BULLETIN OF THE TORREY BOTANICAL CLUB
VOL. 83, NO. 3, pp. 196–206 MAY, 1956

PRODUCTION OF ZOOSPORES FROM GERMINATING OOSPORES OF PYTHIUM MAMILLATUM MEURS

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In an earlier paper (Drechsler 1952) I described the production of swarm spores from germinating oospores of *Pythium ultimum* Trow and *P. debaryanum* Hesse, two fungi widely familiar as parasites causing damping-off, root rot, and stem rot in many crop plants. Such germinative development has since been observed also in *P. mamillatum* Meurs (1928), a fungus of rather similar pathogenic character, which from its morphological resemblances to *P. debaryanum* must be considered a close relative of that species. These resemblances, however, might easily escape attention, for owing to the presence of many protuberances on its oogonia, *P. mamillatum* presents a bristling appearance strongly suggestive of such less intimately related spinose congeners as my *P. acanthicum* and my *P. anandrum*. To show the usual variations in the fungus held referable to Meurs' species, units of asexual and sexual reproductive apparatus selected largely at random in maize-meal agar plate cultures of two isolations are herein figured in numbers sufficient, it is hoped, to make up tolerably representative assortments. One of the isolations (figs. 1 and 2) was obtained early in August, 1926, from a discolored rootlet of a tomato (*Lycopersicon esculentum* Mill.) plant removed from a field in Arlington, Virginia. The other (figs. 3 to 5) came from a discolored rootlet of a sheep sorrel (*Rumex acetosella* L.) plant dug up near Beltsville, Maryland, on April 29, 1949. Both isolations correspond well to a culture of *P. mamillatum* contributed by Meurs to the Centraalbureau voor Schimmelcultures at Baarn, Netherlands, and now being maintained also in the American Type Culture Collection, Washington, D. C., under the designation ATCC11121.

A mycelium of *Pythium mamillatum* actively growing in a Petri plate of

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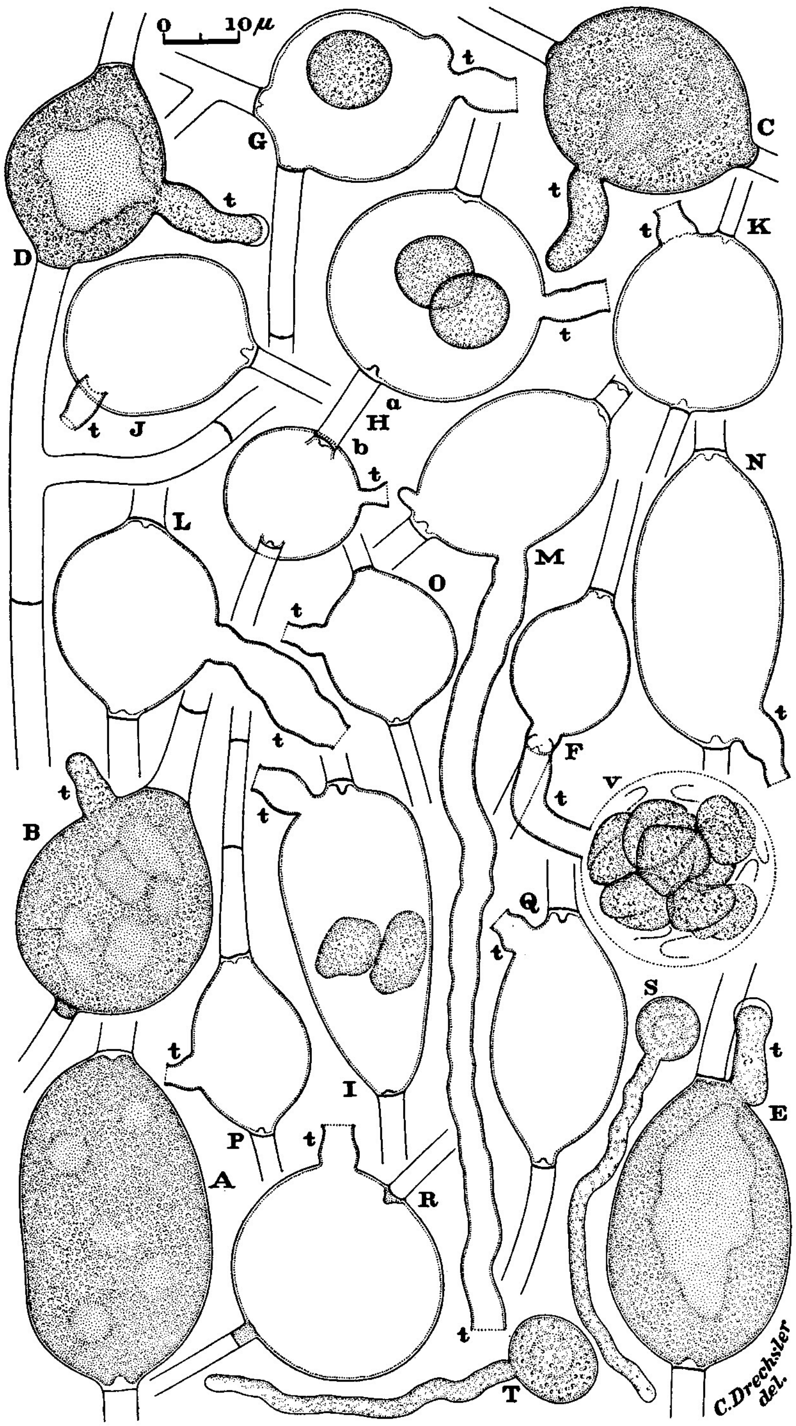
FIG. 1. Asexual reproductive apparatus of *Pythium mamillatum* (tomato strain) produced on irrigated slabs excised from a maize-meal agar plate culture; drawn at a uniform magnification with the aid of a camera lucida; ×1000. A. Large sporangium. B, C. Two sporangia, each putting forth an evacuation tube, t. D, E. Two sporangia ready to discharge their contents, each bearing an evacuation tube, t, with a hyaline cap of dehiscense. F. Sporangium showing empty evacuation tube, t, and vesicle, v, containing 8 motile zoospores. G, H (a, b), I–R. Sporangia after conversion of their contents into swarm spores; t, empty evacuation tube. S, T. Encysted zoospores, each with a vegetative germ tube.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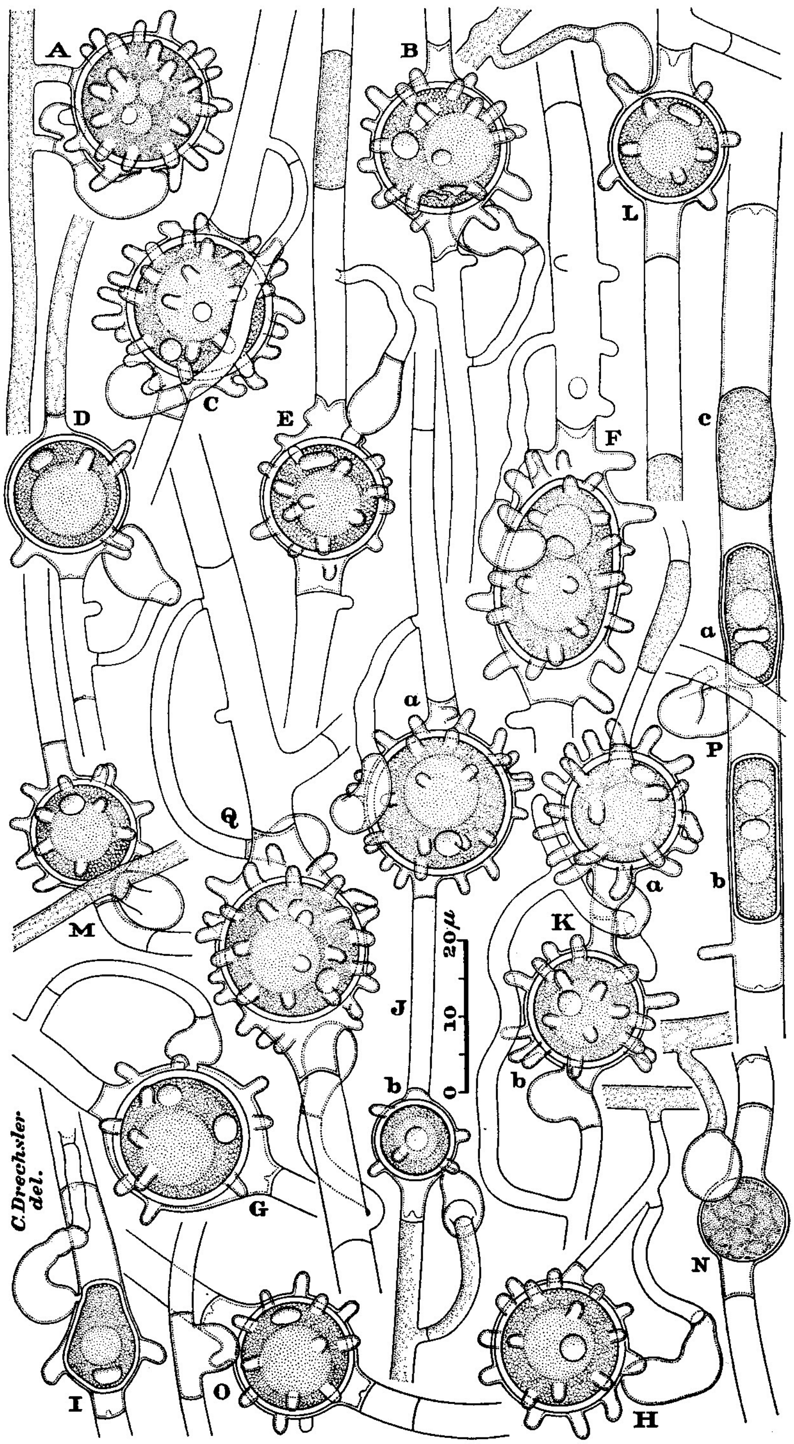
maize-meal agar presents much the same orderly appearance whereby *P. debaryanum* may often be distinguished, in young cultures, from *P. ultimum;* its radially oriented, straightforward, main hyphae giving off relatively short, irregularly ramified branches at moderate intervals. Like *P. debaryanum,* again, but unlike *P. acanthicum* and most other spinose congeners, the fungus in a few days produces a rather copious white aerial mycelium, which later often gradually collapses in large areas of the substratum as a fibrous mat. The tubular membranes of the empty matted filaments are usually found only rather indistinctly visible, so that in much material the hyphae are disclosed to view mainly through the numerous convex cross-walls contained in them. Numerous convex septa also appear in the procumbent and submerged hyphae, being laid down to mark successive stages of evacuation as the protoplasm is withdrawn from extensive portions of the filament for the production of conidia and oospores.

The conidia formed in maize-meal agar cultures of *Pythium mamillatum* (fig. 1, A; fig. 3, A) differ little in their globose or elongate-ellipsoidal shape, in their dimensions, or in their germinative behavior from the conidia of *P. debaryanum.* Under moist conditions at temperatures near 17°C. they develop readily as sporangia, each putting forth an evacuation tube (fig. 1, B-C:t) that after some elongation forms a hyaline cap of dehiscense (fig. 1, D–E: t) which on yielding becomes inflated into a vesicle (fig. 3, B, v) as the protoplasm streams into it from below. Within the vesicle (fig. 1, F, v) the mass of protoplasm is transformed into laterally biciliate zoospores after the manner characteristic of the genus. The number of zoospores produced from sporangia of mycelial origin varies commonly from 5 to 30. Portions of protoplasm that fail to escape are fashioned into zoospores inside the sporangial envelope (fig. 1, G; H, a; I). The empty evacuation tube (fig. 1, F–G: t; H, a–b: t; I–R: t; fig. 3, B–R: t, S, a–b: t) varies considerably in length, in some instances measuring only 2 or 3μ (fig. 3, Q–R: t) and in others measuring over 100μ (fig. 1, M, t). After swimming about for some time the zoospores come to rest and round up (fig. 3, T, a–n). The subspherical cysts usually germinate by extending a vegetative germ hypha (fig. 1, S, T; fig. 3, U–W), though in the absence of nutrients and at suitably low temperatures they sometimes put forth individually an evacuation tube (fig. 3, X) and

FIG. 2. Sexual reproductive apparatus of *Pythium mamillatum* (tomato strain) produced in maize-meal agar plate cultures; drawn at a uniform magnification with the aid of a camera lucida; ×1000. A. Monoclinous reproductive unit with oospore somewhat immature. B–I, J (a, b), K (a, b). Monoclinous reproductive units, each with mature oospore and with oogonium attended by a single antheridium. L–P. Diclinous reproductive units, each with single antheridium; oospore mature in L, M, and O, but immature in N; the cylindrical oogonium of P containing 2 mature cylindrical oospores, a and b, and a vegetative segment or conidium, c. Q. Monoclinous reproductive unit with mature oospore and with oogonium attended by 2 antheridia.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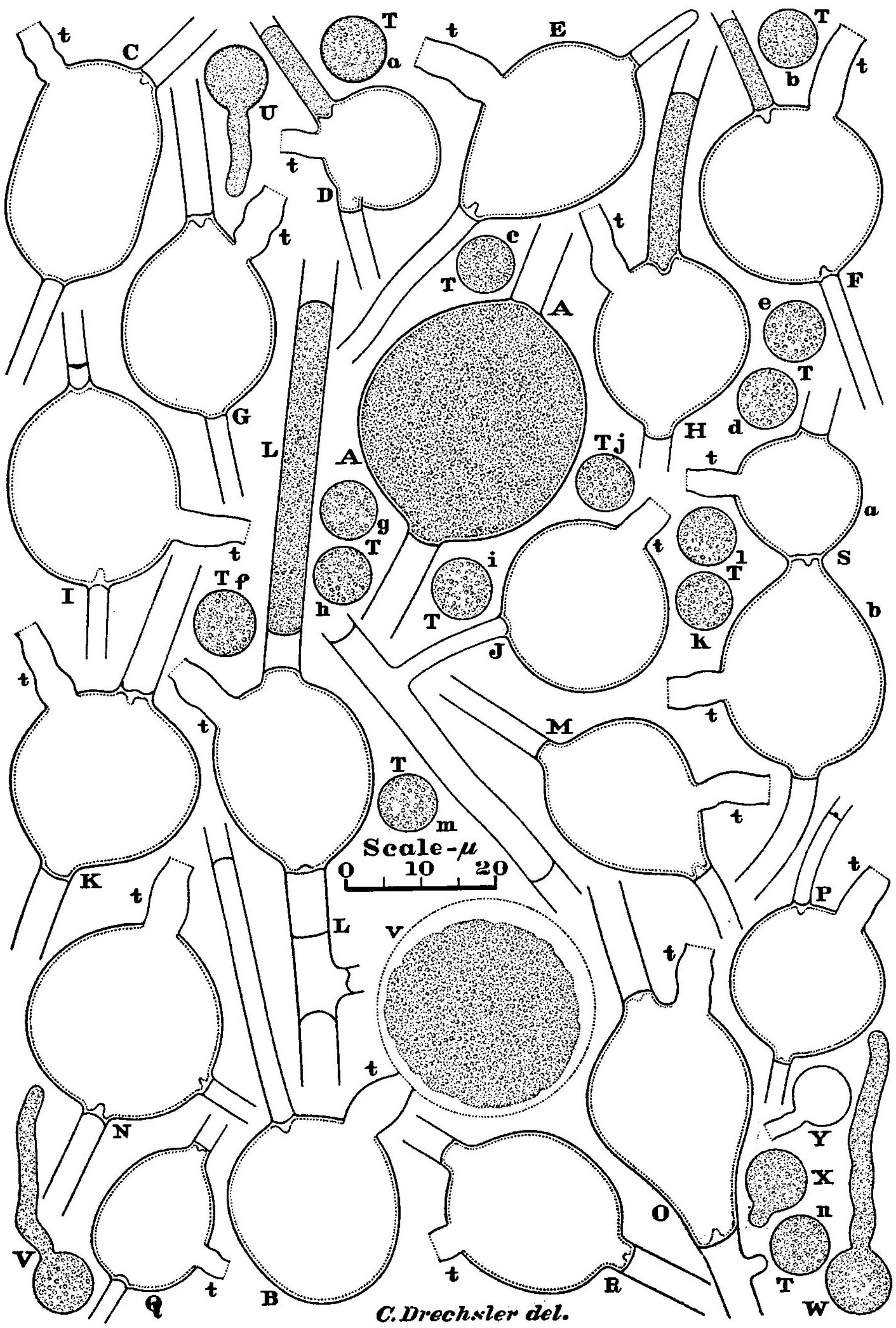


FIG. 3. *Pythium mamillatum* (sheep sorrel strain), × 1000. A. Large sporangium. B. Newly emptied sporangium; t, evacuation tube; v, vesicle. C–R, S (a, b). Empty sporangia; t, evacuation tube. T. Encysted zoospores, a–n. U–W. Encysted zoospores germinating vegetatively. X. Zoospore cyst with short evacuation tube. Y. Zoospore cyst after escape of protoplast.

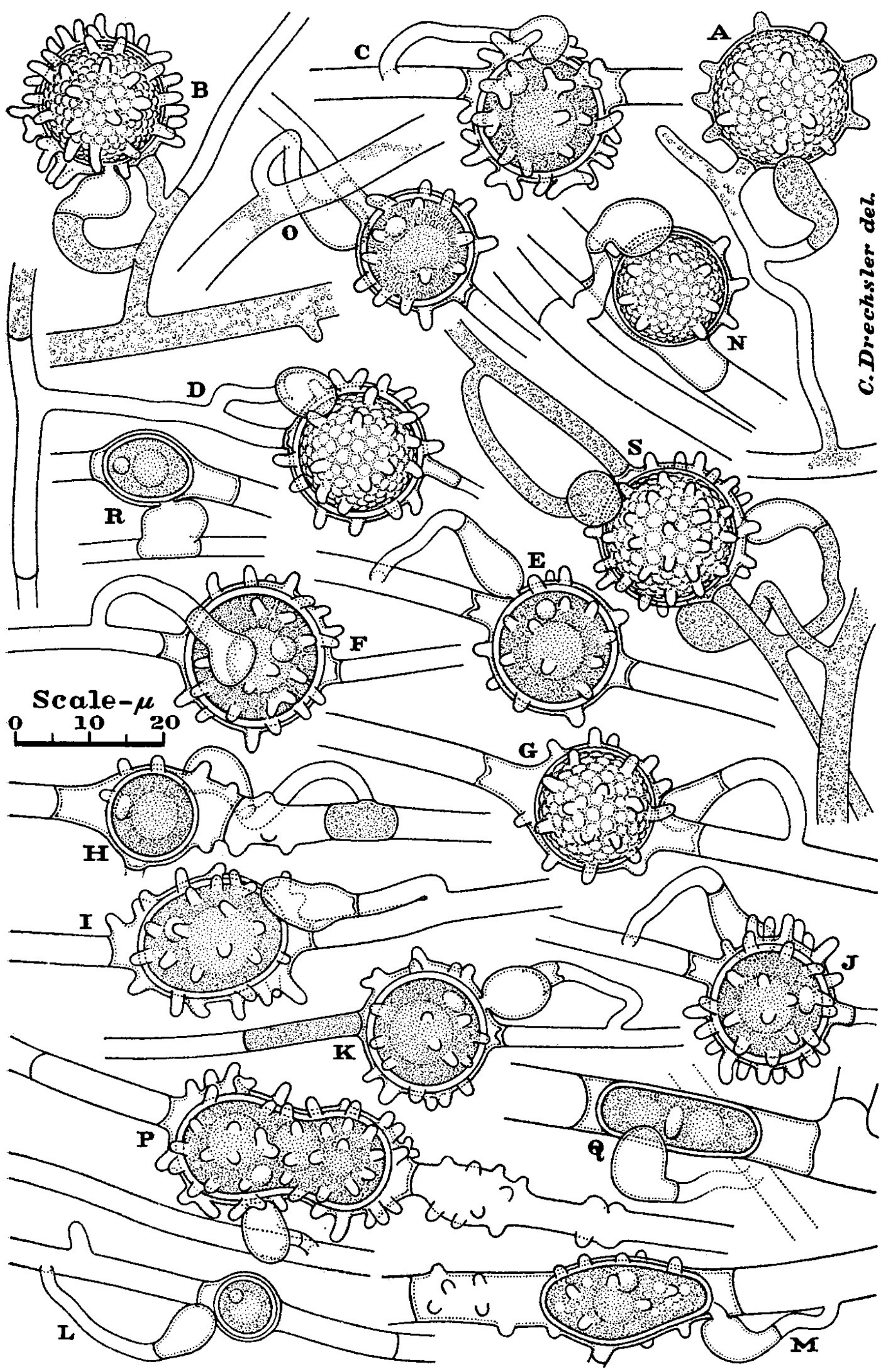


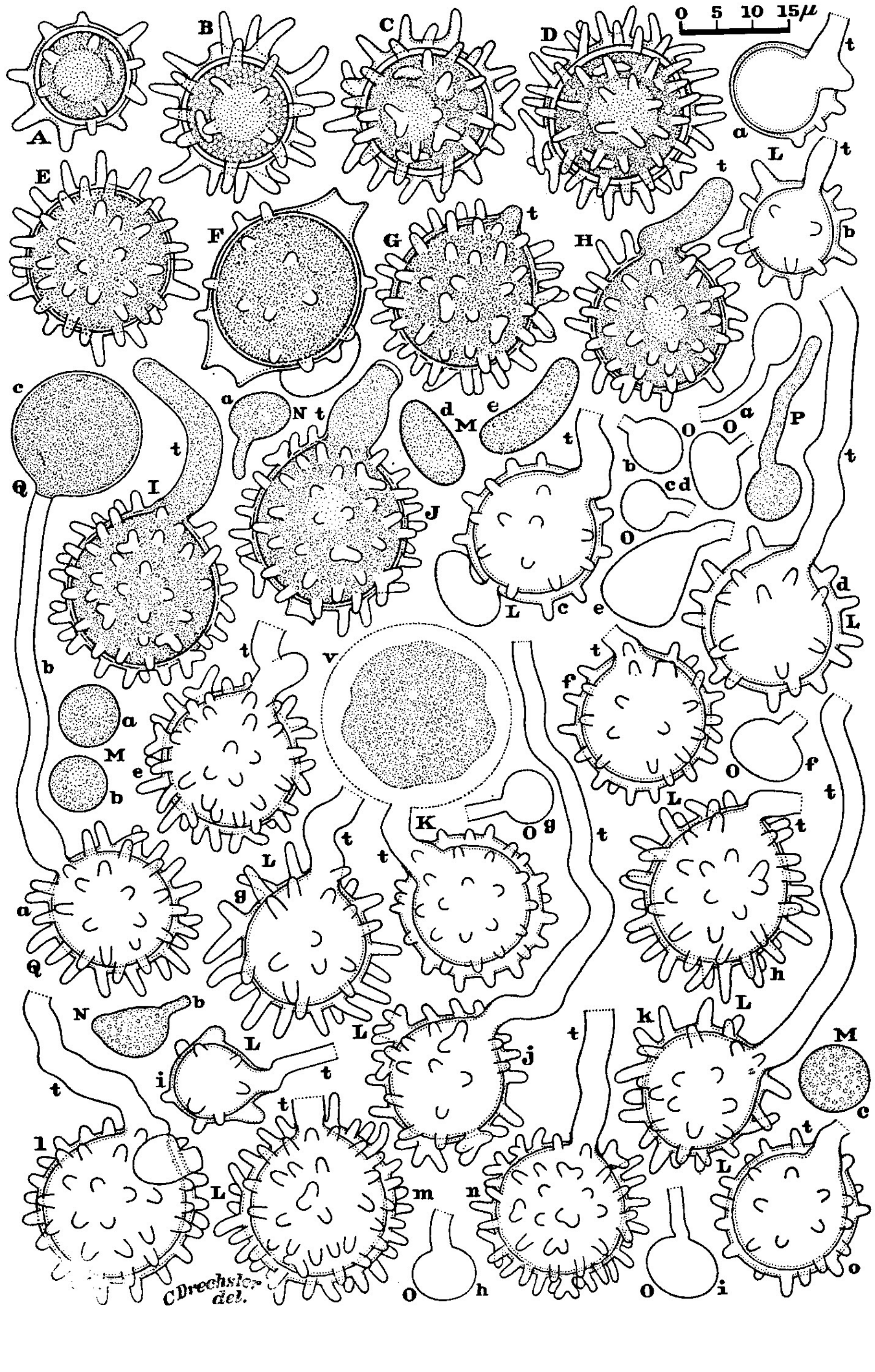
FIG. 4. Sexual reproductive apparatus of *Pythium mamillatum* (sheep sorrel strain), ×1000. A–M. Monoclinous units with single antheridia. N–R. Diclinous units with single antheridia. S. Unit with 3 antheridia.

become emptied (fig. 3, Y) as the protoplasm streams out to be converted into a secondary motile zoospore.

In the positional arrangement of its parts the sexual reproductive apparatus (fig. 2, A–Q; fig. 4, A–S) of *Pythium mamillatum* shows general parallelism with that of *P. debaryanum.* Sometimes the oogonium and antheridium are borne terminally on short branches arising separately from the same hypha (fig. 2, A; fig. 4, A, B). Much more often in monoclinous reproductive units an intercalary (fig. 2, B–G; I; J, a, b; K, a; fig. 4, C–M) or terminal (fig. 2, H) oogonium is fertilized by an antheridium borne terminally on a branch arising from the oogonial hypha at a distance of 5–40μ from the oogonium. Occasionally an oogonium is fertilized by an antheridium formed adjacent to it (fig. 2, K, b). Diclinous reproductive units appear neither exceptional nor abnormal. In such units the antheridium may be borne terminally on a branch (fig. 2, L–N; fig. 4, N–Q), or may consist of an intercalary portion of hypha together with a somewhat massive lateral protuberance in contact with the oogonium (fig. 2, O; fig. 4, R). Cylindrical hyphal segments which develop somewhat tardily as oogonia in forming one (fig. 4, Q) or two cylindrical oospores (fig. 2, P, a, b), in addition, perhaps, to a cylindrical conidium (fig. 2, P, c), are often found to have been fertilized by an antheridium from a neighboring hypha. Oogonia of such belated development are usually altogether smooth (fig. 4, Q) or are only very sparingly furnished with protuberances (fig. 2, P). Pronouncedly undersized oogonia in either monoclinous (fig. 4, L) or diclinous reproductive units (fig. 2, N; fig. 4, R) likewise appear usually devoid of protuberances.

While many oogonia produced by *Pythium mamillatum* in maize-meal agar cultures are fertilized by a single antheridium, more than a few are found attended by two (fig. 2, Q) or three (fig. 4, S) antheridia. With respect to the number of male cells supplied to an oogonium the fungus would

FIG. 5. Germination of oospores taken from 60-day-old maize-meal agar plate culture of *Pythium mamillatum* (sheep sorrel strain); parts A–D showing specimens as found in the aging culture, parts E and F showing specimens after irrigation for 24 hours, parts G–Q showing specimens after irrigation for 48 hours; drawn with the aid of a camera lucida at a uniform magnification; ×1000. A, B. Oospores with single refringent body. C, D. Oospores with 2 refringent bodies. E, F. Oospores in which the wall is reduced in thickness and surrounds a protoplast of coarsely granular texture throughout. G, H. Oospores, each with a growing germ tube, t. I, J. Oospores nearly ready to discharge contents, each having an evacuation tube, t, with a hyaline cap of dehiscense. K. Oospore soon after discharging its contents; t, evacuation tube; v, vesicle. L. Empty oospore envelopes, a–o, each with open evacuation tube, t, extending through the oogonial wall. M. Encysted zoospores, a–e. N. Encysted zoospores, a and b, each bearing a short evacuation tube. O. Empty zoospore cysts, a–i, after escape of protoplast through open evacuation tube. P. Encysted zoospore with vegetative germ tube. Q. Empty oospore, a, with empty germ hypha, b, terminating in a globose sporangium, c.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thus seem to differ little from *P. debaryanum,* in which I have observed no instances where an oogonium was attended by more than four antheridia although Matthews (1931), as also Middleton (1943), includes among some first-hand illustrations of Hesse's species a sexual reproductive unit with six antheridia, all applied to the oogonium in profile view. The illustrations of *P. mamillatum* given by Matthews and by Middleton show more pronounced tapering of the oogonial protuberances than was evident in Meurs' isolation (ATCC11121) or than would seem present in any American isolation referable to Meurs' species.

Ooospores of *Pythium mamillatum* formed in maize-meal agar cultures have for the most part shown relatively little reorganization of contents after the cultures had been stacked under a battery jar for 60 days at laboratory temperatures near 20°C. Some among them would then still retain the internal structure characteristic of the resting state (fig. 5, A),—each containing a large homogeneous reserve globule, a parietal granular layer, and an oblate ellipsoidal refringent body. In others the parietal layer seemed largely composed, not of irregular granules, but of rounded particles arranged circularly (fig. 5, B) as in resting oospores of *Aphanomyces* and of some related saprolegniaceous genera. In still others the reserve globule had become noticeably smaller, while the parietal layer had not only increased in volume but now displayed two refringent bodies as well as scattered vacuolar inclusions of rounded or of irregular shape (fig. 5, C, D). When such oospores were sparingly irrigated in Petri dishes at temperatures near 17°C., with the shallow layer of distilled water being changed from time to time, they acquired in 24 hours a uniformly granular texture throughout the interior, and the wall became thinner and more tightly appressed to the oogonial envelope (fig. 5, E, F). Under wet conditions the oospores thus acquired much the same appearance as asexually formed conidia, having undergone during the first day of irrigation the same changes that the oospores of *P. ultimum* undergo directly in the stale substratum. When irrigation was continued a second day many oospores extended individually a protrusion that soon erupted from the oogonial envelope (fig. 5, G, t) and then elongated externally as a germ hypha (fig. 5, H, t). Virtually all remaining oospores extended germ hyphae during the third 24-hour period of irrigation.

Once the germ tube has emerged from the oogonial envelope its further development would seem governed mainly by environal conditions. At the high temperatures usually prevailing in laboratories in summer the germ hyphae in my irrigated material commonly grew out vegetatively to form small mycelia. Vegetative growth took place in some instances also at temperatures near 17°C., presumably because nutrients were still present here and there in effective quantity. In most instances, however, at these lower temperatures the germ hypha ceased to elongate and formed a hyaline cap of dehiscense (fig. 5, I–J : t). This cap became inflated into a vesicle (fig. 5, K, v) in which the undifferentiated protoplasm discharged from the oospore was converted into motile swarm spores. The empty membranous envelopes showed variations in the lengths of evacuation tubes (fig. 5, K, t; L, a–o : t) similar to the variations in lengths of evacuation tubes on sporangia of mycelial origin. The empty oospore wall appeared of equal or slightly greater thickness than the oogonial envelope, from which it was only rather slightly separated in some instances (fig. 5, L, h, n), and more widely in other instances (fig. 5, L, d, o).

The zoospores produced from germinating oospores in numbers varying usually from 4 to 12, like those produced generally in somewhat greater number from ordinary sporangia, commonly measured 7.5 to 10μ in diameter after they had rounded up (fig. 5, M, a–c). Oversized elongated zoospores (fig. 5, M, d, e) were observed mainly in portions of irrigated material not adequately supplied with water for complete cleavage. Some encysted zoospores soon extended a short evacuation tube (fig. 5, N, a, b) and became emptied (fig. 5, O, a–i) by discharging their contents to be transformed into secondary motile zoospores. Others pushed out individually a vegetative germ hypha (fig. 5, P) capable of growing into a mycelium.

Some oospores of *Pythium mamillatum* (fig. 5, Q, a) germinated by putting forth a germ hypha (fig. 5, Q, b) that soon ceased to elongate and thereupon gave rise to a terminal conidium or sporangium (fig. 5, Q, c). Such germination took place at midsummer temperatures as well as at lower temperatures suitable for development of swarm spores. Sporangia produced externally by oospores without intervention of a vegetative stage commonly yield 4 to 12 swarm spores. Their progenies, as might be expected, are approximately equal to the progenies of oospores that become directly converted into sporangia, and accordingly are less numerous than the progenies issuing from the larger of the sporangia formed asexually on well nourished mycelium.

SUMMARY

1. *Pythium mamillatum* appears intimately related to *P. debaryanum* as it resembles that species in the branching habit of its mycelium, in the shape and size of its sporangia, and in the positional relationships of its oogonia and antheridia.

2. A germinating oospore of *Pythium mamillatum* may produce swarm spores by undergoing conversion into a sporangium, or by giving rise to a globose sporangium outside the oogonial envelope.

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