized by adjacent antheridia. C, Fertilized oogonium showing young oospore with thin wall. D, E, Portions of hypha, each with two oogonia, whereof one, a, shows an oospore with very thin wall, while the other, b, contains an oospore with a thicker wall. F, G, Oogonia, each with a young thin-walled oospore. H, I, Oogonia containing oospores with thicker walls. J, Mature monoclinal sexual reproductive unit. K, Somewhat immature monoclinal unit. L, Portion of hypha with oogonium, a, and conidium, b; the former having been fertilized by two antheridia, c and d. M–O, Mature monoclinal sexual units.



Such production of 2 fertilization tubes, instances of which are also observable now and then in monoclinal lateral antheridia that arise in immediate proximity to the oogonium, may be regarded as exceptional. Departures from the more usual trends of development result in miscellaneous variations. In monoclinal terminal reproductive units, for example, the adjacent antheridium, usually of cylindrical shape, sometimes widens markedly toward the broad base of the oogonium (fig. 6, J), its conformation then representing a frustrum of an inverted cone. Occasionally, again, in monoclinal apparatus a lateral (fig. 4, E, a) or an intercalary (fig. 2, M) oogonium may be found supplied, as in *P. debaryanum*, with an antheridium borne on a short branch arising 10 to 15 μ away. In scattered instances an oogonium formed laterally on a wide hypha will, on conversion of the adjoining portion of the hypha into a male cell, come to be sessile on an intercalary antheridium (fig. 4, D, b).

Development within the oogonium follows the course usual in members of the genus. At the time of fertilization the protoplasmic mass is drawn away from the oogonial envelope (fig. 4, A, B). After the antheridium has contributed most or all of its granular contents, the fertilized protoplast assumes a more accurately spherical shape and lays down a thin colorless peripheral membrane (fig. 4, C; D, a; E, a; F; G). The coarsely lumpy protoplasmic mass now undergoes some little further contraction as it secretes a second wall layer thicker than the first (fig. 2, A; B. fig. 4, D, b; E, b; H; I). A homogeneous reserve globule appears in the center of the young oospore, its progressive enlargement constraining the lumpy mass into a parietal layer (fig. 4, K). Thereupon when the lumps become resolved into small granules, and a single subspherical or slightly flattened refringent body emerges clearly in the granular material, the oospore reaches its fully mature resting condition (fig. 2, C; D, e, g; E-M. fig. 4, J; L, a; M-O. fig. 6, A-D; H-K; N-U). In this condition the oospore regularly fails to germinate when it is placed in pure water. During prolonged storage at a low temperature—at 5° C., for example—such dormant oospores reveal

FIG. 5. Germination of oospores from 90-day-old maize-meal-agar plate cultures of *Pythium ultimum* (sweetpotato strain); all parts drawn to a uniform magnification with the aid of a camera lucida; $\times 1000$ throughout. A, Oospore in early stage of after-ripening. B, After-ripened oospore. C, Oospore beginning to germinate. D, two adjacent oogonia, a and b; from the oospore in the oogonium, a, the germ tube, t, has grown out; the oospore in oogonium, b, has emptied out its contents through the evacuation tube, t. E, Oospore with germ tube surmounted by cap of dehiscence. F, Discharged oospore; t, evacuation tube; v, vesicle with protoplasmic mass undergoing cleavage. G, Discharged oospore; t, evacuation tube; v, vesicle with ten full-fledged zoospores. H-U, Empty envelopes of oogonium, oospore, and evacuation tube, t. V, Encysted zoospores, a-e. W, Encysted oospores, a and b, germinating vegetatively. X, Encysted zoospores, a and b, each with papilla of dehiscence. Y, Zoospore cyst envelopes, a and b, after escape of secondary motile zoospore. Z, Oospore with long vegetative germ hypha.

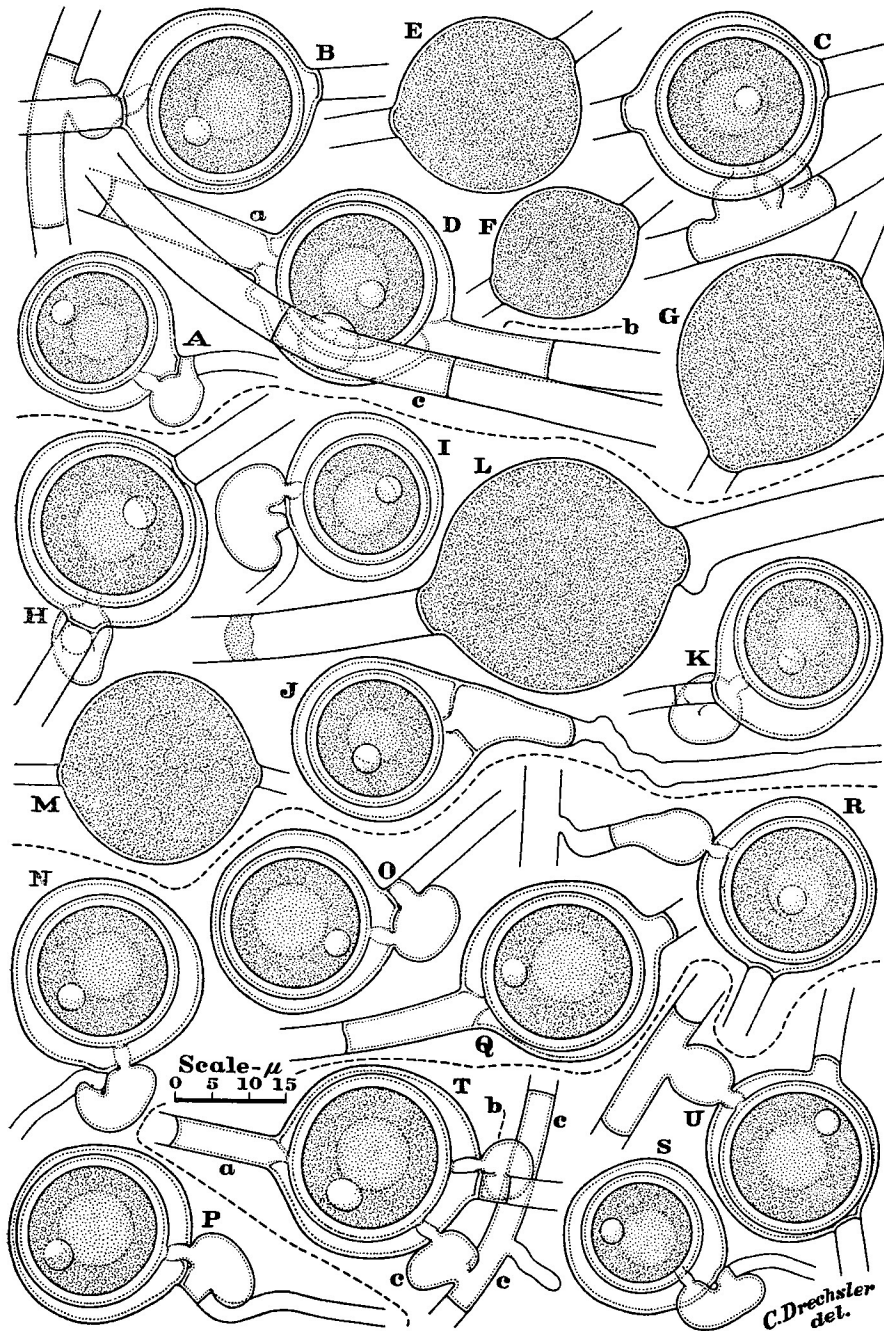


FIG. 6. *Pythium ultimum*, drawn to a uniform magnification from maizemeal-agar plate cultures, with the aid of a camera lucida; $\times 1000$ throughout. Explanation of all parts and sources of all cultures given in text.

little change in their internal organization, and show correspondingly little increase in their capacity for prompt germination. However at temperatures ordinarily prevailing in laboratories, the changes of after-ripening become clearly noticeable in scattered oospores within about 25 or 35 days, and after 90 days by far the greater number of oospores will have lost their durable appearance and their inert behavior.

In the earlier stages of after-ripening the oospore of *Pythium ultimum* often contains 2, 3, or 4 refringent bodies (fig. 3, A; fig. 5, A) which sometimes are noticeably smaller than the single refringent body present at maturity. As their size and their luster diminish the plural refringent bodies are gradually lost to view in the protoplasmic mass surrounding them. Meanwhile the protoplasmic mass has gained in volume not only by resorbing in large part the thick yellowish inner layer of the oospore wall, but also by intermixing and merging with the material in the reserve globule (fig. 3, B; fig. 5, B). Consequently the after-ripened oospore is a rather thin-walled globose body filled with densely granular protoplasm in which about a dozen subspherical vacuoles, mostly 2 to 3.5 μ wide, are indistinctly visible. In its shape, as also in the character of its contents, it closely resembles conidia of mycelial origin (fig. 4, L, b; fig. 6, E-G, L, M) though its endogenous character is manifest in the presence of the normally persisting oogonial envelope. With respect to size, oospores, whether in a dormant or in an after-ripened condition, are generally somewhat less widely variable than conidia of mycelial origin. In most cultures they fail to attain the largest dimensions observable among asexual conidia.

When placed in fresh water the after-ripened oospore puts forth a broad protuberance which on reaching the oogonial envelope (fig. 3, C; fig. 5, C) soon pushes its way through that membrane to elongate externally (fig. 3, D, t; fig. 5, D, a: t). At temperatures near 10° C. the elongating germ tube frequently forms a hyaline cap of dehiscence (fig. 5, E, t) on reaching a length of 5 to 40 μ , though occasionally a germ hypha as much as 100 μ long is found apically modified for discharge (fig. 3, E, t). The protoplasmic contents migrate through the germ hypha (fig. 3, F, t. fig. 5, F, t; G, t) into a vesicle (fig. 3, F, v. fig. 5, F, v; G, v) and are there fashioned into laterally biciliate motile zoospores in the manner characteristic of the genus. After the swimmers have escaped from the evanescent vesicle, the empty evacuation tube (fig. 3, G, a-s: t; u: t. fig. 5, H-U: t) is found terminating abruptly at its open mouth, usually without any indication of being reflexed. Frequently the reduced wall of the oospore remains undivided throughout and may be shown advantageously as a double-contoured envelope slightly thicker than the oogonial membrane loosely surrounding it (fig. 5, H-U). Yet with respect to structure the reduced oospore wall would seem represented somewhat better as a triple-contoured envelope (fig. 3,

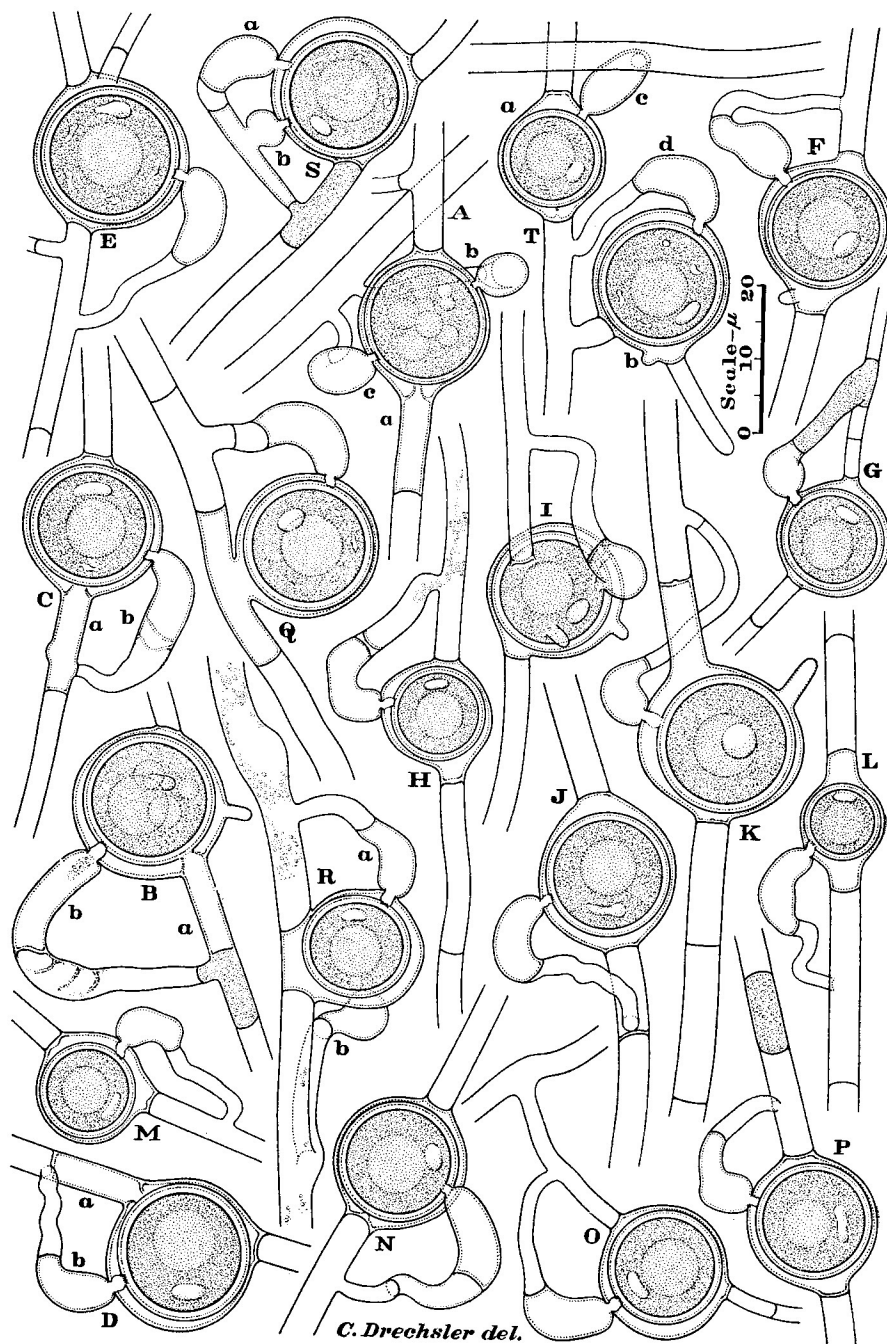


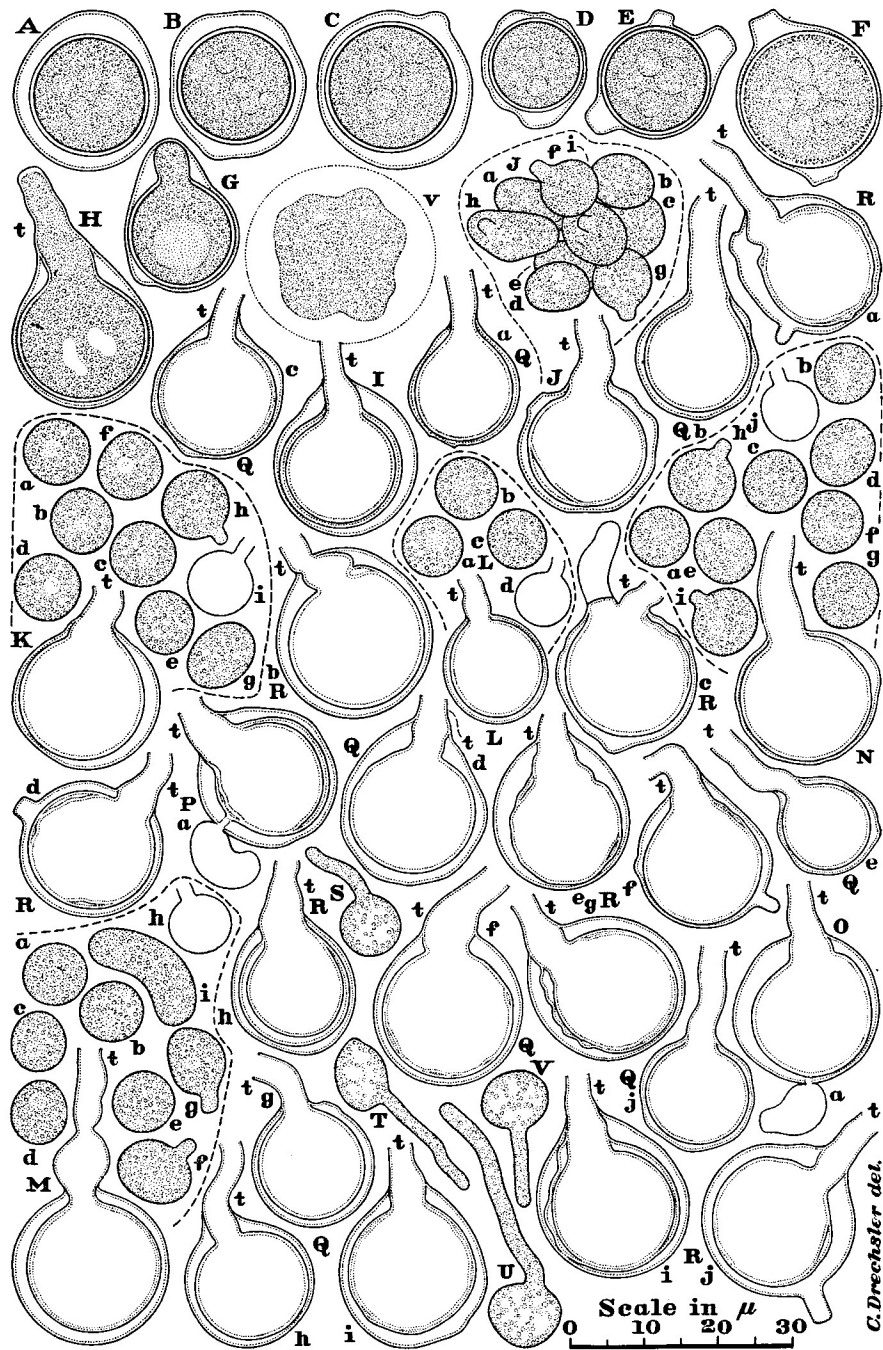
FIG. 7. Sexual reproductive apparatus of *Pythium debaryanum* drawn from maize-meal-agar plate cultures at a uniform magnification with the aid of a camera lucida; $\times 1000$ throughout. Explanation of all parts given in text.

G, a-s, u) in which a thin colorless outer layer is distinguished from a thin, slightly yellowish, inner layer. Near the base of the evacuation tube the outer layer is sometimes found locally separated from the inner one (fig. 3, D; G, b, d, f, h, i, k, m-q, s, u) apparently as the result of tensions earlier exerted by the growing tube.

After-ripened oospores of approximately average size commonly yield 8, 9, or 10 motile zoospores (fig. 5, G), while those of greater or smaller volume give rise to swimmers in correspondingly larger or smaller numbers. Following a variable period of motility the zoospores normally round up into globose cysts mostly 8 to 10 μ , rarely up to 13 μ , in diameter (fig. 3, H; fig. 5, V, a-e). Some time later they usually germinate by putting forth a vegetative germ hypha (fig. 3, I, J; fig. 5, W, a, b), but occasionally where a temperature near 10° C. is maintained they will form a papilla of dehiscence (fig. 5, X, a, b) and discharge their protoplast to develop externally into a secondary swimmer. Each instance of such repetitional development leaves behind an empty subspherical cyst envelope that bears an evacuation tube open at its tip (fig. 5, Y, a, b).

As a rule when the germ hypha from an after-ripened oospore of *Pythium ultimum* has reached a length well in excess of 100 μ (fig. 1, A; fig. 5, Z) it is little likely to serve as an evacuation tube, but will almost certainly continue growth, whether under cold or under warm conditions, as a vegetative filament. In pure water such a germ hypha will often attain a length of 2 or 3 millimeters and may then, after some little branching, give rise to a conidium or in occasional instances to 2 or 3 conidia. The globose asexual reproductive bodies thus formed, either in intercalary (fig. 1, E, a-y) or in terminal positions (fig. 1, E, z), commonly measure 7.5 to 20 μ in transverse diameter. They are, of course, rather markedly smaller than the general run of ordinary conidia developed on well nourished mycelium in vigorous cultures. If the water in which they have been newly formed is replaced at intervals and if they are kept at a temperature near 10° C., a substantial proportion of them (fig. 1, F-N) will develop as zoosporangia by putting forth an evacuation tube (fig. 1, F-N:t) and then discharging their contents into a vesicle to be fashioned into motile zoospores.

In *Pythium debaryanum* production of zoospores is a commonplace phase of development and might, indeed, be nearly as familiar among students of plant diseases as Hesse's binomial, if only during winter our laboratory buildings were not heated so inexorably for tropical comfort. At the bracing temperatures between 16° and 18° C., which can sometimes be maintained in individual rooms by shutting off all radiators and closing all inside doors, ordinary conidia of the fungus will usually give rise to zoospores in ample abundance within a few hours after they have been placed



in a shallow layer of water, directly following their removal from maize-meal-agar cultures 10 to 20 days old. In an agreeably cool laboratory, zoospore production will often intrude spontaneously. Thus, in my experience, when thin slabs excised from agar cultures were examined under the microscope in sparingly moistened covered preparations, numerous conidia around the margin would frequently begin germinating within 30 minutes and in about 2 hours would be liberating active zoospores. More prolific zoospore development may be obtained through shallow irrigation of slabs excised from young Lima-bean-agar plate cultures.

The sexual reproductive apparatus of *Pythium debaryanum*, when considered collectively, is not difficult to distinguish from that of *P. ultimum*. In monoclinal units adjacent antheridia of cylindrical shape (fig. 7, A-D: a) are usually infrequent, and often are to be discovered only after some search. Most commonly antheridia of monoclinal origin are borne terminally on a branch arising at a distance from the oogonium about equal to the diameter of that organ (fig. 7, B-D: b; E-Q; R, a, b; S, a; T, d). On the whole the hyphal connection between the apposed sex organs would seem to correspond well to that shown in the relevant figures given by Hesse (1874, Tafel II, figs. 13, 14). Occasionally a male branch bearing a terminal antheridium may supply also an intercalary antheridium (fig. 7, S, b) provided with a protuberant part in contact with the oogonium. In diclinal reproductive apparatus the antheridium may be sessile (fig. 7, T, c), or may be borne terminally on a branch (fig. 7, A, b, c). As antheridial branches are conspicuously longer and more circuitous on moist agar media than on drier substrata, sexual apparatus produced on irrigated material appropriate for study of zoospore production often has a characteristically rangier appearance than the reproductive units illustrated herein (fig. 7, A-T).

FIG. 8. Germination of oospores from 90-day-old maize-meal-agar plate cultures of *Pythium debaryanum*; all parts drawn to a uniform magnification with the aid of a camera lucida; $\times 1000$ throughout. A-F, Fully after-ripened oospores. G, Oospore in early stage of germination. H, Oospore with short germ tube, t, surmounted by cap of dehiscence. I, Empty envelopes of oogonium, oospore, and evacuation tube, t; v, vesicle containing uncleaved protoplasmic mass. J, Empty envelopes of oogonium, oospore, and evacuation tube, t, together with cluster of nine encysted zoospores, a-i. K-N, Empty membranous envelopes of oogonia and oospores, together with the concomitant encysted zoospores lying around or near the open tip of the evacuation tube, t, in numbers of nine (a-i), four (a-d), nine (a-i), and ten (a-j), respectively. O, Membranes left after germination of oospore by development of zoospores: a, empty antheridium; t, open evacuation tube; the oospore envelope here shows no separation of layers. P, Membranes like those in O, but oospore envelope here showing extensive separation into two layers. Q, Empty membranes of sexual reproductive units, a-j, including envelopes of oogonium, oospore, and evacuation tube, t, left after germination of oospore by production of zoospores; the oospore envelope not extensively separated into two layers. R, Empty membranes of sexual reproductive units, a-j, like those in Q, but oospore envelopes here somewhat more markedly or more extensively separated into two layers. S-V, Encysted zoospores, each germinating by production of a germ tube.

After fertilization is accomplished, development within the oogonium proceeds in *Pythium debaryanum* much as in *P. ultimum*. In the later stages of maturation an oospore may show several reserve globules (fig. 7, A, B). Completely mature oospores reveal the unitary internal organization most usual among members of the genus—a single central reserve globule being surrounded by a densely granular parietal layer within which is imbedded a single refringent body (fig. 7, C-S; T, a, b). The refringent body would seem generally somewhat more strongly flattened in *P. debaryanum* than in *P. ultimum*. The yellowish inner layer of the oospore wall is markedly thinner—commonly by about .4 or .5 μ —in the former species than in the latter. Indeed all associated membranous parts—the oogonial wall as well as the envelope of the antheridium and its supporting branch—appear generally thinner in *P. debaryanum*, are more often discernible only with difficulty, and are wont to vanish from sight earlier than in *P. ultimum*. Contrary to expectations that might be suggested by their less formidable incasement, the oospores of *P. debaryanum* are usually a little slower to show the internal changes which accompany after-ripening. On being stored for 90 days at ordinary room temperatures, maize-meal-agar plate cultures of *P. debaryanum* will often show approximately one-third of the oospores in a fully after-ripened condition, whereas in similar cultures of *P. ultimum* more than three-fourths will be ready for germination.

In *Pythium debaryanum* as in *P. ultimum* oospores capable of prompt germination (fig. 8, A-F) closely resemble conidia of mycelial origin—the wall being markedly reduced in thickness through resorption on its inner side, and the protoplasmic mass appearing of relatively uniform granular texture except for about a dozen globular vacuoles indistinctly visible in the central region. When some fresh water is supplied the oospore forms a protuberance that soon pushes against the oogonial envelope (fig. 8, G) and breaks through that barrier to elongate externally as a germ tube. Under warm conditions, as for example, at 28° C., a mycelium is regularly produced. At lower temperatures—I have used mainly temperatures near 10° and 17° C.—the germ tube likewise often keeps on growing vegetatively, but often, again, it stops growing when it has reached a length between 5 and 15 μ (occasionally when it has reached a length of 20 μ) and becomes modified as an evacuation tube (fig. 8, H, t) by forming a hyaline cap of dehiscence. The protoplasmic materials migrate through the tube (fig. 8, I, t) to collect within the vesicle (fig. 8, I, v) formed by inflation of the hyaline cap, and there are fashioned into laterally biciliate zoospores in the manner usual for the genus. In instances where from localized scarcity of water the zoospores are constrained to encyst in a cluster (fig. 8, J, a-i) or in a looser group (fig. 8, K, a-i; L, a-d; M, a-i; N, a-j) near the empty membranous envelopes, it is evident that oospores of average dimensions commonly yield about 9 or 10 swarmers, while others give rise to greater or

lesser numbers commensurate with their size. The empty membranous parts to which the antheridial envelope (fig. 8, O, a; P, a) is occasionally found attached sometimes shows the oospore wall with rather little or no separation into 2 layers (fig. 8, K-O; Q, a-j), but sometimes, again, fairly extensive separation (fig. 8, I; J; P; R, a-j) may be observed. The empty evacuation tube (fig. 8, I-P: t; Q, a-j: t; a-j: t) terminates abruptly without being widened or reflexed at the mouth, much as in *P. ultimum*, though generally it is shorter than in that species. The zoospores obtained through germination of oospores are indistinguishable, both in their motile and in their encysted condition, from those produced from sporangia of mycelial origin. After encystment they usually germinate by putting forth a vegetative germ hypha (fig. 8, S-V), yet at suitably low temperatures they sometimes form a papilla of dehiscence (fig. 8, J, f-i; K, h; M, f, g; N, h, i) and individually discharge their contents to be converted externally into a secondary motile zoospore. Where repetitional development has taken place, the empty cyst wall (fig. 8, K, i; L, d; M, h; N, j) bears an evacuation tube commonly 1.5 to 2.5 μ long and approximately 2 μ wide.

That after-ripened oospores of *Pythium debaryanum* will readily yield zoospores on germination is not surprising since the ordinary conidia of this fungus can usually be made to function as zoosporangia, whether they were nourished on artificial media, on mature vegetable tissues, or on seedlings of cultivated plants. In *P. ultimum*, however, production of zoospores from after-ripened oospores finds little analogous parallelism in the germinative behavior of the ordinary asexual conidia, though in a morphological sense these are hardly to be distinguished from the generally smaller conidia of germinative origin that so frequently develop as zoosporangia. Superior capabilities for zoospore formation have earlier been noted (Drechsler 1946b) in the after-ripened parthenospores of my *P. anandrum*, yet in that species zoospore development from ordinary asexual conidia is a well ascertained phase of reproduction, and can usually be induced with some patient effort. As has been mentioned previously (Drechsler 1946a), zoospore development in *P. ultimum* would seem adapted more especially for bringing about infection of rootlets in the deeper levels of the soil during the early cool part of the growing season—a frequent type of infection well exemplified in the sweetpotato rootlet rot from which the fungus shown in figures 4 and 5 was originally isolated.

SUMMARY

1. The features distinguishing *Pythium ultimum* and *P. debaryanum* in the formative and mature stages of sexual reproduction are reviewed and illustrated.

2. Oospores of both species become capable of germination after a resting period during which they acquire the structural characters of conidia.

3. At temperatures near 10° C. after-ripened oospores of *P. ultimum* will often germinate by producing zoospores.

4. Conidia formed on germ hyphae extended from after-ripened oospores of *P. ultimum* will likewise frequently develop as zoosporangia.

5. After-ripened oospores of *P. debaryanum* will readily give rise to zoospores at 10° C. as well as at temperatures near 17° C., their germinative behavior not differing noticeably from that of the ordinary conidia produced asexually by the fungus.

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