THREE SPECIES OF PYTHIUM WITH LARGE OOGONIAL PROTUBERANCES

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Among 15 members of the genus *Pythium* that I (5) presented nearly a decade ago as species new to science, were included 2 forms, *P. mastophorum* and *P. polymastum,* that were set forth at the time as being apparently most directly related to *P. megalacanthum* de Bary. Owing to the circumstance that *P. megalacanthum* has since then been cited in connection with injury to various economic plants in different countries, an increased possibility seems in prospect that my 2 forms may become rather variously understood in accordance with diverse applications of de Bary's binomial. To abate in some degree whatever occasion for error may arise, it appears expedient to supplement my earlier diagnoses with illustrated discussion of the more important of the relevant morphological and developmental details,—the illustrations, to facilitate comparison, having been prepared for reproduction at magnifications uniform with similar figures given earlier in papers on congeneric parasites (6, 7, 9). Such discussion likewise is devoted to a third fungus, which has been dealt with as *P. megalacanthum* more extensively in recent literature than any other, and which, besides, in its structural features, presents so obvious a parallelism with both *P. mastophorum* and *P. polymastum* that its intimate relationship to these forms seems unquestionable.

PYTHIUM MASTOPHORUM

Though numerous cultures derived from a wide variety of affected vegetable structures have been examined during the 12 years since *Pythium mastophorum* was isolated from a discolored root of an unthrifty specimen of English daisy, *Bellis perennis* L., collected in the District of Columbia, the fungus has not yet been obtained from diseased material a second time. The species could not well have been overlooked in examinations of planted plate cultures, as, even previous to the development of its reproductive bodies, the characteristically haphazard disposition of its mycelial elements contrasts rather markedly with the more orderly arrangements of hyphae usual for most root-inhabiting oomycetes. Opportunity has been lacking for determining to what extent the fungus may be associated with rootlet decay of the English daisy, as diseased specimens of that ornamental have not again become available for use in the preparation of cultures.

Asexual reproduction of the fungus may be induced by shallow irrigation of well-nourished, actively growing mycelium. Such irrigation is readily accomplished by removing small slabs from a Lima-bean-agar plate culture to a sterile Petri dish and adding sterile distilled water until the liquid moistens, yet does not seriously flood, the upper surface of the slabs. As nutrient substances tend to diffuse out from the rich substratum, thereby encouraging a

continuation of vegetative growth, it has often proved advantageous to re-place the water at intervals. The manipulation required for these changes usually results in the introduction of putrefactive bacteria, which, in the course of 1 or 2 days, multiply so as to attain a concentration decidedly un-favorable for zoospore production by most root-rotting oomycetes. *Pythium mastophorum*, however, would seem little repressed in its asexual reproduc-tive development as long as bacterial contamination remains fairly moderate, for usually, it continues to give rise to zoosporangia during the third and fourth days after the irrigation treatment is begun.

The zoosporangia, usually borne terminally (Fig. 1, A, B), or subter-minally (Fig. 1, C, D, E), though sometimes occupying definitely intercalary positions (Fig. 1, Q, R), are of the subspherical type and have approximately the same dimensions as the homologous bodies, for example, of *Pythium irregulare* Buism. and *P. mammillatum* Meurs. Nevertheless, they would not readily be mistaken for reproductive bodies of the two more familiar para-sites, owing partly to a perceptible tendency for them to assume an oblate ellipsoidal (Fig. 1, A–E) rather than a prolate ellipsoidal shape, and partly to a darker, less translucent consistency of their protoplasmic contents. In the latter respect they suggest somewhat the appearance of young oogonia or globose gemmae belonging to certain relatively delicate members of the Saprolegniaceae.

After an individual sporangium has been delimited from its supporting hypha by the deposition of 1 or 2 septa, it puts forth an evacuation tube, sometimes from an equatorial position (Fig. 1, F, G), sometimes from an apical position (Fig. 1, H, I), and sometimes from a basal position (Fig. 1, P). Sooner or later a large vacuole appears within the sporangium, and a refractive gelatinous cap is formed at the tip of the evacuation tube (Fig. 1, I). These changes presage discharge of the protoplasmic material into a vesicle resulting from inflation of the apical cap. In the vesicle cleavage of the granular material wholly after the fashion usual in the genus *Pythium* brings about its conversion into relatively large biciliate zoospores (Fig. 1, J, JJ, K–N). On being liberated through rupture of the vesicle, the zoo-spores swim about for a variable period in a slow, seemingly deliberate man-ner, and eventually round up into subspherical cysts having an average diameter of approximately 13 μ.

Proliferous development of sporangia has never been observed in any material of *Pythium mastophorum*. Very frequently, indeed, the portions of sporangiferous filaments adjacent to empty sporangia are likewise devoid of contents (Fig. 1, O–X). Irregularities in development of asexual repro-ductive apparatus have been noted in this species as in many other oomycetes: illustrative instances being represented often in excessive contortion of the evacuation tube (Fig. 1, G), in a somewhat branched condition of that tube (Fig. 1, J, M), and in production of a second tube following frustration of the first (Fig. 1, L).

Sexual organs are formed abundantly and promptly when *Pythium mas-tophorum* is grown on maize-meal agar. The young oogonia make their ap-
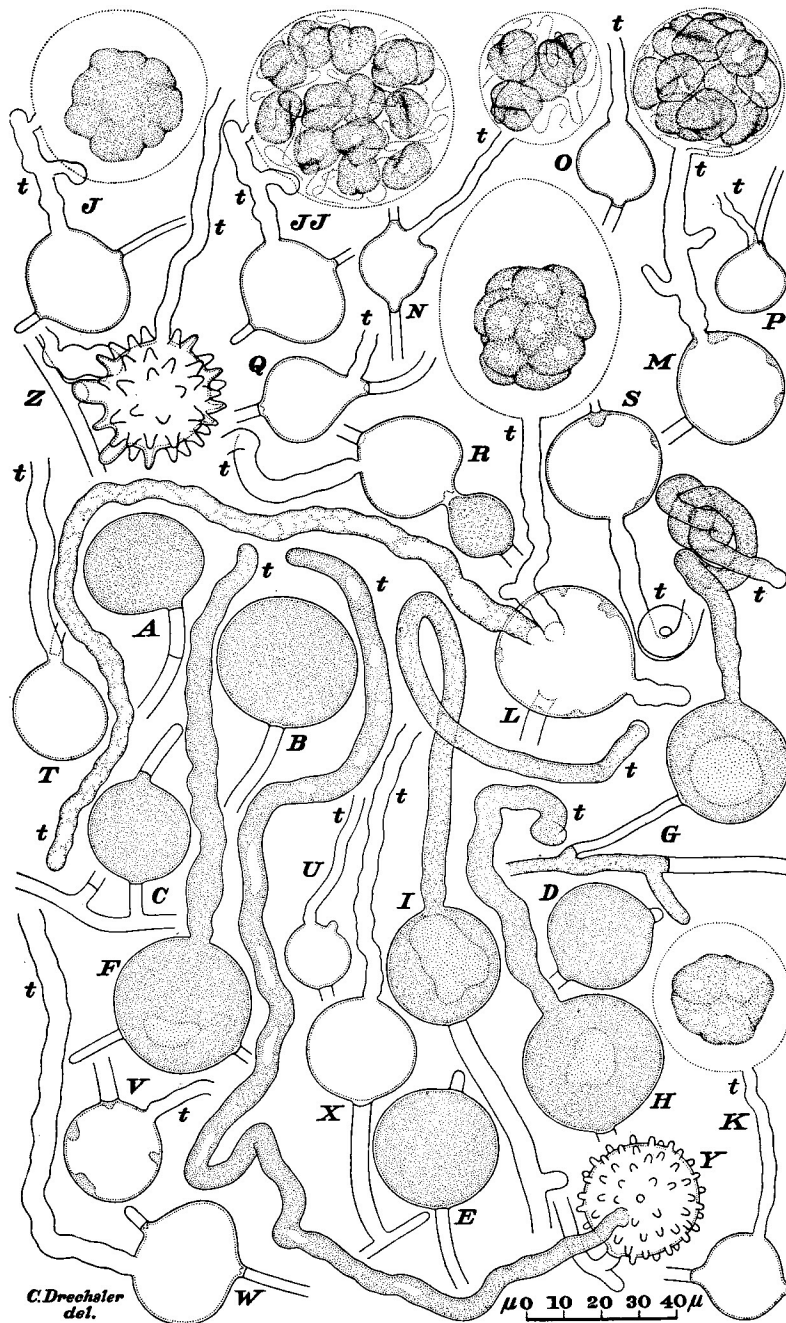
Fig. 1. Asexual reproductive apparatus of *Pythium mastophorum* formed on irrigated Lima-bean agar, and, with the aid of a camera lucida, drawn to a uniform magnification; ×500 throughout. A–E. Full-grown sporangia. F–I. Sporangia, each of which has put forth an evacuation tube. J, JJ. Sporangium showing respectively an early and a late stage in the fashioning of zoospores within the vesicle. K–N. Sporangia showing successively later stages in zoospore development. O–X. Empty sporangia, after escape of zoospores. Y. Oospore germinating by production of a long evacuation tube. Z. Empty oogonium, after germination of oospore by production of zoospores. (t, evacuation tube in F–Z.)

pearance in the substratum as globose bodies, usually borne terminally on short lateral branches that are often somewhat bent or contorted. Like the sporangia, they contain very densely granular protoplasm, the meager translucence of which gives them a darkish aspect. At a relatively early stage in the enlargement of the individual female organ, the usually single antheridium begins to develop in contact with it as a saccate or lobate expansion terminating a filament not closely connected with the oogonial hypha. The 2 sexual structures and the filaments supporting them are usually engaged with one another to a greater or lesser extent, the engagement being promoted at times by the presence of short diverticulations (Fig. 2, A, B, C). Before the oogonium has quite attained definitive size it puts forth protuberances, now in scattered arrangement, now in closer, bristling array. At first these protuberances may be hemispherical (Fig. 2, D, E), but soon they mostly become drawn out distally into broadly conical (Fig. 2, F) or even somewhat mammiform shapes (Fig. 2, G). After withdrawal of protoplasmic contents from them, the protuberances are revealed as characteristically thick-walled modifications with lumina often very markedly narrowed at the apex (Fig. 3, A–D). Following delimitation of both oogonium and antheridium through insertion of basal septa, the latter organ intrudes into the former a rather broad fertilization tube by means of which it delivers up its contents. A familiar sequence of developmental changes brings into existence an oospore that, at maturity, is provided with a wall of moderate thickness, and shows the internal structure most usual among species of *Pythium*,—its single large central reserve globule being surrounded by a granular parietal layer wherein a single refringent body, mostly oblate ellipsoidal in shape, is discernible (Fig. 3, A–D).

Degeneration of sexual apparatus is sometimes virtually absent in maize-meal agar cultures of *Pythium mastophorum*, while at other times, in the same medium, fully half of the oogonia fail to yield good oospores. Frequently only a portion of the granular material within an oogonium is lost, the remainder being utilized in endogenous formation of a smaller secondary oogonium, which is usually furnished with correspondingly fewer and smaller protuberances, and gives rise to a proportionately smaller oospore. Now and then similar partial degeneration in a secondary oogonium is concomitant with endogenous development of a tertiary oogonium, within which an oospore of small dimensions, yet of correct internal structure, may be borne. Except for a brief passage devoted wholly to a description of the secondary and tertiary oogonia, the diagnosis of *P. mastophorum*, for better comparison with related species, avoids consideration of these bodies and of the oospores formed in them, its statements on ordinary dimensions of oogonium, antheridium, and oospore being based on measurements of completely normal structures found in maize-meal-agar cultures that showed practically no degeneration of sexual apparatus. Normal and abundant sexual development of the fungus is accompanied generally by a dimensional variability that, in comparison with variability in some congeneric forms, may be re-
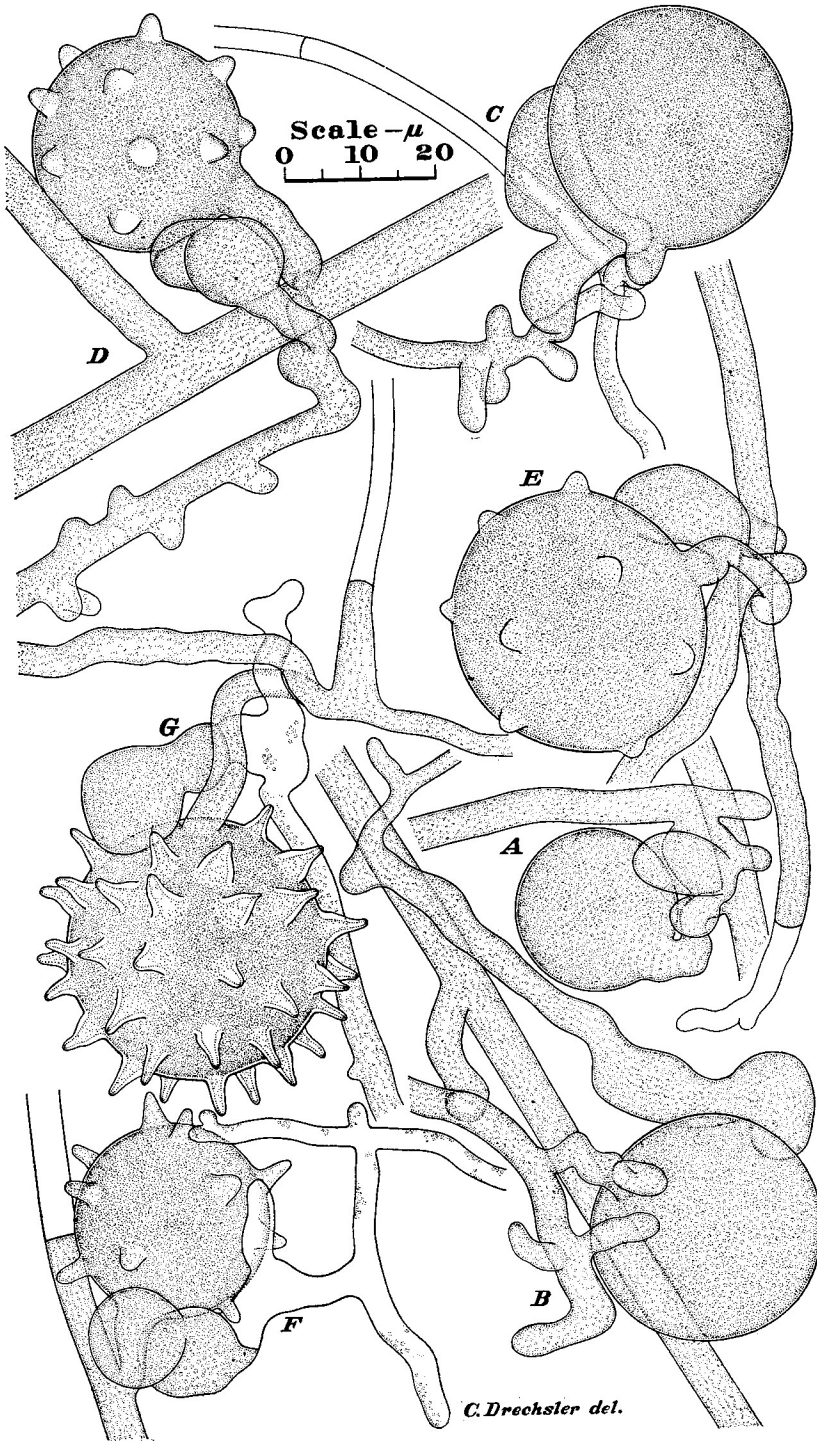
FIG. 2. A–G. Immature units of sexual apparatus of *Pythium mastophorum* formed in maize-meal-agar plate cultures, and, with aid of a camera lucida, drawn to a uniform magnification; × 1000 throughout.
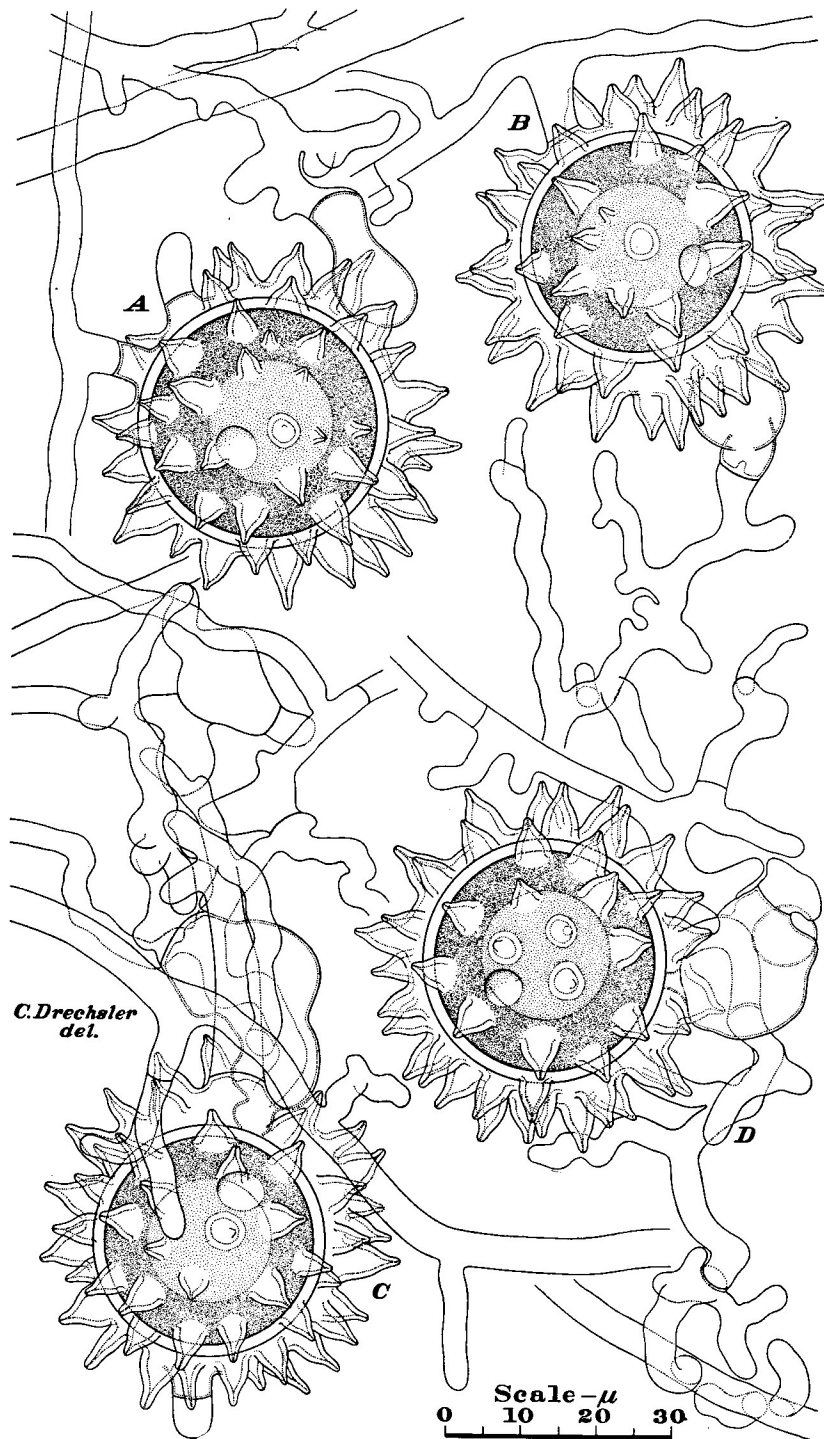
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Scale—μ
0   10   20   30

FIG. 3.   A–D. Mature units of sexual apparatus of *Pythium mastophorum* formed in maize-meal-agar plate cultures, and, with the aid of a camera lucida, drawn to a uniform magnification; ×1000 throughout.

garded as moderate. Thus the 200 values for diameter of oogonium (exclusive of protuberances) from which was computed the average for this dimension given in the diagnosis—values obtained by measuring specimens selected at random—showed, when expressed to the nearest micron, a distribution as follows: 25 μ, 1; 30 μ, 3; 31 μ, 6; 32 μ, 15; 33 μ, 26; 34 μ, 27; 35 μ, 43; 36 μ, 24; 37 μ, 24; 38 μ, 15; 39 μ, 9; 40 μ, 6; 41 μ, 1. And the 200 values for oospore diameter from which was computed the average for that dimension—values obtained by measuring the oospores contained within the 200 oogonia—showed, when expressed to the nearest micron, the following distribution: 21 μ, 1; 22 μ, 1; 24 μ, 4; 25 μ, 5; 26 μ, 6; 27 μ, 23; 28 μ, 29; 29 μ, 46; 30 μ, 48; 31 μ, 28; 32 μ, 5; 33 μ, 3; 35 μ, 1.

Since *Pythium mastophorum* is usually rather reluctant to form zoospores, it is somewhat surprising that, when asexual reproduction occurs in irrigated preparations, a considerable proportion of such oospores as happen to have been produced simultaneously with the asexual sporangia likewise germinate by production of zoospores. The events associated with this type of germination follow the sequence earlier set forth in my account of *P. acanthicum* Drechsl. (9, p. 402).

PYTHIUM POLYMASTUM

Pythium polymastum came into my hands in a pure culture received with 13 other fungous cultures in May, 1925, from the late Doctor G. P. Clinton, in whose laboratory at the Connecticut Agricultural Experiment Station, New Haven, Conn., it was stated to have been isolated on April 1, 1921, from lettuce, *Latuca sativa* L. Although decaying roots of lettuce obtained from sickly plants in greenhouses both at the Arlington Experiment Farm, Arlington, Va., and at the U. S. Horticultural Station, Beltsville, Md., have on several occasions during more recent years been examined microscopically, the characteristic sexual bodies of the fungus could not be recognized in any affected tissues. The fungus has never been obtained when diseased lettuce roots of such origin were planted on agar media in Petri dishes; nor, indeed, has it come to light following similar treatment of discolored roots or rootlets from numerous other phanerogamic plants undertaken by me during a period of 18 years. To my knowledge the species has not been isolated from any natural source a second time. For some years, however, Clinton's original strain, as also the one and only strain of *P. mastophorum* hitherto brought into pure culture, has been widely available to mycologists at the Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Growing in pure culture on maize-meal agar, *Pythium polymastum* shows approximately the same moderate rate of mycelial extension as *P. mastophorum;* and reveals, too, the same characteristically random disposition of its vegetative filaments. Although its vegetative hyphae exceed in width those of the form isolated from roots of the English daisy, the difference with respect to coarseness is not a conspicuous one. Because of their meager display of delicate branches both species must be reckoned among the coarser members of the genus *Pythium*.
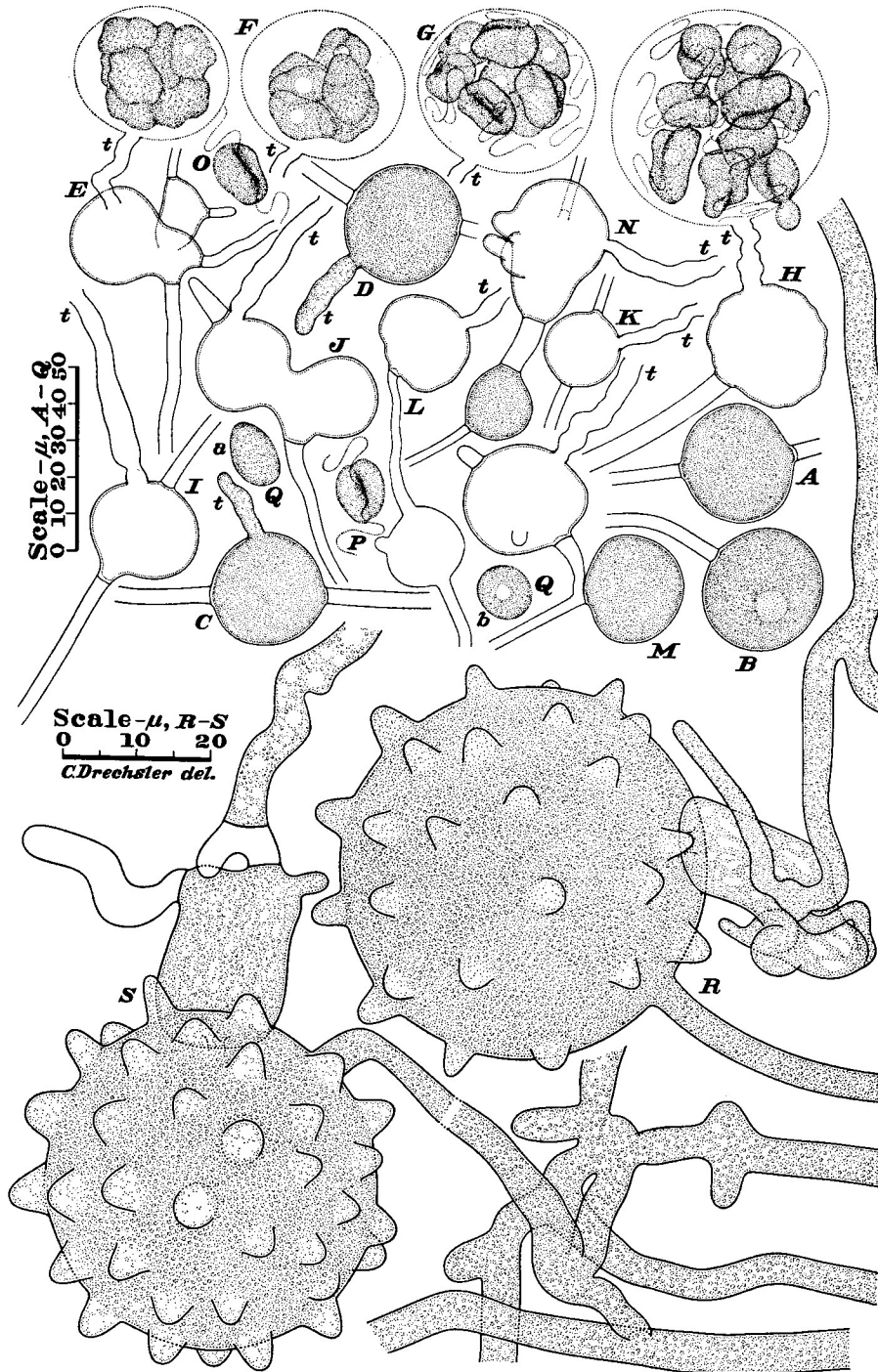
FIG. 4. *Pythium polymastum*, drawn with aid of a camera lucida. A–Q. Asexual reproductive apparatus formed on irrigated Lima-bean agar; ×500: A, B, Full-grown sporangia; C, D, Sporangia, each with an elongating evacuation tube; E, F, G, Sporangium, showing 3 successive stages in the fashioning of its zoospores; H, Sporangium, showing a zoospore in act of escaping; I–N, Empty sporangia; O, P, Motile zoospores; Q, a, b, Zoospores rounding up; t, evacuation tube in C–N. R, S. Immature units of sexual apparatus formed in maize-meal agar; ×1000.

Zoospore production is not readily induced in *Pythium polymastum*, but has nevertheless been obtained several times by carefully irrigating slabs excised from Lima-bean-agar plate cultures well permeated with young mycelium. The sporangia, like those of *P. mastophorum*, appear rather tardily as somewhat opaquely darkish bodies occurring in either intercalary (Fig. 4, A) or terminal (Fig. 4, B) positions. For the most part they are of subspherical or oblate ellipsoidal shape, yet irregular specimens are so frequent that they can hardly be considered exceptional. In some instances, for example, the sporangium is composed of 2 connected lobes (Fig. 4, E, J), or, again, it consists of a main swollen structure together with a number of dome-shape diverticula (Fig. 4, N). The evacuation tube (Fig. 4, C–N: t) may arise from an equatorial position (Fig. 4, C, D) or from a position closer to the basal or distal end (Fig. 4, I, J). On attaining a length sometimes less (Fig. 4, H, L) but at other times greater (Fig. 4, I) than the diameter of the sporangium, it yields at the tip to permit escape of the sporangial contents into a vesicle, where they undergo transformation into biciliate zoospores wholly through the development generally characteristic of species of *Pythium* (Fig. 4, E, F, G). Escape of the swimming spores ordinarily ensues from extensive disintegration of the vesicle as is usual in congeneric forms; though a few instances came under observation where a relatively small opening served as exit for one or several of the motile spores (Fig. 4, H) before the vesicular membrane—evidently one of more than customary toughness—gave way extensively. The empty sporangial envelope not infrequently shows a relaxed undulant contour (Fig. 4, H, L), but, even where irregularity of outline is not clearly noticeable (Fig. 4, E, I, J, K, M, N), some reduction in volume may be presumed to have accompanied loss of contents.

The zoospores of *Pythium polymastum*, after swimming about in a stately manner (Fig. 4, O, P), come to rest (Fig. 4, Q, a) and round up into cysts (Fig. 4, Q, b) with an average diameter of 15 to 16 μ. They are, indeed, the largest zoospores I have ever seen in any species of *Pythium*. Despite their extraordinary dimensions they seem clearly exceeded in size by the zoospores of *P. megalacanthum*, which, according to de Bary's account (2), round up into cysts with an astonishing average diameter of 18 to 20 μ. Since zoospores generally appear little given to pronounced dimensional variability, it is believed that a difference of 3 or 4 μ in average width of these bodies would in itself set apart Clinton's fungus as a species different from that of de Bary, notwithstanding certain obvious resemblances, which had led me at first to hold the two identical,—an error whereby the fungus under consideration came to be cited as *P. megalacanthum* in a paper by Harter and Whitney (10). Additional ground for objecting to the earlier tentative identification derives from the absence of any proliferous tendency in the zoosporangial development of *P. polymastum* under cultural conditions that evoked unmistakable proliferation in *P. anandrum* Drechsl.,—assuredly a fungus not disposed to produce zoosporangia at all abundantly.

When cultivated on maize-meal agar, *Pythium polymastum* usually begins its sexual reproduction 3 or 4 days after active mycelial growth has been
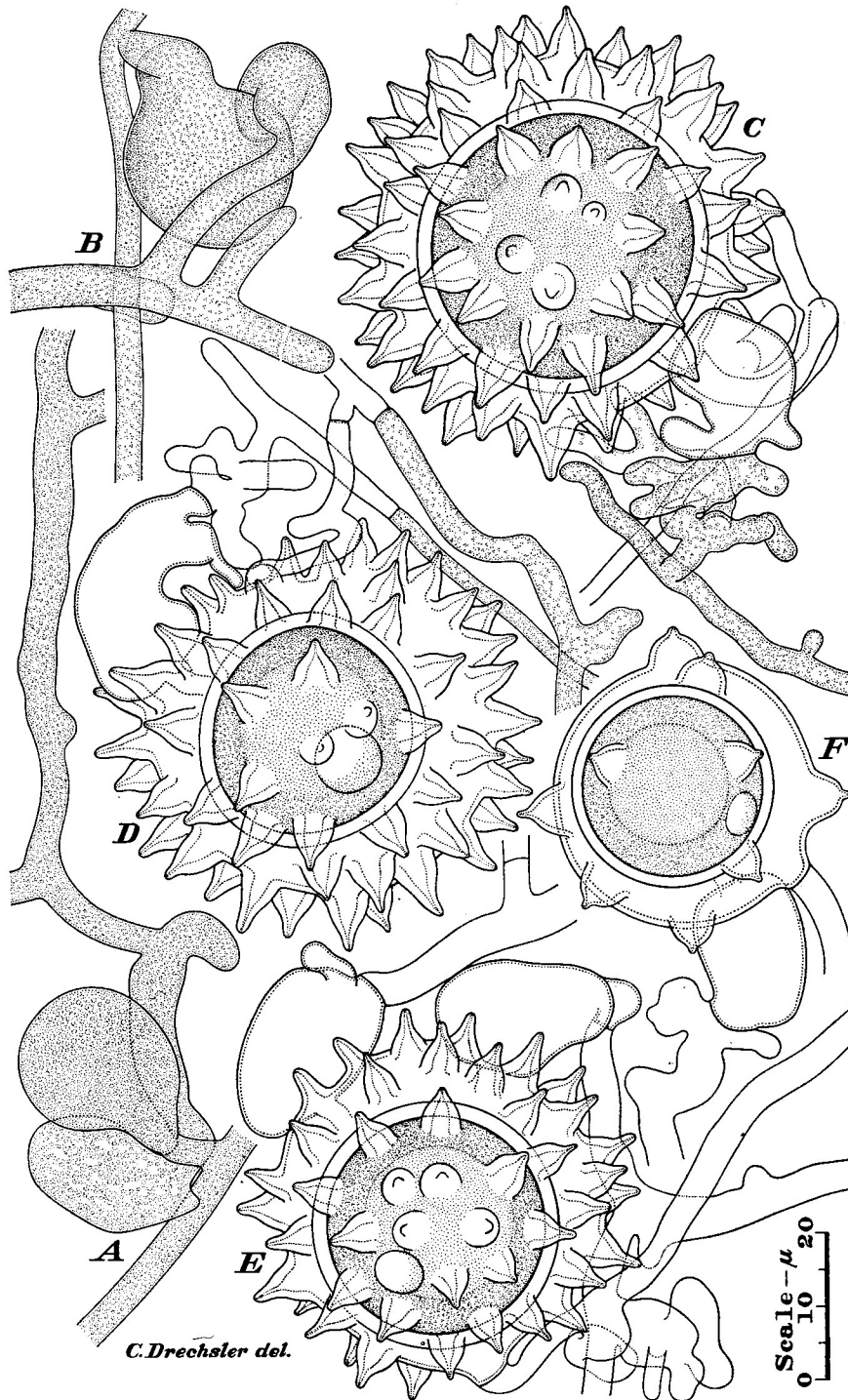
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FIG. 5. Sexual apparatus of *Pythium polymastum* formed in maize-meal-agar plate cultures, and, with aid of a camera lucida, drawn to a uniform magnification; ×1000 throughout. A, B. Units at a very early stage of development. C–F. Mature units.

Scale—μ

30

20

10

0

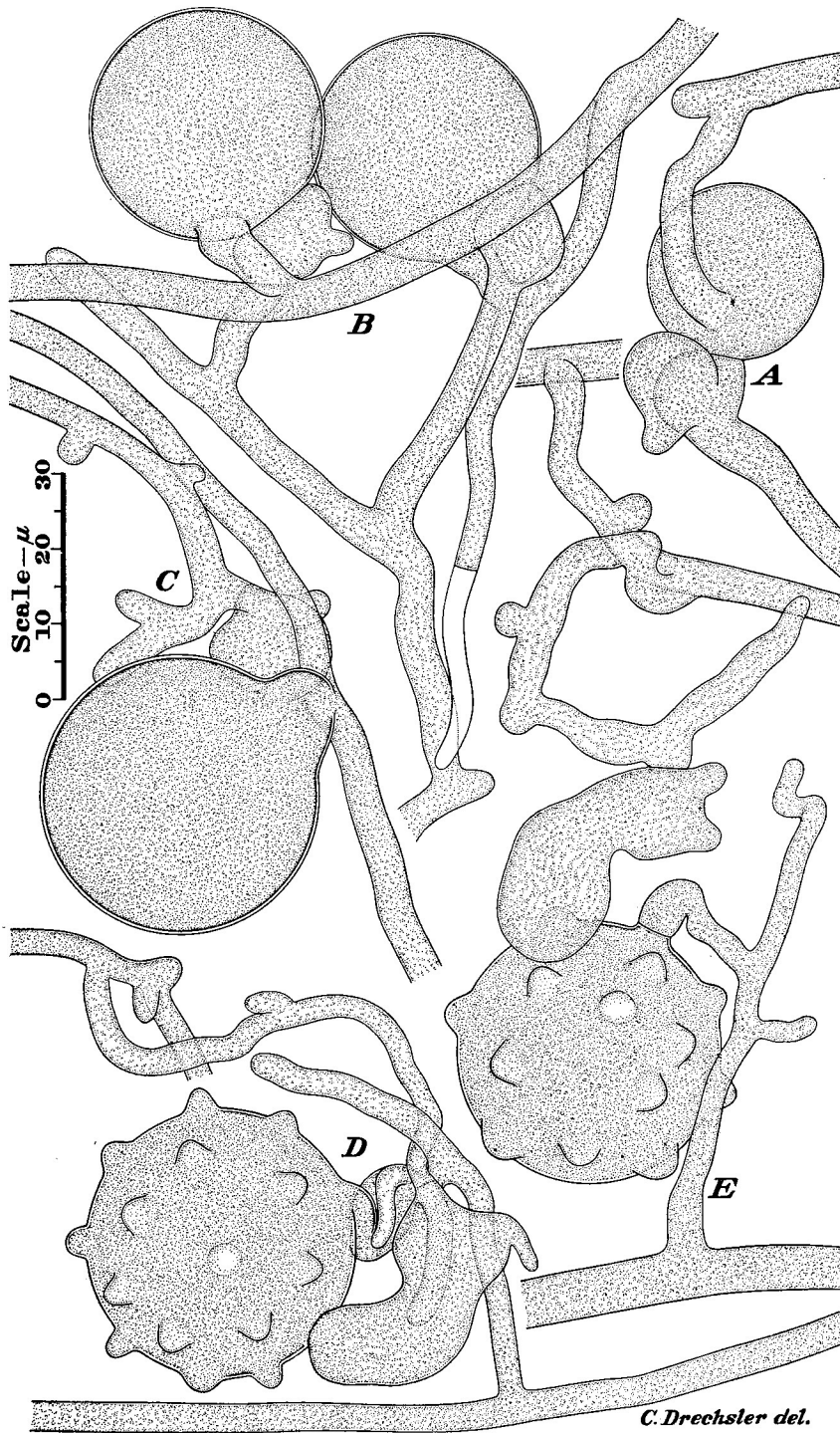*B*

A

C

D

E

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FIG. 6.   A–E. Immature sexual apparatus in various stages of development, found in maize-meal-agar cultures, and, with the aid of a camera lucida, drawn to a uniform magnification; × 1000 throughout.

initiated. The oogonia first become visible as somewhat opaquely darkish globose bodies that are either borne terminally on relatively short branches (Fig. 5, A, B; 6, A, B) or are sessile and lateral on longer mycelial filaments (Fig. 6, C). From a very early stage the female structure is found in contact with a young antheridium, which consists often of a bulbous enlargement borne terminally or laterally on a hyphal element not closely connected with the oogonial filament (Fig. 5, A). Occasionally, 2 antheridia are present (Fig. 5, E). The sex organs remain continuous with the mycelium as they increase in size through migration of protoplasmic material into them. During the final stages of expansion the oogonium puts forth a variable number of rather wide proturbances (Fig. 4, R, S; 6, D), and then is delimited by the deposition of a basal septum (Fig. 6, E). At about the same time (Fig. 6, E), or frequently even a little earlier (Fig. 4, R, S), the antheridium, whose growth has in many instances been accompanied by a greater or lesser development of lobes, protuberances, or diverticulations, also is delimited by the laying down of a basal cross-wall. Soon the processes through which the single oospore is brought into being are inaugurated by noticeable contraction of the female protoplast away from its sculptured enveloping wall, intrusion of a stout fertilization tube into the oogonial chamber, and transfer of the antheridial contents.

The withdrawal of protoplasmic material from the oogonial envelope brings into clearer view marked variations among the protuberances with respect to shape, thickness of wall, and width of lumen. In some instances these excrescences are represented by narrow, cylindrical, thin-walled digitations 10 to 30 μ in length, which, for the most part, are bluntly rounded at the tip, yet which, again, may become expanded terminally and bear smaller secondary protuberances along the expanded portion; in other equally numerous instances, they are represented by small cusps with greatly reduced lumina. As, however, the more bizarre variations in design of the protuberances seem to be associated almost always with serious oogonial deterioration, their significance must be discounted generously in any sound characterization of the species. In cultures wherein degeneration is reduced in such measure that most units of sexual apparatus yield good oospores the oogonial protuberances appear more usually to be of conical or mastoid shape; the lumen, in most instances, being fairly wide in the broad, thick-walled basal part, but becoming conspicuously narrowed in the apical prolongation (Fig. 5, C–F). The number of protuberances present on oogonia with good oospores varies greatly: less than a dozen being visible in the upper and equatorial aspects of some comparatively smooth envelopes (Fig. 5, F), though, usually, scores are crowded together in the corresponding portions of the more elaborately sculptured envelopes (Fig. 5, C–E).

Despite their relatively large dimensions, the oospores of *Pythium polymastum* show the internal organization familiar among species of *Pythium* other than *P. helicoides* Drechsl. and its allies. At early maturity the parietal protoplasmic layer surrounding the homogeneous central reserve globule

reveals a rather finely granular texture (Fig. 5, C–F).   Examination after 6 or 8 weeks of storage at a temperature of approximately 5° C. shows usually a perceptible increase in size of the reserve globule, with an equal decrease in thickness of the parietal layer.   The parietal layer, moreover, now shows coarser texture, its constituent granules having attained a diameter of about 0.5 µ; so that, except mainly for a less regular arrangement of the granules, the resulting appearance is reminiscent of the oospores of many saprolegniaceous forms, including all terricolous species of *Aphanomyces*. As in various congeneric forms, many oospores, after a prolonged period of dormancy, show imbedded in the parietal layer from 2 to 4 refringent bodies of somewhat smaller dimensions than the single large subspherical or oblate ellipsoidal refringent body found at early maturity (Fig. 5, C–F).

The fungus appears somewhat exacting in its nutritional requirements for proper sexual reproduction.   Some culture media, including maize-meal-decoction agar, that allow abundant development of good oospores by the several *Pythium* species commonly associated with damping-off, will permit development of good oospores by *P. polymastum* in perhaps only about 5 or 10 oogonia out of every hundred such structures produced.   Addition to an agar medium of a considerable amount of very finely ground maize meal usually brings about marked reduction in oogonial degeneration, with the result that as many as half of the units of sexual apparatus produced may then form oospores with correctly organized contents.   Partial degeneration of oogonia, with endogenous development of smaller secondary oogonia that function successfully in forming good, though usually rather small, oospores, occurs in cultures of *P. polymastum* much as in cultures of *P. mastophorum*.

The information on oogonial dimensions submitted in the diagnosis of the species was based on measurements of 200 oogonia produced in a maize-meal-agar culture that had more than ordinary merit with respect to normality of sexual reproduction.   The specimens measured were free even of partial degeneration, all having produced correctly organized oospores without intervention of any secondary oogonium.   Measurements of the 200 oospores in question provided the metric data underlying the statements pertaining to dimensions of the oospore.   In order to remedy in some part the irregular distribution of values that was evident when both sets of measurements were divided into classes having a range of only 1 µ, an equal number of additional measurements have been made with similar precautions against unnecessary incorporation of effects due to degeneration.   The combined 400 measurements show a distribution of values for diameter of oogonium (exclusive of protuberances), expressed to the nearest micron, as follows: 26 µ, 2; 27 µ, 1; 28 µ, 4; 29 µ, 1; 30 µ, 1; 31 µ, 3; 32 µ, 4; 33 µ, 8; 34 µ, 4; 35 µ, 8; 36 µ, 12; 37 µ, 10; 38 µ, 10; 39 µ, 18; 40 µ, 16; 41 µ, 16; 42 µ, 22; 43 µ, 13; 44 µ, 37; 45 µ, 27; 46 µ, 29; 47 µ, 27; 48 µ, 29; 49 µ, 33; 50 µ, 20; 51 µ, 12; 52 µ, 13; 53 µ, 8; 54 µ, 4; 55 µ, 5; 56 µ, 1; 58 µ, 1; 59 µ, 1.   The 400 oospores contained within the oogonia measured showed a distribution of values for diameter, expressed to the nearest micron, as follows: 21 µ, 1; 22 µ, 4; 23 µ,

2; 24 µ, 3; 25 µ, 8; 26 µ, 7; 27 µ, 9; 28 µ, 10; 29 µ, 6; 30 µ, 15; 31 µ, 15; 32 µ,
26; 33 µ, 20; 34 µ, 25; 35 µ, 34; 36 µ, 41; 37 µ, 48; 38 µ, 40; 39 µ, 30; 40 µ, 20;
41 µ, 15; 42 µ, 11; 43 µ, 5; 44 µ, 3; 45 µ, 1; 46 µ, 1. From the 2 sets of
measurements averages of 44 µ and 35 µ have been computed for diameter
of oogonium and diameter of oospore, respectively.

Oospores of correct internal structure, taken from maize-meal-agar cul-
tures that with some protection against excessive desiccation had been stored
for 2 or even 3 years, were repeatedly found capable of germination. To be
sure, during my earlier experience with the species, difficulty was at times
encountered when oospores of unimpeachable organization, taken from cul-
tures only a few months old, failed to germinate, or to show any other sign
of continued life. The secret of such puzzling failure was eventually found
to lie in the thermal requirements of *P. polymastum;* its oospores being unable
to germinate and its vegetative mycelium being killed outright at the high
summer temperatures often prevailing for somewhat extended periods in
Washington, D. C. Recourse to artificial cooling during the summer months
showed that in oospores of this as of related forms correct internal structure
always betokens capability of germination.

PYTHIUM MEGALACANTHUM DE BARY SENSU BUISMAN

A fungus more impressive in its dimensions than *Pythium polymastum*
was made known by Buisman (3) in 1927 in a paper devoted to root rots
affecting various economic plants in The Netherlands. Its spiny subspherical
oogonia had been first observed by her during the summer of the previous
year, on examining roots of some flax (*Linum usitatissimum* L.) plants from
Groningen affected with typical "vlasbrand," and again later in the same
season, on examining flax roots from Friesland. The phycomycete was
readily isolated and grown in pure culture. When mycelium from a pure
culture was applied to healthy roots of flax seedlings grown in nutrient solu-
tion, softening of the main root ensued after several days. Subsequently, as
the infection advanced into the lateral roots, these became glassy and limp.
Roots of plants artificially inoculated showed in their tissues very numerous
large spiny oogonia, which bodies reminded Buisman of the illustrations of
the oogonia of *P. megalacanthum* given by de Bary. The phycomycete from
flax roots seemed to her to correspond very well with the description sub-
mitted by de Bary for that species. Although she recognized that the oogonia
of her fungus, ranging in diameter from approximately 30 to 70 µ, often
exceeded the measurements (36 to 45 µ) given by de Bary, she held that,
owing to wide variability in the structures concerned, the differences noted
were of no importance. Her failure to obtain zoosporangia she held might
come from refractoriness of the particular strain represented in her single
culture. In fine, she identified the fungus in flax roots as *P. megalacanthum.*

In 1928 van der Meer (15) published the results of successful inoculation
experiments, which not only fully corroborated Buisman's discoveries con-
cerning the causal relationship of the large spiny fungus to "vlasbrand,"

but which, in addition, indicated this fungus to be the sole primary parasite responsible for the disease. Because of failure to obtain typical symptoms of the disease in plants artificially inoculated with *Thielavia basicola* (B. & Br.) Zopf or with *Asterocystis radicis* de Wild., she denied the primary pathogenicity of these 2 organisms, and held them capable only of slightly accentuating the destructive action of *Pythium megalacanthum*. Extensive investigations reported by Diddens (4) 3 years later likewise revealed the spiny oomycete as the sole cause of true "vlasbrand," though conceding some feeble parasitism to *T. basicola* in the causation of a slight root rot. Active parasitism, incidentally, was attributed by Diddens also to *P. debaryanum* Hesse and *P. irregulare* Buis., these fungi, like *P. megalacanthum*, having been found by her capable of causing serious root rot in water cultures, even if in pot cultures they caused only a kind of damping-off quite different from the typical "vlasbrand" there brought on by the spiny form alone.

Although van der Meer's conclusion as to *Pythium megalacanthum* being solely responsible for flax scorch would thus seem to have been amply corroborated by Diddens, it was nevertheless very soon disputed by Marchal (12, 13, 14), who found that in Belgium in 1930 flax plants with typical "brûlure" contained none of the spiny oogonia distinctive of *P. megalacanthum*, but consistently revealed in their affected roots numerous resting spores characteristic of *Asterocystis radicis*. Accordingly, the Belgian investigator, who had set forth the latter organism as the cause of "brûlure" in 1901, still continued 3 decades later to regard it as responsible for the disease in Flanders. He was inclined to believe, furthermore, that flax scorch is a pathological condition, which, like "damping off," might well be brought about by different root parasites in different regions,—in The Netherlands, preponderantly by *P. megalacanthum;* in Flanders, mainly by *A. radicis;* elsewhere, perhaps by species of *Thielavia* and *Hypochnus*. A similar view of the disease as a complex one, seems, indeed, to have been entertained previously by Buisman and apparently underlay also Schilling's account (20) of "Flachsbrand," which appeared in 1928. Recently, in a Belgian publication, Vanderwalle and van den Bruel (24) have characterized *P. megalacanthum* as only a saprophyte occurring on dead plants, without giving any grounds why they considered erroneous the body of evidence submitted by the several Dutch investigators in favor of its causal connection with flax scorch.

Regardless of its disputed pathogenic relationship, the flax-root fungus, a culture of which was received from the Centraalbureau voor Schimmelcultures, is one of the most striking of oomycetes. Growing on maize-meal agar its mycelium, whether examined with the naked eye or under a microscope, shows a general resemblance to the mycelium of *Pythium mastophorum* and of *P. polymastum*. The hyphae, mostly 3 to 7 μ in width, pursue somewhat random courses through the substratum, giving off branches rather sparingly and at rather wide angles. Aerial development, for the most part, is meager, resulting usually in a scant arachnoid covering, or in scattered fleecy wisps. Appressoria of the sickle-shape type (Fig. 7, A) are produced here and there
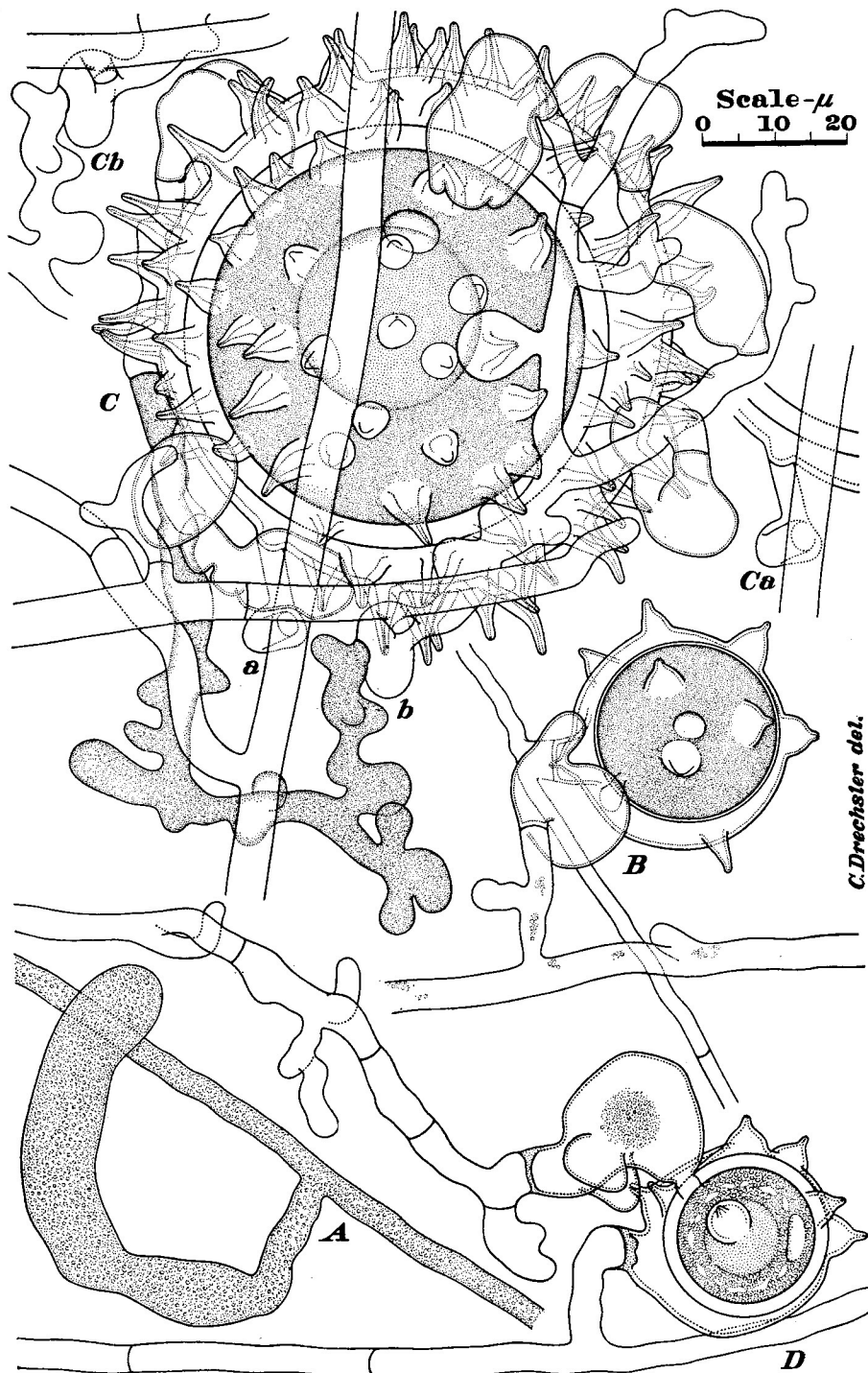
FIG. 7. The flax-root fungus, drawn from maize-meal-agar cultures; × 1000. A. Appressorium. B. Small immature sexual unit. C. Large mature sexual unit; attachment of oogonial stalk, a, and of antheridial hypha, b, being shown also separately. D. Small mature sexual unit, about 60 days old.

in contact with the hard surface of the container, where they are borne terminally on short lateral branches given off by the main hyphae. As their functional frustration sometimes leads to repetitional development, connected systems of curved elements are to be seen, though in lesser numbers than in some more familiar related forms.

After a mycelium of the fungus has been growing for 3 or 4 days it begins to produce sexual reproductive structures. The oogonia make their appearance as smooth globose bodies, for the most part borne terminally on straight or slightly curved lateral stalks usually between 5 and 20 μ long (Fig. 8, A, C, D), though some supporting stalks of more pronounced curvature may attain lengths of approximately 30 or 40 μ (Fig. 8, B; 9, A, a). The beginnings of a male complement, arising commonly from a hypha without close mycelial connection with the oogonial thread, soon become applied to the oogonium or to the hyphal elements supporting it. Sometimes the young female organ becomes inwrapped by a branching filament destined to bear one or several antheridia (Fig. 8, A). However, on the whole, contact of the growing oogonium itself with the young male structures would seem less extensive than the contact of the male parts with the oogonial stalk (Fig. 8, B, C) and an adjacent portion of parent filament (Fig. 8, D). Lobulate excrescences and diverticulations are often present on the antheridia or on the antheridial hyphae to promote intrication of male and female elements.

On attaining approximately its definitive size, the oogonium puts forth protuberances that, like the homologous modifications in *Pythium mastophorum* and *P. polymastum*, are often hemispherical or conical (Fig. 7, C; 9, A, a; B, a; 10, A, a; B, a) or somewhat mammiform (Fig. 7, B, D), not to mention the more extravagant variations in shape frequently associated with eventual degeneration of the oogonial contents. In units of sexual apparatus having normal development the protuberances measure from 1.5 to 12 μ (average about 7 μ) in length, and from 2 to 8 μ (average about 4.7 μ) in basal width; the walls surrounding them measuring 0.6 to 1.8 μ in thickness at the base to become often rather markedly thinner toward the apex. The number of protuberances visible in the upper and equatorial aspects of an oogonium varies from 5 in small specimens to about 110 in large specimens, the average lying perhaps between 55 and 60. A fairly extensive portion of the oogonial wall adjacent to the supporting stalk and representing, in most instances, from one-twelfth to one-sixth of the subspherical envelope, remains free of protuberances.

The sculptured oogonium is delimited by deposition of a thick septum usually at a distance of several microns from the large spherical part, thereby including in the female organ, as it were, an adjacent portion of its stalk. Sometimes, indeed, the delimiting cross-wall may be placed at the very origin of the stalk, so that the oogonium comes to be sessile on the parent filament (Fig. 10, B, a).

Fertilization is accomplished sometimes by a single antheridium (Fig. 7, B, D; 9, A, a; B, a; 10, B, a), but very frequently 2 (Fig. 10, A, a) or 3
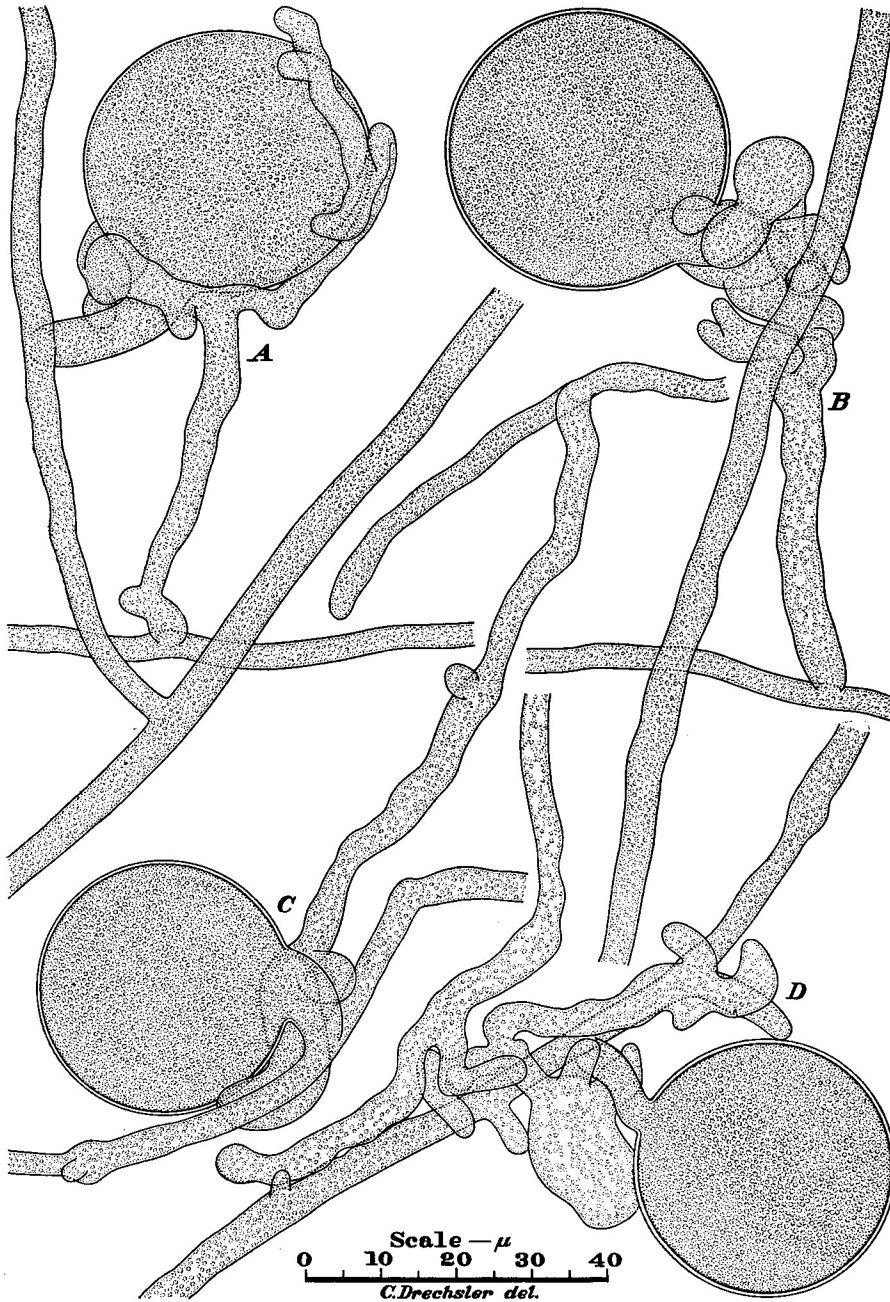
Scale — μ
0   10   20   30   40

C.Drechsler del.

FIG. 8. Young units of sexual apparatus of the flax-root fungus, *Pythium megala-canthum* de Bary *sensu* Buisman, formed in a maize-meal-agar culture, and, with the aid of a camera lucida, drawn to a uniform magnification; ×1000 throughout. A. Unit in which the growing oogonium is being more or less enveloped by a branching antheridial filament, before any male organ becomes distinguishable. B, C, D. Units in each of which a young antheridium became recognizable as a terminal enlargement of an antheridial branch; each unit showing moderate intrication of male and female parts.
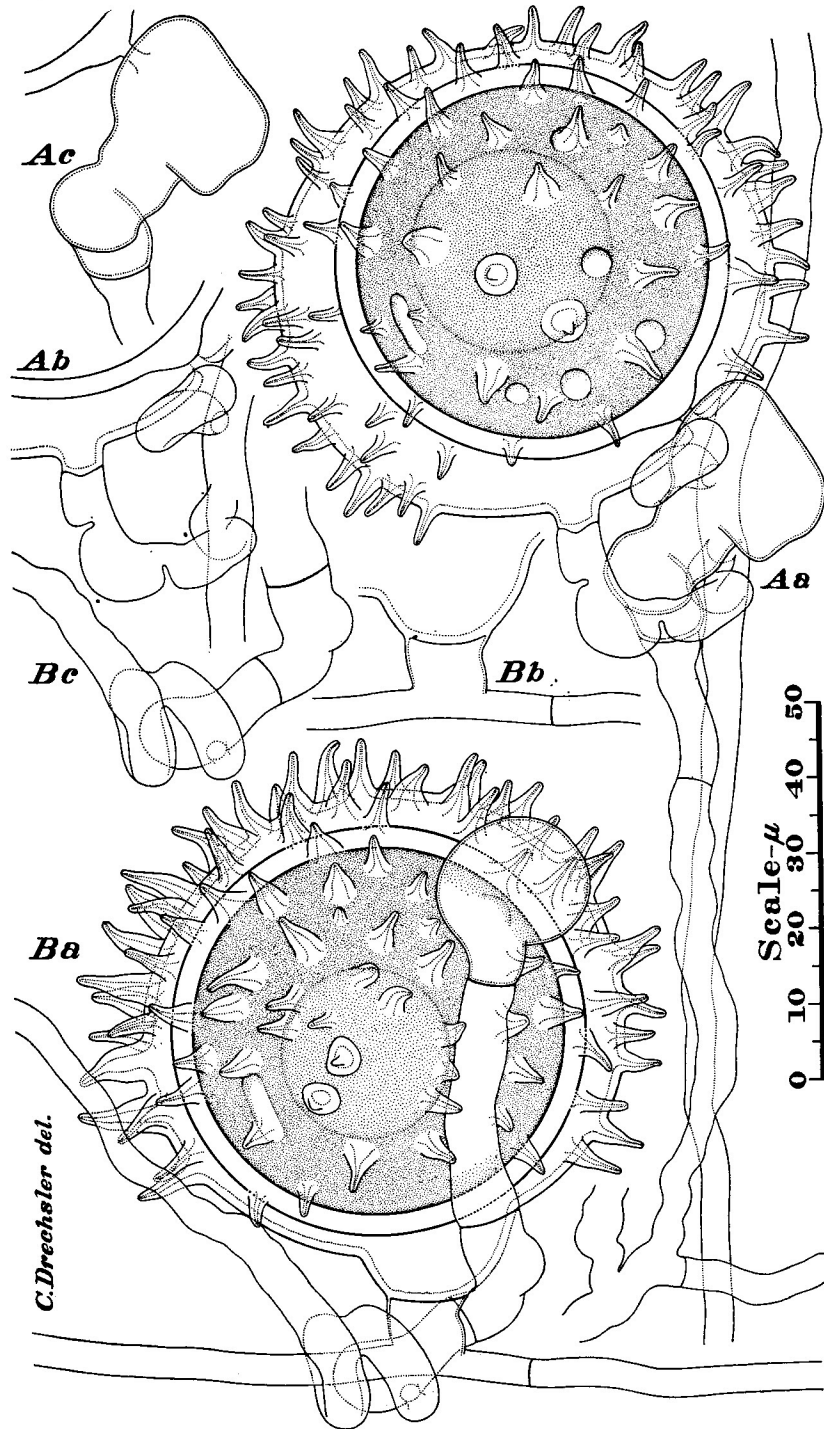
FIG. 9. A, a, B, a. Two mature sexual units of the flax-root fungus, formed in maize-meal agar, and drawn with aid of camera lucida; × 1000. A, b, B, b. Their respective oogonial attachments, drawn separately. A, c, B, c. Their somewhat intricate antheridial parts drawn separately.
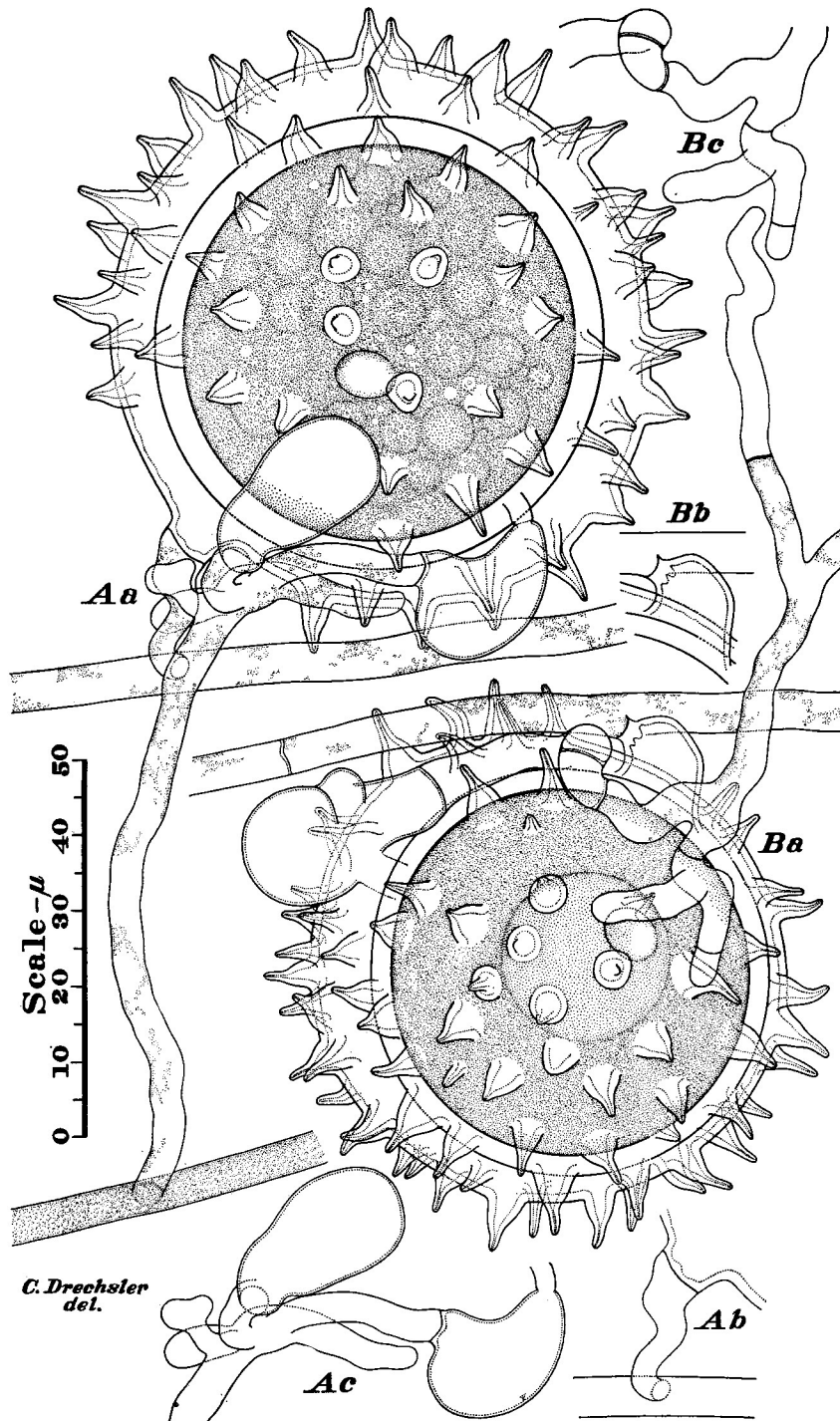
FIG. 10. A, a, B, a. Two units of sexual apparatus of the flax-root fungus formed in maize-meal agar, and drawn with aid of camera lucida; × 1000. A, b, B, b. Their respective oogonial attachments. A, c, B, c. Somewhat intricated portions of their respective male components, drawn separately.

antheridia may participate in the process, and as many as 7 have been found present (Fig. 7, C). Plural male organs appear in most cases to be supplied from a single mycelial filament. In shape the antheridia show considerable variety, swollen clavate and broadly saccate types predominating. They measure usually 18 to 40 μ (average approximately 27 μ) in length and 9 to 18 μ (average approximately 14 μ) in width. Transfer of antheridial contents into the oogonium is accomplished by means of a fertilization tube measuring 2 to 5.5 μ in length and 2 to 5 μ in diameter.

Development subsequent to fecundation is marked by the same sequence of changes as in most congeneric forms. After rounding up into a spherical shape (Fig. 7, B) the fertilized protoplast surrounds itself with a smooth wall (Fig. 10, A, a) 1 to 4.5 μ, mostly 2 to 4 μ (average 3 μ) in thickness. At early maturity the oospore, colorless or slightly yellowish, reveals a single reserve globule, 10 to 30 μ (average 23 μ) in diameter, which is surrounded by a parietal granular layer in which is imbedded a single refringent body, subspherical or strongly flattened (Fig. 7, C, D; 9, B, a; 10, B, a). Oospores in cultures several weeks or several months old show additional maturation by a perceptibly larger reserve globule, by a more coarsely granular structure of the parietal layer, and often by the presence of plural refringent bodies (Fig. 9, A, a).

Buisman noted that oogonia produced in cultures of the flax-root fungus were often empty, owing to degeneration of their protoplasmic contents. And, indeed, when the fungus is grown on potato agar or on agar media prepared with filtered maize-meal decoction, oogonial degeneration often takes place in such measure that only a few of the sexual units give rise to good oospores. Addition of a considerable quantity of very finely ground maize meal to an agar medium often brings about marked improvement, with the result that fully half of the sexual apparatus may at maturity contain oospores of correct internal structure. Partial degeneration, accompanied by endogenous development of smaller, secondary oogonia that then bear correspondingly smaller oospores, occurs also in cultures of this species. However, only oogonia of primary origin, each containing an oospore of irreproachable internal organization without any intervention of secondary development, were selected in making measurements intended for comparison with measurements of allied forms. Four hundred specimens, otherwise taken at random in maize-meal-agar cultures of more than ordinary merit with respect to normality of sexual development, gave a distribution of values for oogonial diameter, expressed to the nearest micron, as follows: 25 μ, 3; 27 μ, 1; 29 μ, 1; 30 μ, 1; 31 μ, 3; 32 μ, 4; 33 μ, 1; 34 μ, 3; 35 μ, 3; 36 μ, 3; 37 μ, 5; 38 μ, 2; 39 μ, 1; 40 μ, 4; 41 μ, 1; 42 μ, 1; 43 μ, 3; 44 μ, 4; 45 μ, 1; 46 μ, 1; 47 μ, 3; 49 μ, 1; 50 μ, 2; 51 μ, 4; 52 μ, 6; 53 μ, 6; 54 μ, 5; 55 μ, 10; 56 μ, 10; 57 μ, 14; 58 μ, 13; 59 μ, 33; 60 μ, 31; 61 μ, 28; 62 μ, 27; 63 μ, 24; 64 μ, 32; 65 μ, 25; 66 μ, 13; 67 μ, 14; 68 μ, 10; 69 μ, 10; 70 μ, 6; 71 μ, 5; 72 μ, 1; 73 μ, 5; 74 μ, 2; 75 μ, 5; 76 μ, 1; 77 μ, 3; 78 μ, 1; 79 μ, 2; 81 μ, 1; 82 μ, 1. The 400 oospores contained within these oogonia

gave values for diameter, expressed to the nearest micron, with distribution as follows: 20 μ, 2; 21 μ, 2; 22 μ, 1; 23 μ, 3; 24 μ, 2; 25 μ, 1; 26 μ, 5; 27 μ, 4; 28 μ, 3; 29 μ, 5; 30 μ, 1; 31 μ, 4; 32 μ, 3; 33 μ, 9; 34 μ, 2; 35 μ, 2; 36 μ, 1; 37 μ, 5; 38 μ, 2; 39 μ, 3; 40 μ, 2; 41 μ, 8; 42 μ, 8; 43 μ, 8; 44 μ, 10; 45 μ, 9; 46 μ, 19; 47 μ, 20; 48 μ, 35; 49 μ, 32; 50 μ, 20; 51 μ, 35; 52 μ, 25; 53 μ, 22; 54 μ, 23; 55 μ, 12; 56 μ, 11; 57 μ, 10; 58 μ, 6; 59 μ, 9; 60 μ, 6; 61 μ, 3; 62 μ, 2; 63 μ, 1; 64 μ, 1; 65 μ, 2; 66 μ, 1. From the 2 sets of values were computed averages of 59.1 μ and 47.5 μ for diameter of oogonium and diameter of oospore, respectively.

Such attempts as I have made to induce the development of zoosporangia through careful irrigation of slabs removed from vigorous agar plate cultures of the flax-root fungus, failed like the earlier efforts of Buisman and Diddens directed to a similar end. As Diddens well pointed out, absence of knowledge concerning a zoosporangial phase in the life history of the fungus renders inadvisable any change in assignment from that originally made by Buisman; though, at the same time, Buisman's identification is left with much less factual support than might be desired. It seems worthy of emphasis that in de Bary's account of his *Pythium megalacanthum* is described an asexual reproductive phase that, besides displaying specific characters of ordinary distinctiveness in the shape, position, and proliferous development of the sporangia, reveals a character of extraordinary distinctiveness in the unusually large size of the encysted zoospores. Until swimming spores of comparable size, and sporangia of proliferous tendency, have been demonstrated in the fungus under consideration, its identification with de Bary's species must be regarded as very uncertain. The reflection may be permitted that should the dimensional proportionality between oospores, on the one hand, and zoospores on the other, that is evident in *P. mastophorum* and *P. polymastum,* extend to the flax-root fungus, the zoospores of that fungus, if such bodies be indeed existent, would fulfill approximately the requirements of size indicated in de Bary's account. The fact that instances of sporangial proliferation have not yet been observed in either *P. mastophorum* or *P. polymastum* argues somewhat against a likelihood of such development in an intimately related form; yet in view more especially of the frequently terminal position of the sporangium in the 2 species named, proliferation in these species and in others closely related to them would at least not seem wholly precluded on morphological grounds.

Comparison of the sexual stage produced by the flax-root fungus in maize-meal agar cultures, with the sexual stage described for *Pythium megalacanthum* by de Bary, discloses neither such convincing correspondence in structural and developmental details as would confirm a supposition of identity, nor such out-and-out disagreement as would definitely imply separateness. In *P. magalacanthum,* according to de Bary, the mycelial connection between an oogonium and the antheridium fertilizing it, is never a close one; and certainly in the flax-root fungus as well as in *P. mastophorum* and *P. polymastum,* the male organ likewise is borne on a branch only distantly con-

nected with the oogonial filament. A marked difference is apparent, how-
ever, in respect to the developmental stage of the oogonia at which the an-
theridia are applied to them. Referring to the oogonia of *P. megalacanthum*,
de Bary (1) wrote: "Haben dieselben ihre volle Grösse erreicht, so treten an
die meisten auch Antheridien heran." In the flax-root fungus, by contrast,
and, as has been noted, also in *P. mastophorum* and *P. polymastum*, the
antheridia develop more or less simultaneously with the oogonia after con-
tact of the opposite sexual elements or of the supporting hyphae has been
established at an early stage. Concerning the characteristic intrication of
parts, frequently, even if not constantly, evident in sexual apparatus of the
3 mamelonated fungi herein discussed, de Bary made no statement.

The oogonial protuberances of *Pythium megalacanthum* were set forth
in the original accounts of that species as being of generally conical shape,
from 6 to 9 µ in length, mostly broad at the base, straight or slightly curved,
and fairly acute or more bluntly rounded at the apex. Approximate agree-
ment with regard to these details is readily apparent in numerous oogonia of
the flax-root fungus. If, as Diddens correctly pointed out, the spines here
often attain lengths exceeding 9 µ (Fig. 7, C), it is equally true that fre-
quently they fail to attain a length of 6 µ (Fig. 9, B). In de Bary's accounts,
however, no allusion is made to dome-shaped and to mammiform variations
of the oogonial protuberances, or to any conspicuous thickness of wall espe-
cially in the basal portion of the individual spine, or to any marked attenua-
tion of the lumen in the frequently prolonged apical portion,—particulars in
which are conveniently displayed the insignia of interrelationship distin-
guishing the small group that includes *P. mastophorum*, *P. polymastum* and
the flax-root fungus as its known members. Of course, de Bary's failure to
record such further details cannot be considered conclusive evidence that
they were alien to his fungus, for often they are not expressed well in mate-
rial kept more or less submerged in water.

Moreover, since species of *Pythium* are somewhat inclined to produce
smaller oogonia and smaller oospores under aquatic conditions than in fairly
dry, solid substrata, one should be prepared to allow for substantial differ-
ences between the measurements of these bodies submitted by earlier authors,
and homologous masurements taken from firm agar cultures. Nevertheless
the range in values for oogonial diameter in *P. megalacanthum* given by de
Bary, 36 to 45 µ, and his approximate value for diameter of oospore, 27 µ,
indicate main dimensions so much smaller than those of the flax-root fungus
that the differences could be dismissed as of no importance only if satisfactory
agreement prevailed with respect to most other features. Assuredly in size
of oogonium and of oospore both *P. mastophorum* and *P. polymastum* con-
form much more closely to de Bary's description of *P. megalacanthum* than
does the flax-root fungus. It seems worthy of mention, too, that the single
figure of de Bary's showing an oognium of *P. megalacanthum* with a mature
oospore (2, Plate 5, Fig. 12), reveals a reserve globule larger in proportion to
the oospore, and a parietal layer correspondingly narrower in proportion to

the oospore, than is usual in the flax-root fungus, or, for that matter, in *P. mastophorum* and *P. polymastum*. Finally, de Bary neither mentions in his text nor illustrates in his figures any marked inequality in the distribution of spines over the oogonia of *P. megalacanthum;* whereas, in the flax-root fungus, protuberances are generally lacking in a fairly extensive region of the oogonium, adjacent to or surrounding the oogonial stalk.

A few years ago I (8) briefly recorded my having observed large oogonia with somewhat mastoid, thick-walled protuberances in the tissues of discolored roots of potted cineraria (*Senecio cruentus* DC.) plants from a greenhouse near Alexandria, Virginia. Undoubtedly these oogonia belonged to a species of *Pythium* intimately related to *P. mastophorum*, *P. polymastum*, and the flax-root fungus. A similar taxonomic relationship may with tolerable plausibility be attributed to a fungus that Nicolas and Aggéry (16) observed earlier in France in brown discolored rootlets of yellowish stunted specimens of parsley, *Petroselinum hortense* Hoffm. In the parenchyma and near the vessels of the affected rootlets, the fungus was present for the most part as spherical thick-walled "oeufs" 28 to 36 µ in diameter exclusive of their spirally arranged "épines trapues" that measured 3 to 4.7 µ both in basal width and in length. Associated with these "oeufs" were present, though in lesser quantity, some spherical thin-walled bodies designated as sporocysts. When produced on extramatrical mycelium coming from affected rootlets bathed in water, the sporocysts were found to be terminal or intercalary in position, and to measure 10.4 to 47 µ in diameter. The form and dimensions of the "oeufs" being held to approximate the form and dimensions ascribed by de Bary to the oogonia of *P. megalacanthum,* the parsley fungus was referred to that species despite a "petit difference" recognized in lesser dimensions of the "épines trapues" relative to the longish conical spines set forth by de Bary. While, perhaps, the dimensional difference in question can plausibly be dismissed on the ground that it might derive from the variability normal to fungi, there yet remains unreconciled an apparent difference in shape of the protuberances. For, although de Bary stated that the spines of his fungus were much given to variation in form, nothing in his text or in his illustrations specifically indicates a hemispherical form as having been noted among the variations. In any case hemispherical protuberances similar to those figured by Nicolas and Aggéry actually are found on many oogonia of *P. mastophorum*, *P. polymastum* and the flax-root fungus, not only while the organs concerned are still immature (Fig. 2, D, E; 4, R, S; 6, D, E), but also after they have reached maturity.

Of the several passages found in additional writings where the binomial *Pythium megalacanthum* was mentioned in relation to fungi encountered at first hand, some are wanting in confirmatory observational information, while others convey observational information that neither sustains the thesis that an organism conspecific with de Bary's was concerned, nor, again, in any instance encourages a presumption that some member of the mamelonated series under consideration might have been involved. From an early inci-

dental comment of Schroeter's (21, p. 232) it appears that this writer believed he once found de Bary's organism in stems of *Veronica hederifolia* in Breslau, though, for want of details, it is not evident on what grounds the belief was entertained. Under the name *Pythium megalacanthum,* Sideris (23) much more recently published several microphotographs of sexual apparatus pertaining to a fungus presumably isolated in Hawaii from roots of the pineapple, *Ananas sativus* Schult. From the scale of magnification indicated in the legend accompanying the microphotographs, the oogonia shown would seem to measure 27 to 33 μ in diameter, exclusive of the numerous spines, about 3 to 4 μ long, borne on them. The oospores loosely contained in 2 of the spiny envelopes give measurements for diameter of approximately 24 and 27 μ, and seem to be provided with smooth walls despite a characterization of the oospores of the species in the author's analytical key as "large, terminal, with long spines," and despite, too, the inclusion of the species in a subsection supposedly distinguished by spiny oospores. According to the key the Hawaiian fungus develops epigynous antheridia whose broad saccate shape undergoes little change in consequence of fertilization. As to further details, it would appear that germination of asexual reproductive bodies takes place frequently by emission of vegetative hyphae, rarely by formation of zoospores; and that the hyphae of the fungus are irregular, the "colony faintly developed with small superficial whitish specks."

Under the name *Pythium megalacanthum,* too, was reported (11) from France in 1934 a fungus that, after having been isolated the previous year frem melon plants affected with wilt and collar-canker, had been found capable of bringing forth the original symptoms in inoculation experiments performed by Labrousse. The next year Petri (17), in Italy, recorded the isolation from infected roots of the orange, *Citrus limonum* Risso, of 2 species of *Pythium,* whereof one with spiny oogonia measuring 33 to 35 μ in diameter inclusive of the spines, 4 to 5 μ long, borne on them, was held perhaps referable to *P. megalacanthum.* According to a very recent account by Salmon and Ware (19) specimens of watercress originating from unproductive beds in Sussex, England, and bearing spores with thick spiny walls in the soft parts of the collapsed main stem, were reported by Cook to have yielded cultures of only a single fungus,—a species of *Pythium* that would probably prove to be *P. megalacanthum.* Very recently, also, van Poeteren (18) reported that at Zouderwoude, The Netherlands, active decay in cuttings of various ornamentals, including members of the genera *Chrysanthemum,* *Pelargonium* and *Primula,* was found attributable to *P. megalacanthum;* his report, very regrettably, however, offering no comment as to how closely the causal agent in question might resemble the spiny flax-root fungus prominent in Dutch phytopathological literature.

In considerations touching the taxonomic affiliations of *Pythium megalacanthum,* the possibility can hardly be ignored that the fungus dealt with by de Bary may have been a form alien to the series under discussion. As was intimated earlier (9, p. 420) it may perhaps have been a form more

closely related to *P. anandrum,* which, like de Bary's species, combines spiny oogonia with proliferous sporangia,—a combination not frequent in the genus *Pythium,* and hitherto not demonstrated for any of the 3 species distinguished by thick-walled oogonial ornamentation. The same infrequent union of morphological features is found in an interesting fungus recently described by Shanor (22) as *Phytophthora stellata.* Though the antheridia of this fungus usually have a close mycelial connection with the oogonia fertilized by them, the mere presence of these organs provides a detail wherein the parallelism with de Bary's species is borne out better than in the parthenogenetic *P. anandrum.* Other such details are evident in the conformation of the acutely pointed, broad-based, conical spines figured by Shanor, and in the usual development of a distinct evacuation tube by the sporangia of *P. stellata* preliminary to discharge. On the other hand, it must be admitted that in diameter of oogonium (15.5 to 22.4 μ) and length of oogonial spines (up to 3.6 μ) *P. stellata* shows little—less than *P. anandrum*—of the dimensional impressiveness that led de Bary to characterize his *P. megalacanthum* as "eine sehr stattliche Form."

SUMMARY

The diagnoses of *Pythium mastophorum* and *P. polymastum* published earlier are supplemented by an illustrated discussion of the morphology and development of these fungi. From the general similarity in habit and structural detail revealed by them, the 2 species are considered to be intimately related to each other. In the same intimate relationship is rather obviously embraced also the extraordinarily large oomycete that under the binomial *P. megalacanthum* has been made known by several Dutch investigators as causing flax scorch in The Netherlands. While agreeing with de Bary's original description of *P. megalacanthum* in some particulars, the sexual apparatus of the flax-root fungus disagrees with that description in other and no less important particulars. Not any of the later literature wherein de Bary's binomial is mentioned in connection with a plant disease can be held to contain very convincing evidence that the fungus to which this binomial was originally attached has actually been rediscovered in recent times. The true *P. megalacanthum* may even prove to be alien to the *mastophorum* series herein treated, perhaps having closer affiliations with such spiny proliferous forms as *Pythium anandrum* and *Phytophthora stellata.*

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