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Production of zoospores from germinating oospores of Pythium butleri.

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With plates VII—XIV.

Subramanian (1919) in the descriptive account of his *Pythium butleri* set forth the oospores of that fungus as germinating by a germ tube, not by zoospores. Germination of oospores by production of a vegetative germ hypha was likewise described and illustrated by Ramakrishna Ayyar (1929) in his account of an evidently conspecific isolation he obtained from *Opuntia dillenii* (Ker.) Haw. and referred to *P. aphanidermatum* (Eds.) Fitzp. Edson (1915) in presenting his *Rheosporangium aphanidermatum* had mentioned earlier that in some directly observed instances the germinating oospores invariably pushed out germ tubes which later developed into vegetative mycelium. More recently Balakrishnan (1948) noted that oospores of his *P. indicum*, a closely related species, germinated similarly by emission of a germ tube. No reference to oospore germination is found in the account Meurs (1934) gave of his *P. deliense*, or in the brief characterization of the fungus that Sideris (1931) designated as *Nematosporangium aphanidermatum* var. *hawaiiensis*. The statement of Butler (1907) setting forth oospore germination in his *P. indigoferae* as "rapid, by a hypha, not by zoospores" has some pertinent interest here; for, although the published illustrations of that fungus give no decisive indication of its more intimate affinities, Sideris, who had available for study a presumably relevant organism isolated from cucumber (*Cucumis sativus* L.) roots by McRae in India, treated *N. indigoferae* (Butl.) Sideris as a close relative of *N. aphanidermatum* (Edson) Fitzp. and *N. butleri* (Subr.) Sideris. Later Meurs and also Middleton (1943), after firsthand comparison of cultures — neither author mentioned the host from which was originally obtained the isolation of *P. indigoferae* studied by him — likewise treated *P. indigoferae* as a near relative of *P. aphanidermatum*, with which species both authors considered *P. butleri* to be identical.

It has become rather widely accepted that Carpenter (1921) proved the sameness of *Rheosporangium aphanidermatum* and *Pythium butleri* by showing that both were morphologically identical with a fungus he had isolated from sugarcane (*Saccharum offici-*

narum L.) in Hawaii. As Carpenter's illustrations of sexual reproductive apparatus reveal far different antheridial morphology than was figured by Edson and by Subramaniam, the sugarcane fungus manifestly represented a species different from the species described in the United States and in India; so that the proof of identity on which Fitzpatrick (1923) relied in reducing ***P. butleri*** to synonymy with ***P. aphanidermatum*** would seem quite worthless. By good fortune, however, the conclusion reached by Carpenter and Fitzpatrick was much more meritorious than the proof; since the general parallelism in antheridial morphology set forth by Edson and Subramaniam gives sound reason for holding ***P. aphanidermatum*** and ***P. butleri*** to be intimately related, if not actually identical.

The suggestion conveyed in some writings that Carpenter and Fitzpatrick compared authentic cultures of ***P. aphanidermatum*** and ***P. butleri*** is not supported in any statement by either author. Little nomenclatorial significance can be ascribed to some more recent comparisons of cultures designated by the two binomials, for in 1922 when several *Pythium* isolations I had obtained from cucumber and watermelon (*Citrullis vulgaris L.)* fruits needed to be identified Edson informed me that he had lost all cultures of his fungus several years earlier and that all cultures sent to other workers had similarly long been extinct. On examining very kindly a number of isolations which from resemblances in antheridial morphology seemed referable to his species Edson acknowledged that they showed general similarity to ***P. aphanidermatum*** but that their production of swollen and lobulated parts was much more extensive than any homologous development he had ever seen before. He expressed the same opinion on other isolations of like morphology that were obtained during the ensuing 5 years from various herbaceous crop plants including garden pea (***Pisum sativum*** L.), kidney bean (***Phaseolus vulgaris*** L.). common tomato (***Lycopersicon esculentum*** Mill.), tobacco (***Nicotiana tabacum*** L.), common eggplant (*Solanum melongena* L.), sweetpotato (*Ipomoea batatas* (L.) Lam.) and Kentucky blue-grass (***Poa pratensis*** L.). His permanent stained slides of asexual reproductive apparatus, about 20 in number, which he allowed me to examine, showed, indeed, much less outward modification of zoosporangia than was commonly displayed in irrigated material of my own isolations. The individual sporangium figured by Edson (1915, pl. 44, fig. 7) was easily recognized, being considerably larger and more richly branched than any other that came under observation. Sporangia of relatively meager outward differentiation like those seen in Edson's slides were later found to be produced commonly in 5 isolations, of which 3 had been obtained in

1927 from sugarcane roots, while the other 2 had originated from diseased tobacco seedlings in Sumatra. All 5 isolations were further distinguished by generally smaller dimensions and some reluctance in zoospore formation. Accordingly the proposal was then made (Drechsler, 1934) to recognize *P. aphanidermatum* as being exemplified in the less robust isolations, whereas *P. butleri* would be held represented in the larger and more strongly lobulated isolations. As, unfortunately, for a long period after 1926 Edson was not available for consultation, no opinion on the 5 less robust isolations, whether favorable or adverse to my proposal, could be obtained from him. Unfortunately, too, his stained slides of *P. aphanidermatum,* representing the last type material of the species, are no longer extant, having been lost before 1942. Restrictions on the transportation of pathogenic cultures and materials have discouraged any survey to determine the distribution of the less robust species, so that it remains undecided whether the fungus could at all likely have been present in the Illinois soil from which Edson obtained his original isolation of *P. aphanidermatum.*

In view of the uncertainties relating to *P. aphanidermatum,* it seems preferable to use the binomial *P. butleri* for the more robust form. As Subramaniam's diagnosis specified oospore germination by a germ tube — Edson likewise saw only such germination — some doubt might arise as to the identity of isolations in which oospores can with unusual readiness be induced to germinate abundantly by the formation of swarm spores. Accordingly the main morphological features of the robust species, as displayed in a few isolations from Virginia and Maryland, are herein briefly set forth firsthand, and some observations are given on its seasonal occurrence that reveal temperature relationships of moment in determining the manner of oospore germination.

Among the species of *Pythium* occurring outdoors in Maryland and adjacent states, *P. butleri* is conspicuous for its frequently copious production of aerial mycelium. With its strong capacity for aerial development would seem associated at times some little tendency toward the epiphytic growth habit characteristic more especially of *P. indigoferae.* Thus after 15 days of unusually wet weather beginning on July 14, 1945, *P. butleri* developed very luxuriantly on vegetable plots near Beltsville, Maryland, producing large wefts of cottony mycelium on numerous crookneck squash (*Cucurbita pepo* L.) fruits attached to vigorous plants at heights 10 to 25 centimeters above the ground (Pl. VII, A—E). Here and there the bulky mycelial masses served in extending the fungus upwards or sideways, for wherever they encountered a neighboring fruit or a succulent petiole the new structure was closely invested by hyphae and penetrated by means

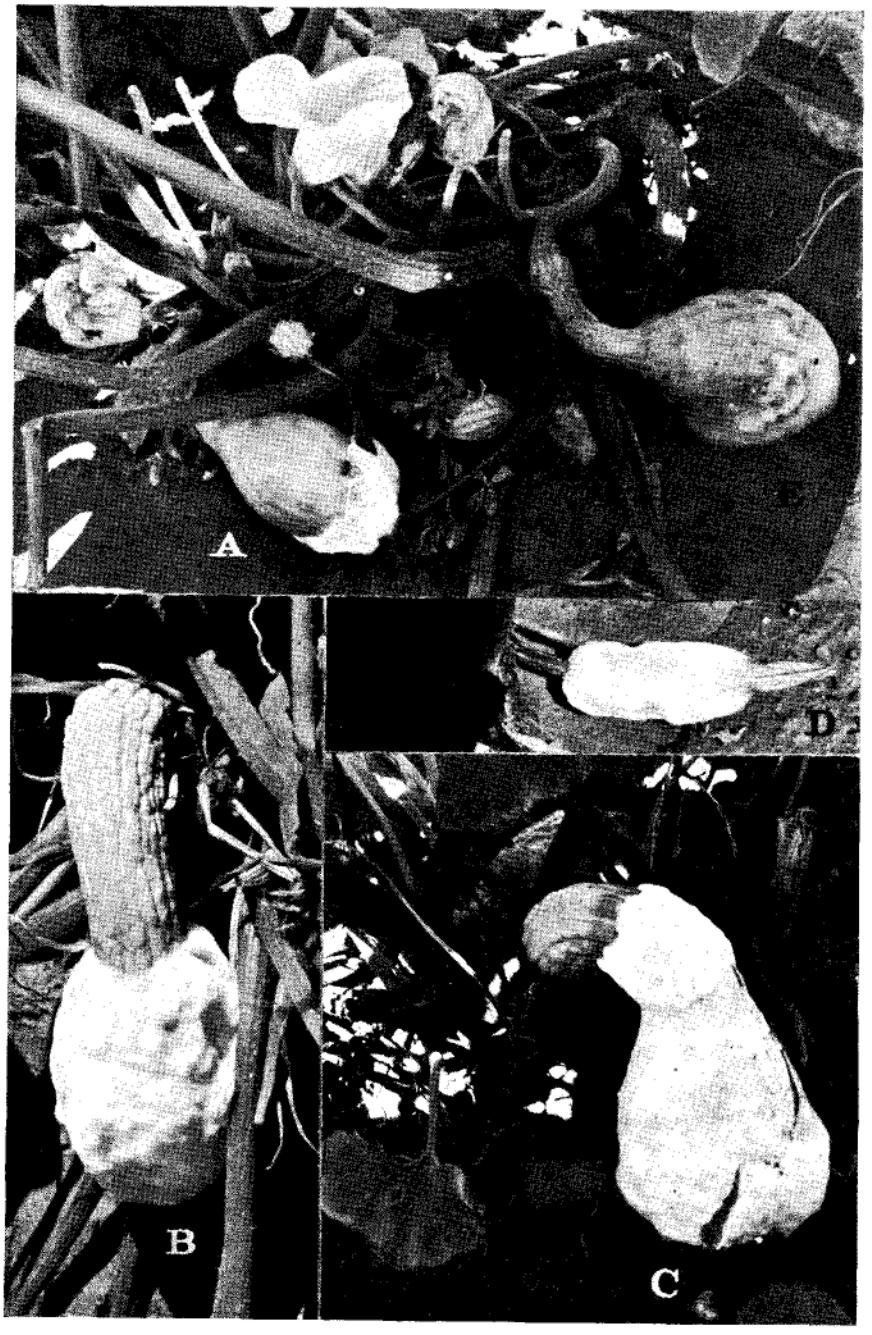
of numerous appressoria similar to those produced in somewhat clustered arrangement (Pl. VIII, A, B) on the floor of a maize-meal-agar plate culture. In dwarf varieties of the kidney bean wefts of aerial mycelium were likewise effective in spreading the fungus from pod to pod 10 to 25 centimeters above the ground. Conspicuous development of aerial mycelium was noted (Drechsler, 1952) also on kidney beans attacked by *P. ultimum* under very moist conditions in a field in Delaware. However, in its capacity to spread well above ground by spanning gaps between neighboring host structures with aerial mycelium *P. butleri* seems equalled only by my *P. myriotylum*, a species not so far known to occur outdoors in Maryland or Delaware, though recurring repeatedly during June and July as causal agent of bean stem-rot in greenhouses near Beltsville, in which temperatures sometimes above 40° C. then apparently inhibit development of all congeneric parasites.

Despite its discomfiture under glass in summer *Pythium butleri* occurs widely outdoors in the middle and northern latitudes of the eastern United States during periods of high temperature and high humidity. In central Maryland it is usually more active in attacking vegetable crop plants during June, July, and August than any other member of the genus, though in these months it may be noticeably injurious only during 15 to 30 days of especially hot wet weather. Late in August the fungus often forms conspicuous cottony wefts on full-grown cucumbers attached to moribund vines in poorly drained areas. Soon afterwards, however, with the onset of cooler weather early in September, it usually vanishes from sight outdoors and until the following June is commonly found active only in greenhouses. An exceptionally early appearance of the fungus in April, 1929, when it attacked tomato seedlings outdoors in many localities in Maryland, would seem to have been attributable to extraordinarily warm, wet spring weather, and more especially, perhaps, to a six-day period from April 4 to April 9 unprecedented so early in the year for prolonged high temperatures. Where *P. butleri* attacks small seedlings or portions of older plants composed mainly of firm rather than of succulent tissues, or where the surrounding air becomes relatively dry, it puts forth little or no external mycelium, so that the injury it causes looks much like the injury of such familiar congeneric parasites as *P. ultimum* Trow and *P. debaryanum* Hesse. Although containing a large mass of very succulent tissue highly favorable for the growth of *P. butleri,* watermelons penetrated by the fungus at the blossom end usually show no aerial mycelium as they undergo complete destruction; for the hard stone-cell layer directly under the epidermis serves here as an effective barrier against external development even in a humid atmosphere.

The adaptation of *Pythium butleri* to high temperatures becomes evident also in its ready production of zoospores in our relentlessly overheated laboratories, where similar development in *P. ultimum* and *P. debaryanum* is wholly precluded. Lavish use of fuel in winter is not usually sufficient to prevent zoospore formation in *P. butleri* either from sporangia of mycelial origin or from germinating oospores. If sizable masses of young vegetative mycelium, such as may be obtained conveniently by cutting pieces of softened tissue from newly infected cucumber fruits or by excising slabs from vigorous lima-bean-agar plate cultures, are transferred to a shallow layer of distilled water, numerous stout hyphae with many irregularly swollen branches grow out from the irrigated material to form a fringe 2 to 3 millimeters wide. At temperatures from 33 to 37° C., often prevailing during summer in laboratories near Beltsville, many of the hyphal outgrowths give rise in a few hours to intricate complexes of distended lobulate branching elements, without however, producing motile zoospores. Zoospores have been found produced rather sparingly in irrigated preparations that were being examined under a microscope at a room temperature of 32° C. At laboratory temperatures between 18 and 30° C. they develop more abundantly, often being liberated in spectacular profusion. In many instances the asexual reproductive unit, or sporangium, that discharges its contents into a terminal vesicle (Pl. VIII, C, v) for transformation into motile swarm spores (Pl. VIII, D, v) consists of several communicating lobulate branches. In many other instances it is composed of a portion of widened axial hypha together with one or several distended lateral branches (Pl. VIII, E, a—f), each sporangial unit after evacuation showing an open discharge tube (Pl. VIII, E, a—f: t). Here and there an empty hypha that in view of its relatively uniform, moderate width (4 to 6 μ) and of its commonplace sparse branching, had evidently not undergone any outward modification, is found divided into segments, mostly 40 to 150 μ long, which, from the open evacuation tube (Pl. VIII, F, a—c:t; G, a:t) borne laterally on each, must have served as zoosporangia. Sometimes, indeed, an empty filamentous segment comes under observation even as a few zoospores — mostly 2 to 5 in number — are being formed in a vesicle at the tip of the lateral discharge tube (Pl. VIII, G, b:t) borne on it. Whatever the shape of their parent sporangium, the liberated swarm spores, after coming to rest and rounding up, often germinate by emission of 1 or 2 germ hyphae (Pl. VIII, H, a—i), especially if the temperature is above 33° C. or if nutrient substances are present in considerable quantity. At lower temperatures (18 to 25° C.) and in the absence of food materials they often discharge their contents through a narrow evacuation tube (Pl. VIII, I, a—l) for conversion into a

secondary motile zoospore. In some very prolific irrigated preparations, where large numbers of encysted zoospores often cohere in floating masses (Pl. VIII, J) visible to the naked eye as delicate scum, empty cyst envelopes and cysts provided individually with a projecting evacuation tube show that repetitional development takes place abundantly at the periphery of the separate masses.

The range of temperatures, including those above 33° C., suitable for development of the distended hyphal branches that later undergo conversion into sporangia, is suitable also for sexual reproduction. In many sexual units all parts are supplied from the same mycelial thread (Pl. IX, A—C:a; Pl. X, A—B:a; Pl. XI, A—E:a; Pl. XII, A—C:a) though the hyphal connection between oogonium and antheridium varies greatly in length. Rather commonly the two branches (Pl. IX, A—C:b, c; C:g, h; Pl. X, A:b, c; Pl. XI, A:b, c; C—F: b, c; Pl. XII, A—B:b, c) supporting, respectively, the oogonium and its attendant antheridium (Pl. IX, A—C:d, e; C, i, j; Pl. X, A:d, e; Pl. XI, A:d, e; C—F:d, e; Pl. XII, A—B:d, e), together with any other intervening portions of hyphae, have an aggregate length of 75 to 225 μ. *Monoclinous units in which the combined length of the two* branches (Pl. XI, B, b, c; Pl. XII, C, b, c) bearing the oogonium and antheridium (Pl. XI, B, d, e; Pl. XII, C, d, e), together with the length of other connecting hyphal parts, is less than 75 μ, are not usually abundant. In exceptionally compact monoclinous units formed on stout hyphae (Pl. X, B, a) a portion of hypha (Pl. X, B, b) only a few microns long may separate an intercalary antheridium (Pl. X, B, c) from the base of the laterally attached oogonium (Pl. X, B, d) it has fertilized. A large proportion of sexual reproductive units are of diclinous origin, showing no evident mycelial connection between the wide hypha (Pl. IX, C, a; Pl. X, C—G:a; Pl. XII, D—G:a) supplying the oogonium and the neighboring hypha (Pl. IX, C, l; Pl. X, C—G:b; Pl. XII, D—G:b) supplying the antheridium. Very commonly in diclinous as also in monoclinous apparatus a lateral branch (Pl. IX, C, g; Pl. X, D—F:e; Pl. XII, D—F:c) of varying length bears the oogonium (Pl. IX, C, m; Pl. X, D—F:e; Pl. XII, D—F:d) terminally, though occasionally an oogonium (Pl. X, G, c) is formed in laterally intercalary position; the attendant antheridium occupying usually an intercalary (Pl. X, D—F:d; Pl. XII, D—F:e) or a subterminal (Pl. IX, C, n; Pl. X, G, d) position. In instances where a wide hypha (Pl. IX, D, a; Pl. X, C, a; Pl. XII, G, a) gives off a branch (Pl. IX, D, c; Pl. X, C, c; Pl. XII, G, c) bearing two oogonia (Pl. IX, D, d, f; Pl. X, C, e, f; Pl. XII, G, d, e) close together a neighboring hypha (Pl. IX, D, b; Pl. X, C, b; Pl. XII, G, b) may directly supply two adjacent attendant antheridia (Pl. IX, D, e, g; Pl. XII, G, f, g) or may give off a branch (Pl. X, C, d) bearing two attendant antheridia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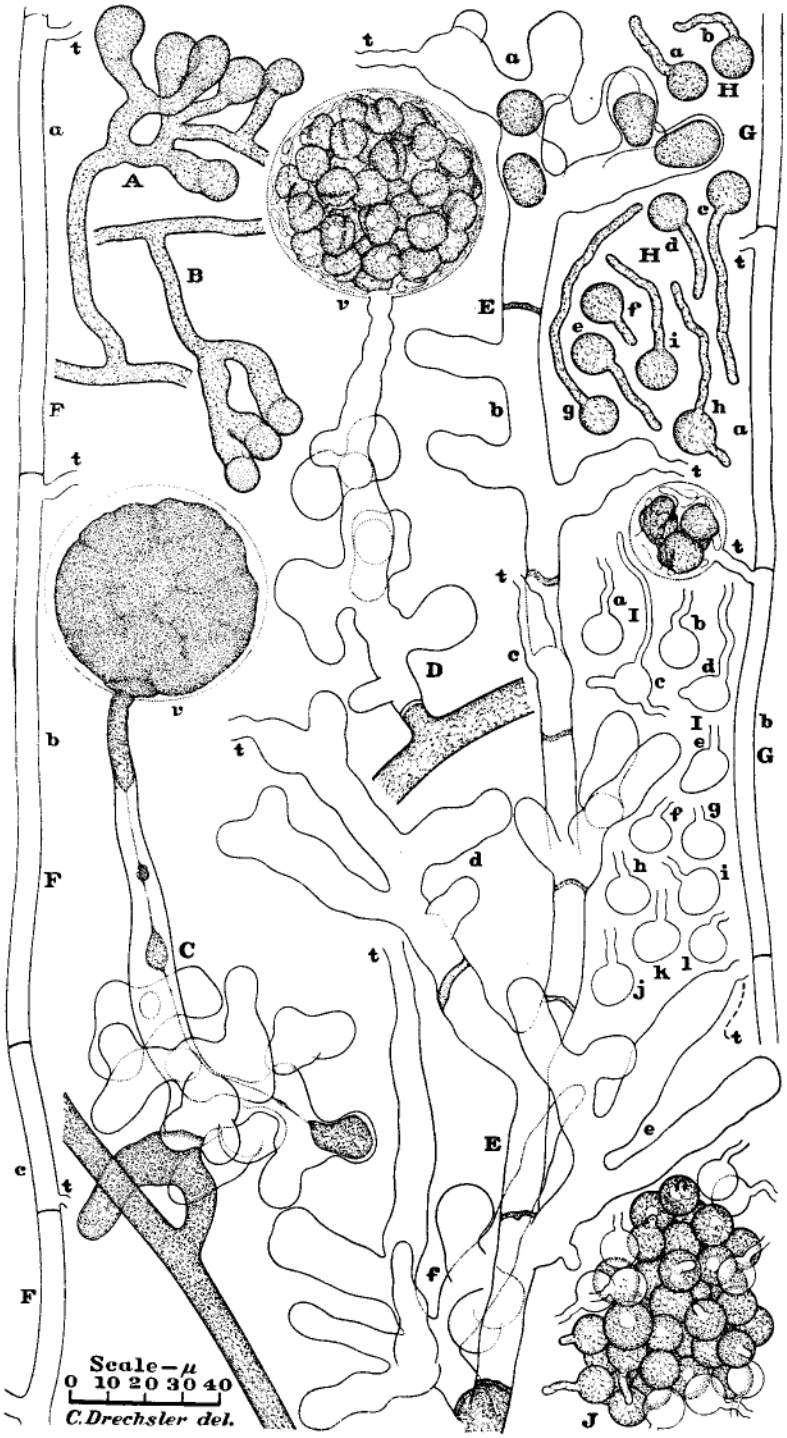
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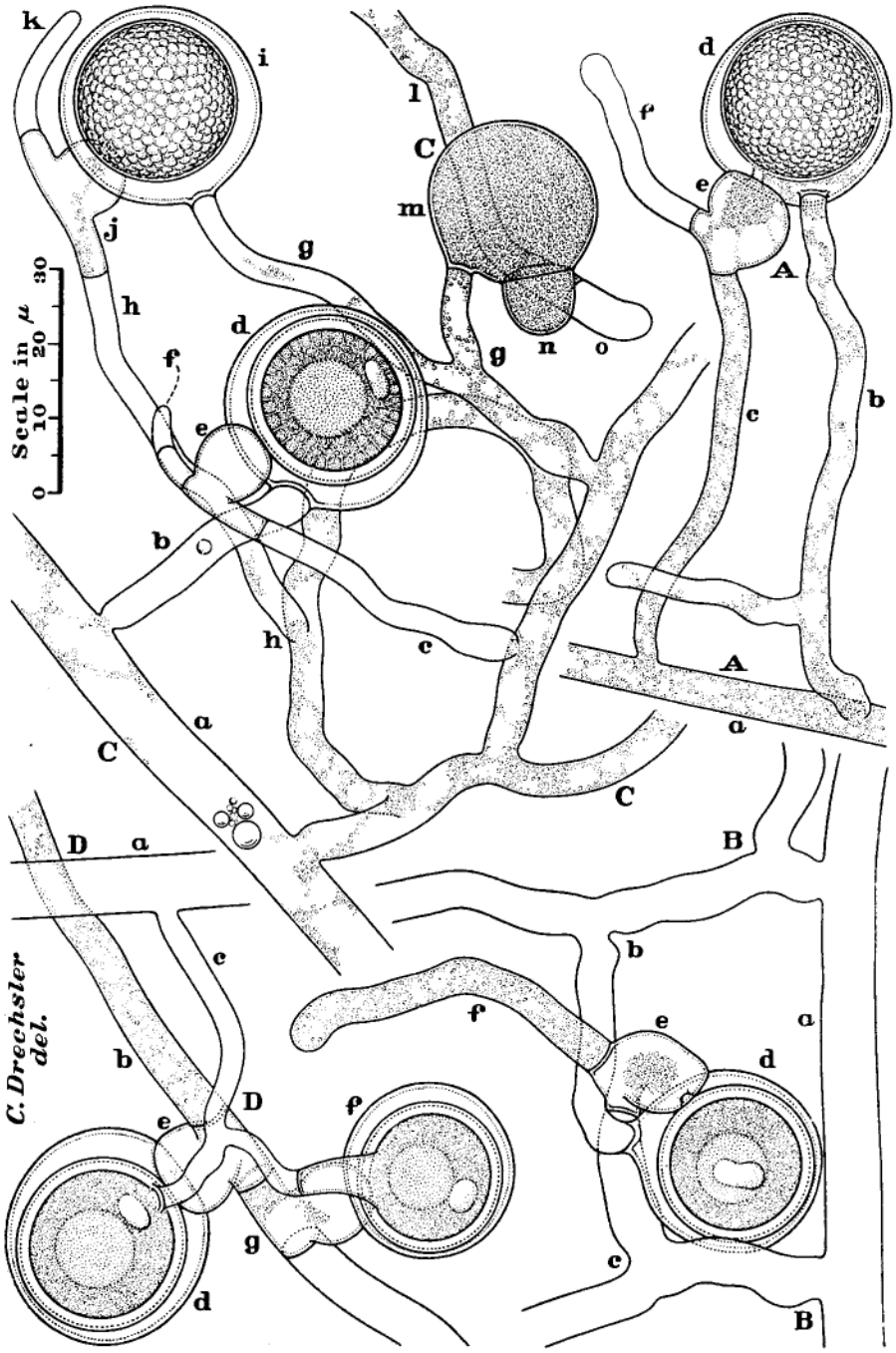
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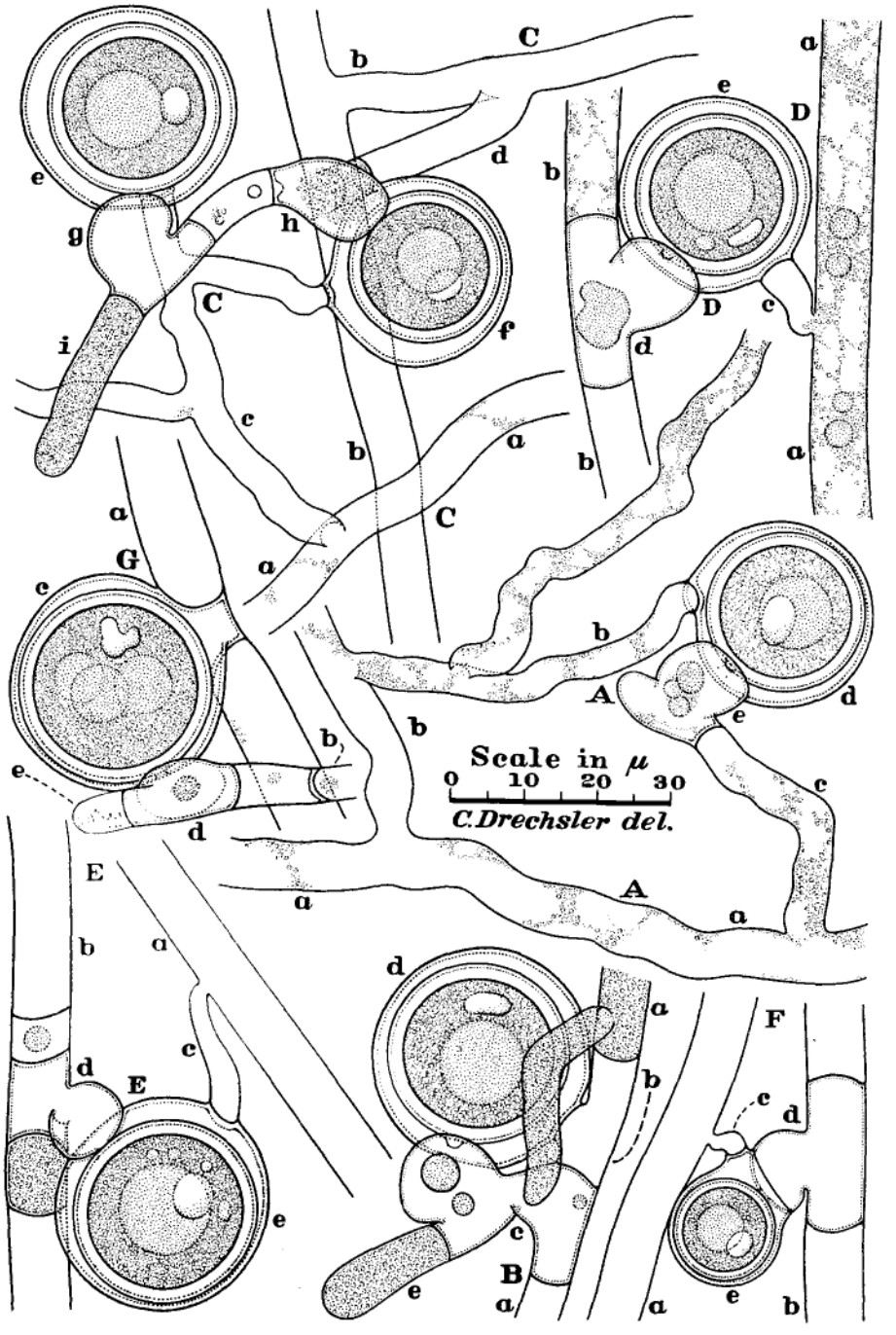
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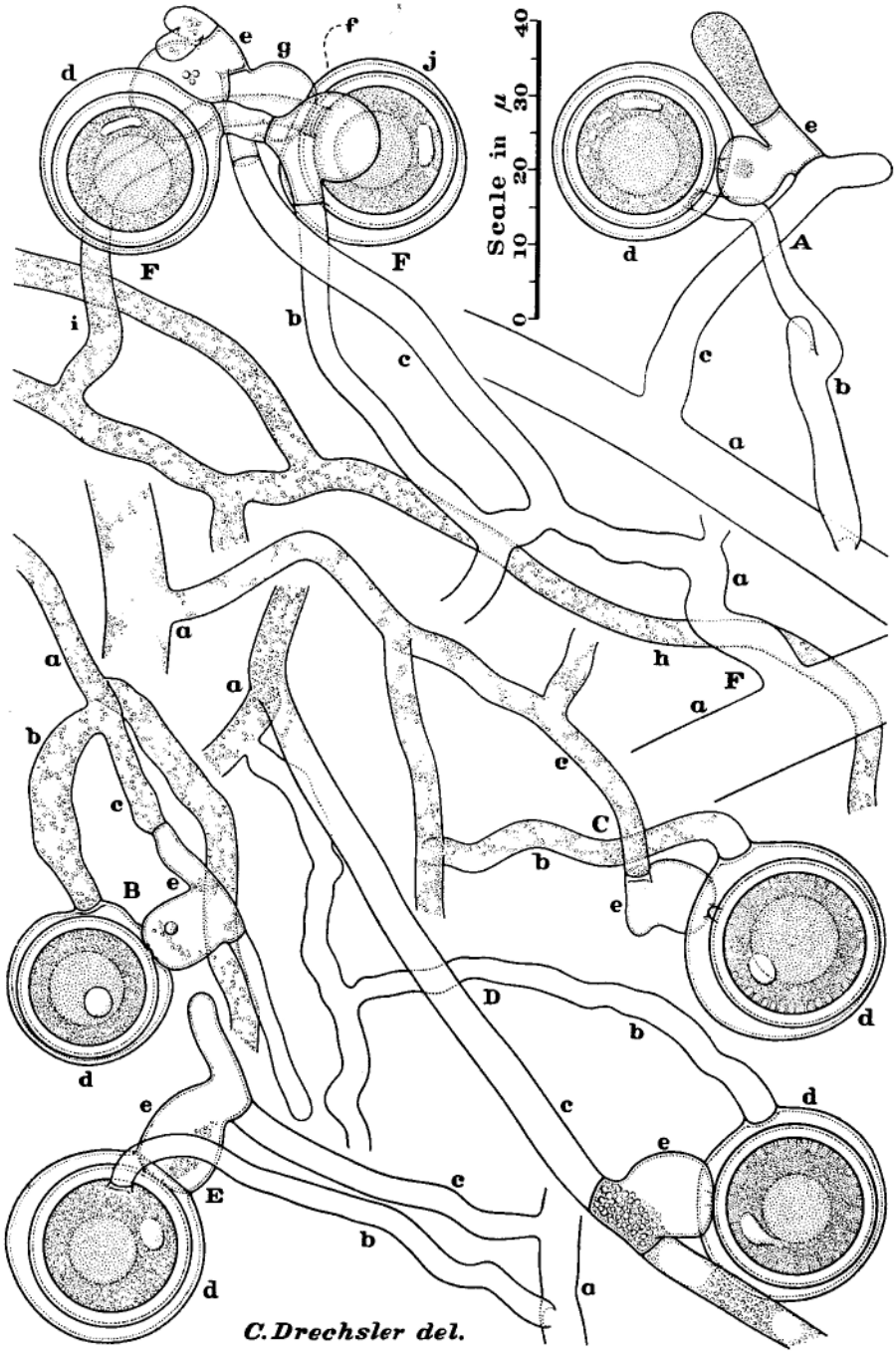
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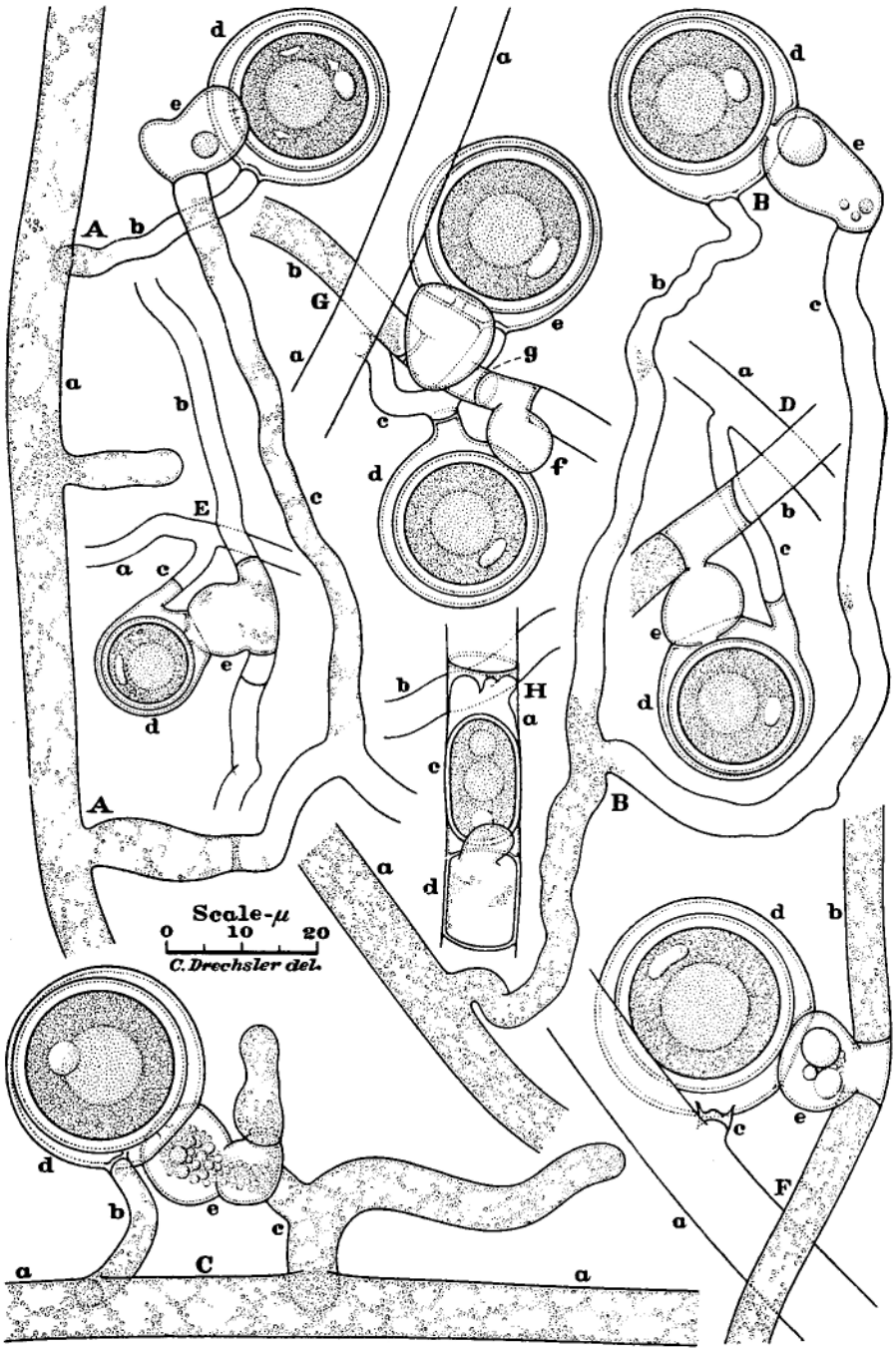
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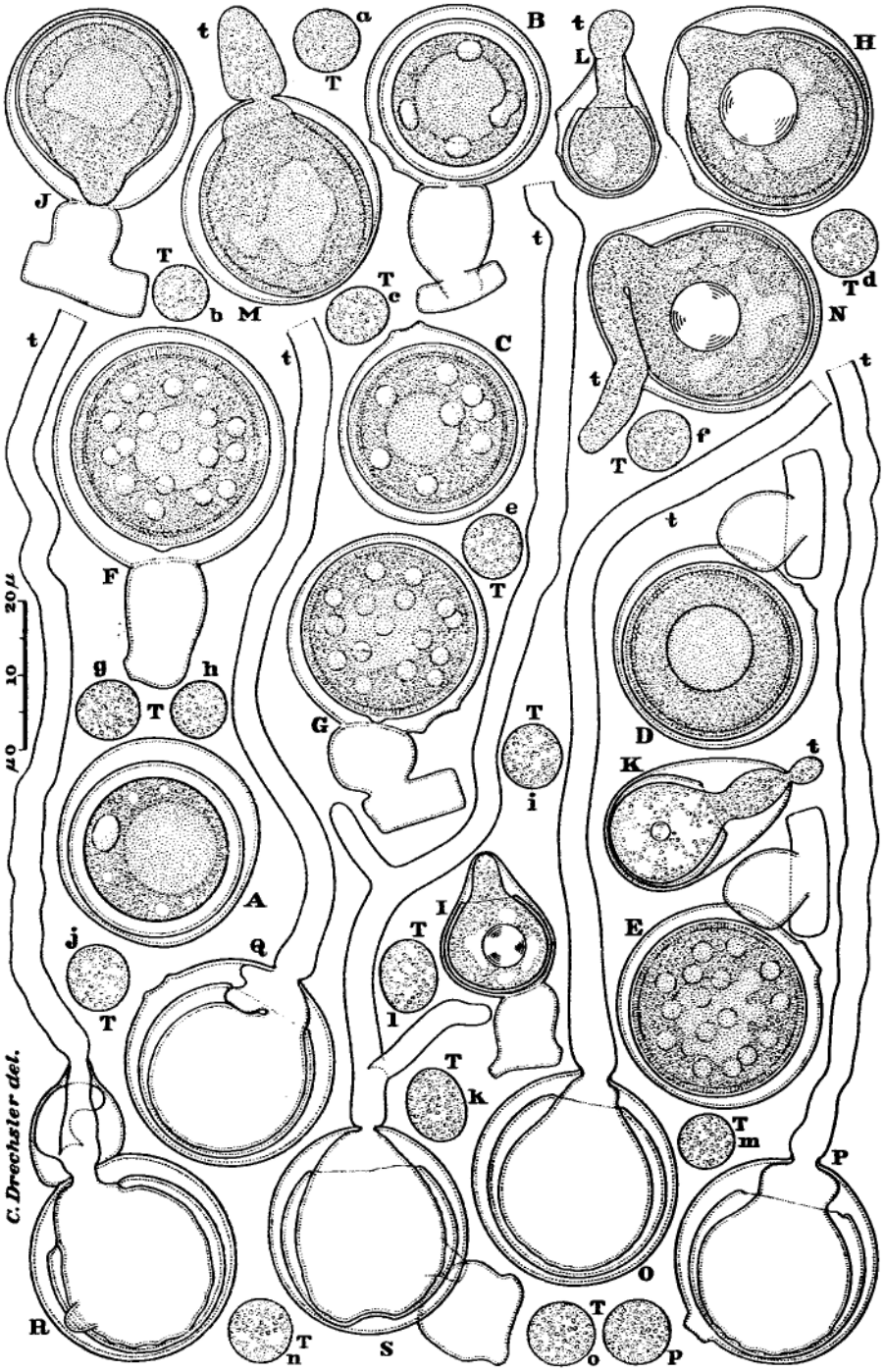
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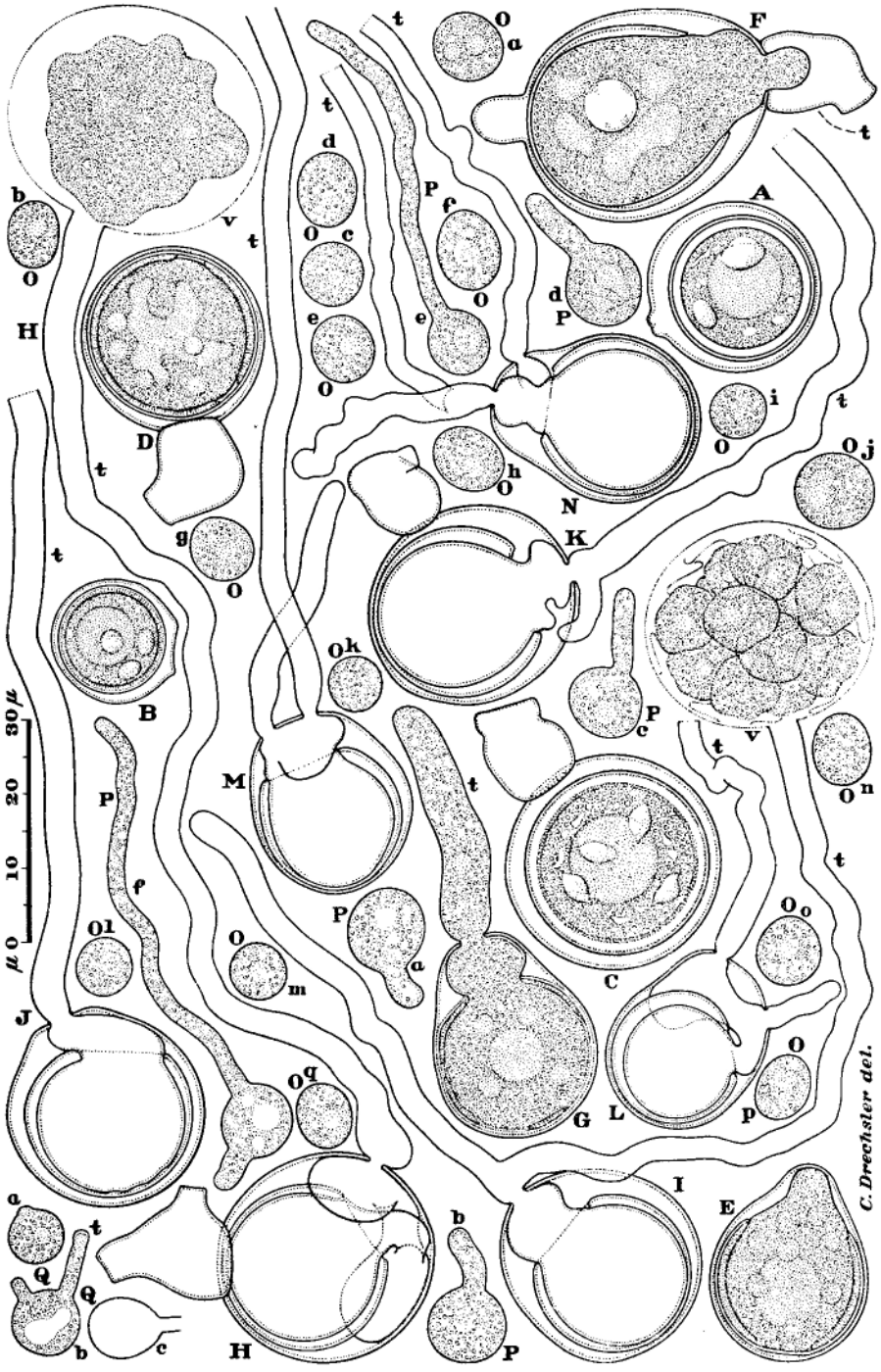
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(Pl. X, C, g, h) only a little apart. How two hyphae may contribute unequally to grouped sexual units is illustrated in the reproductive apparatus shown in Plate XI, F, where one main hypha (Pl. XI, F, a) with 2 sexual branches (Pl. XI, F, b, c) contributes a terminal oogonium (Pl. XI, F, d) and 3 intercalary antheridia (Pl. XI, F, e—g), while the other main hypha (Pl. XI, F, h) with a single sexual branch (Pl. XI, F, i) supplies only an oogonium (Pl. XI, F, j). In aging cultures some rather short cylindrical hyphal segments (Pl. XII, H, a) containing isolated remnants of protoplasm may develop as oogonia; after fertilization from a neighboring hypha (Pl. XII, H, b) or from an adjacent segment (Pl. XII, H, d) has taken place, they form individually an elongated oospore (Pl. XII, H, c). Some relatively small living hyphal segments (Pl. IX, B, f; Pl. X, B, e; C, i) become isolated distad of subterminal antheridia, though in many instances such terminal segments (Pl. IX, A, f; C, f, k, o; Pl. X, G, e) contain little or no protoplasm.

In *Pythium butleri* the fully mature oospore (Pl. XII, A—F:d) shows the internal organization found in most other members of the genus, its single reserve globule of homogeneous consistency being surrounded by a densely granular parietal layer in which is imbedded a flattened or oblate ellipsoidal refringent body. Newly formed oospores of such resting structure commonly fail to germinate when placed in distilled water. As in related species, capacity for germination would seem to require certain modifications of inward structure that first become sparingly noticeable in maize-meal-agar cultures about 15 days after planting, and later are recognizable in steadily increasing numbers of oospores. It seems probable that the tendency of oospores formed at the same time in the same tract of substratum to undergo these modifications after resting periods of variable duration — some oospores undergoing them after a few weeks, others after a few months, and still others after many months — may be helpful in reëstablishing the fungus in successive years despite the caprices of spring and summer weather. After storage for 225 days in a stack under an inverted battery jar at indoor temperature ranging from 18 to 37° C. the maize-meal-agar plate cultures from which was taken the material that with appropriate irrigation yielded the specimens shown in Plates XIII and XIV, showed about one-fifth of all oospores still in a resting condition (Pl. XIII, A).

An initial stage of after-ripening is recognizable in oospores that contain 2 refringent bodies (Pl. XIV, A), and somewhat more advanced stages in oospores displaying 3 (Pl. XIV, B) or 4 (Pl. XIII, B; Pl. XIV, C) refringent bodies. At a later stage when the number of refringent bodies has increased to 8, the reserve globule in some instances (Pl. XIII, C) still retains a subspherical shape. Often no

refringent bodies are discernible in oospores (Pl. XIII, D) examined in newly mounted portions of agar substratum that had dried out badly, the parietal layer in such material presenting a very finely granular texture throughout, and thereby appearing nearly as homogeneous as the sharply defined, accurately spherical reserve globule it surrounds. More than a dozen refringent bodies may come into view when the dried substratum has been immersed in distilled water for an hour, being made visible as the parietal layer acquires a coarsely granular texture (Pl. XIII, E); the mass of reserve material meanwhile diminishing in volume as its boundary becomes irregular and less distinct. The distribution of cellular components here revealed after immersion in water is similar to that normally achieved by oospores in moist substratum through continued multiplication of refringent bodies and gradual encroachment of the parietal granular layer on the reserve globule (Pl. XIII, F, G; Pl. XIV, D).

Soon after the multiplication of refringent bodies has begun, the thick, slightly yellowish inner layer of the oospore wall darkens perceptibly. The layer gradually turns somewhat opaque, its substance appearing more and more like that of the adjacent protoplasm (Pl. XIII, C—G; Pl. XIV, B, C). In one region, or perchance in two regions (Pl. XIV, D), the inner layer now vanishes completely. A protrusion of the protoplast pushes its way through a resulting gap into the oogonial chamber (Pl. XIII, H—J; Pl. XIV, E). After growing straightforward or sideways, often rather massively, within the oogonial chamber the protrusion presses through the oogonial envelope to emerge externally as a germ tube (Pl. XIII, K—N:t). In instances where the protrusion uses the fertilization aperture as passageway (Pl. XIV, F, t) it needs further to break through the antheridial wall, should this membrane still remain attached. With the continued elongation of the germ hypha (Pl. XIV, G, t) all refringent bodies become indiscernible. The protoplasm in which they are lost to view shows densely granular, dispersedly vacuolate texture at it continues to digest materials in the diminishing reserve globule and to assimilate materials from the inner layer of the oospore wall.

Whether an elongating germ hypha will grow into an extensive mycelium or will serve as an evacuation tube in the production of zoospores is determined mainly by external conditions. The same conditions that will discourage swarm-spore development from sporangia of mycelial origin — presence of nutrient substances in considerable quantity, temperatures in excess of 33° C. — will likewise discourage zoospore production from germinating oospores, and consequently will constrain germ hyphae to grow out vegetatively. Similarly, conditions favorable for zoospore production from ordinary sporangia — lack of food materials in the surrounding water, tempera-

tures well below 33° C. — seem favorable also for zoospore production from germinating oospores. Accordingly, if irrigated preparations of oospores are largely freed of residual nutrients by changing the water from time to time, germination by the production of swarm spores often increases markedly. Where oospores are tried out that have been taken from old Petri plate cultures contaminated with molds and bacteria, repeated renewal of water should in many instances help zoospore formation also by removing harmful substances elaborated by the alien microorganisms.

Where a germinating oospore is to serve as a sporangium the germ hypha ceases to elongate and forms a hyaline cap at its tip. This cap suddenly yields and is inflated into a vesicle (Pl. XIV, H, v) as the protoplasmic contents of the oospore flow into it from the germ hypha, now functioning as evacuation tube (Pl. XIV, H, t). The emptied membranous parts (Pl. XIII, O—S; Pl. XIV, H—N) show a few noteworthy features. Within the persisting colorless layer of the oospore wall is inclosed a smaller envelope, evidently interpretable as sporangial wall, since it appears continous with the wall of the protrusion inside the oogonial chamber as well as with the wall of the evacuation tube (Pl. XIII, O—S:t; Pl. XIV, H—N:t). This inner wall is much more distinct than any homologous membrane observable in *Pythium ultimum* or *P. debaryanum*. As a rule it is widely separated from the persistent outer layer of the oospore wall near the region where it enters the oogonial chamber, and appears clearly discrete throughout the antipodal region. The sporangial protrusion within the antheridial chamber sometimes shows rather massive lobulated development (Pl. XIV, H, L—M) and may then extend two separate germ hyphae through the oogonial wall (Pl. XIV, L—M). Usually only one hypha serves as evacuation tube (Pl. XIV, L—M:t), but in some instances two hyphae are found open at the tip (Pl. XIV, N, t, t). Outside the oogonial envelope an evacuation tube may give off one (Pl. XIV, I, K, N) or two (Pl. XIII, S, t) branches. In instances where the evacuation tube has pushed through the fertilization orifice, it may give off a branch in the antheridial chamber (Pl. XIII, R). Functional evacuation tubes produced in shallowly irrigated preparations mostly vary in length from 25 to 225 μ. The longer tubes are generelly produced by the larger oospores. Unbranched tubes are usually longer than branched ones, and single functional evacuation tubes seem generally longer than plural tubes.

After the undifferentiated mass of protoplasm from the oospore has been received into the vesicle (Pl. XIV, H, v) it undergoes cleavage and conversion into laterally biciliate zoospores (Pl. XIV, I, v) in the usual manner. From 4 to 18 swarm spores are commonly liberated, the number depending mainly on the size of the parent

oospore. Following a period of active motility they come to rest and round up (Pl. XIII, T, a—p; Pl. XIV, O, a—q). In many instances they germinate soon afterwards by putting forth one (Pl. XIV, P, a—e) or two (Pl. XIV, P, f) germ tubes. In other instances, however, a zoospore (Pl. XIV, Q, a) derived from a germinating oospore produces an evacuation tube (Pl. XIV, Q, b:t) and empties out its contents (Pl. XIV, Q, c) for conversion into a secondary swarm spore.

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Explanation of Plates VII—XIV.

Plate VII: Crookneck squash plants showing 5 fruits, A—E, at heights 10 to 25 centimeters above the ground, spontaneously attacked by *Pythium butleri* and in varying measure enveloped by aerial mycelium of the fungus. The plant bearing fruits A and E shows also rather extensive infection of the thick succulent petioles. Photographed outdoors in a field at the Plant Industry Station near Beltsville, Maryland, on July 29, 1945. Approximately × ¼.

Plate VIII. Asexual reproductive apparatus of an isolation of *Pythium butleri* obtained from a watermelon fruit found affected with buff-colored blossom-end rot in a field in Arlington, Virginia, on August 5, 1922; parts A and B produced in a maize-agar plate culture; parts C—J produced from slabs which after removal from a Lima-bean-agar culture had been placed in a shallow layer of distilled water; × 500 throughout. A, B, Portions

of mycelial hyphae with grouped appressoria formed on glass of Petri dish. C, Lobulate sporangium that has nearly completed the discharge of its protoplasmic contents into a vesicle, v. D, Empty lobulate sporangium showing about 30 motile zoospores within vesicle, v. E, Distended submerged hyphal outgrowth showing 6 empty sporangia, a—f, each with its evacuation tube, t, open at the tip. F, Externally unmodified hypha showing 3 empty sporangia, a—c, each with evacuation tube, t, open at the tip. G, Unmodified hypha showing one empty sporangium, a, with open evacuation tube, t, and another empty sporangium, b, with an open evacuation tube, t, surmounted by a vesicle containing 3 active zoospores. H, Encysted zoospores, a—i, germinating by emission of one or two germ tubes. I, Empty membranous envelopes, a—l, each of which was emptied in the production of a secondary zoospore. J, Cohering group of encysted zoospores, showing 9 that have each given rise to a secondary zoospore, and 8 others that are each extending a slender evacuation tube.

Plate IX. Sexual reproductive apparatus formed in a maize-meal-agar plate culture by an isolation of *Pythium butleri* obtained from a kidney-bean plant found affected with stem rot in a field near Beltsville, Maryland, on June 27, 1949; × 1000 throughout. A, B, Monoclinous reproductive units: a, main hypha; b, oogonial branch; c, antheridial branch; d, oogonium (immature in A, with mature oospore in B); e, antheridium; f, terminal hyphal segment. C, Wide hypha, a, supplying 2 monoclinous reproductive units, b—f and g—k, composed, respectively, of oogonial branch (b, g), antheridial branch (c, h), oogonium (d, i), antheridium (e, j), empty distal segment (f, k); these being connected with a diclinous reproductive unit, g and l—o, composed of oogonial branch (g), antheridial branch (l) oogonium (m), antheridium (n), distal hyphal segment (o); oogonium d contains a nearly mature oospore, oogonium i contains a young oospore, and oogonium m is still unfertilized. D, Two main hyphae, a and b, supplying 2 mature diclinous reproductive units, c—e and f—g; the ramified oogonial branch (c) bears terminally the 2 oogonia (d, f) that are fertilized by the 2 adjacent antheridia (e, g) borne intercalarily in hypha b.

Plate X. Sexual reproductive apparatus formed in a maize-meal-agar plate culture by an isolation of *Pythium butleri* obtained from a kidney-bean plant found affected with stem rot in a field near Beltsville, Maryland, on June 27, 1949; × 1000 throughout. A, Mature monoclinous reproductive unit: a, wide hypha; b, oogonial branch; c antheridial branch; d, oogonium; e, antheridium. B, Mature monoclinous reproductive unit borne compactly on a wide hypha, a; only a short portion of an empty segment, b, separates the laterally intercalary antheridium, c, from the oogonium, d; e, living terminal segment. C, Two main hyphae, a and b, from which are given off, respectively, the ramified oogonial branch, c, and the antheridial branch, d; the oogonia, e and f, are fertilized, respectively, by the intercalary antheridia, g and h; i, living terminal segment; oospores in both oogonia are in fully mature resting state. D—F, Three mature diclinous reproductive units, each of which is supplied from 2 wide hyphae, a and b; c, oogonial branch; d, intercalary antheridium; e, oogonium. G, Diclinous reproductive unit supplied from two main hyphae, a and b; c, laterally intercalary oogonium; d, antheridium; e, short terminal segment.

Plate XI. Sexual reproductive apparatus formed in a maize-meal-agar plate culture by an isolation of *Pythium butleri* obtained from a kidney-bean plant found affected with stem rot in a field near Beltsville, Mary-

land, on June 27, 1949; × 1000 throughout. A—E, Five monoclinous reproductive units: a, parent hypha; b, oogonial branch; c, antheridial branch; d, oogonium; e, antheridium; oospores very nearly mature in C and D, and in fully mature resting state in A, B and E. F, Mature monoclinous reproductive unit, a—e, connected with mature diclinous unit, f—j: a wide hypha (a) supplies 2 sexual branches (b, c) which together bear not only an oogonium (d) and its attendant antheridium (e) but also other antheridia (f, g) that both have fertilized a second oogonium (j) borne on a branch (i) given off by a neighboring main hypha (h); the close hyphal connection between oogonium d and antheridium f being somewhat exceptional.

Plate XII. Sexual reproductive apparatus formed in a maize-meal-agar plate culture by an isolation of *Pythium butleri* obtained from a kidney-bean plant found affected with stem rot in a field near Beltsville, Maryland, on June 27, 1949; × 1000 throughout. A—C, Three fully mature reproductive units: a, parent hypha; b, oogonial branch; c, antheridial branch; d, oogonium; e, antheridium. D—F, Three mature diclinous reproductive units, each supplied from 2 neighboring hyphae, a and b, of which one (a) gives off a branch, c, bearing an oogonium, d, that is fertilized by an antheridium, e, borne intercalarily on the other hypha (b). G, Two mature monoclinous reproductive units supplied from 2 neighboring hyphae, a and b, of which one (a) gives off a branch, c, bearing 2 oogonia, d and e, that are fertilized, respectively, by 2 antheridia, f and g, borne intercalarily on the other hypha (b). H, Mature diclinous reproductive unit formed from remnants of protoplasm in 2 neighboring hyphae, a and b; the ripe oospore, c, shows 2 reserve globules, owing to its elongated shape; a segment, d, adjacent to the cylindrical oogonial segment may have served as an extra antheridium.

Plate XIII. Reproductive apparatus observed in irrigated preparations of oospores taken from a 225-day-old maize-meal-agar plate culture of an isolation of *Pythium butleri* obtained from the crookneck squash fruit shown in Figure 1, B; × 1000 throughout. A, Oospore still in resting condition. B, Oospore showing some after-ripening in that it contains 4 refringent bodies. C, Oospore showing 8 refringent bodies and darkened inner layer of wall. D, Oospore from dried — out portion of old culture; no refringent bodies discernible in the fine — textured parietal layer surrounding the sharply defined reserve globule; inner layer of wall darkened. E, Same oospore after immersion in distilled water for 1 hour; many refringent bodies now visible in coarsely granular parietal layer; reserve globule now of irregular shape. F, G. Oospores in advanced stage of after-ripening: many refringent bodies discernible; reserve globule irregular; inner layer of oospore wall becoming obliterated. H—J, Oospores in early stage of germination, with protoplast protruding into oogonial chamber. K—N, Oospores, each with a germ tube, t, elongating externally. O—S, Empty membraneous envelopes left after escape of motile zoospores from vesicle at open tip of evacuation tube, t. T, Encysted zoospores, a—p, that originated from germinating oospores.

Plate XIV. Reproductive apparatus observed in irrigated preparations of oospores taken from a 225-day-old maize-meal-agar plate culture of an isolation of *Pythium butleri* obtained from the crookneck squash fruit shown in Figure 1, B; × 1000 throughout. A, Oospore showing 2 refringent bodies. B, Oospore in somewhat more advanced stage of after-ripening, showing 3 refringent bodies and darkening of inner layer of its wall.

C, Oospore with 4 refringent bodies. D, Oospore with inner layer of wall dissolved away in 2 regions. E, Oospore in early stage of germination, with protoplast protruding broadly against oogonial envelope. F, Oospore extending germ tube, t, into antheridial chamber. G, Oospore with germ tube, t, elongating externally. H, Oospore that has discharged its protoplasm through an evacuation tube, t, into a terminal vesicle, v. I, Oospore that has discharged its protoplasm through an evacuation tube, t, into a vesicle, v, where 13 motile zoospores have been formed, J—M. Empty membranous envelopes left after liberation of motile zoospores from vesicle at open tip of evacuation tube, t. N. Empty membranous envelopes with 2 evacuation tubes, t, each open at its tip. O, Encysted zoospores, a—q, which originated through germination of oospores. P, Encysted zoospores, a—e, each germinating by emission of a broad vegetative germ tube; f, zoospore putting forth two germ tubes. Q, Three zoospores, a—c, showing different stages of repetitional development: a, narrow evacuation tube beginning to grow out; b, evacuation tube, t, surmounted by hyaline cap; c, empty membrane with open evacuation tube.