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MYCOLOGY

Development of Pythium debaryanum *on wet substratum*

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MYCOLOGY.—*Development of* Pythium debaryanum *on wet substratum*. CHARLES DRECHSLER, United States Department of Agriculture, Plant Industry Station, Beltsville, Md.

In a recent paper (Drechsler, 1952) on swarm-spore development from oospores of *Pythium ultimum* Trow (1901) and *P. debaryanum* Hesse (1874) the two fungi were compared with respect to the morphology of their sexual reproductive apparatus as displayed in Petri plate cultures prepared with moderately firm maizemeal agar. Comparison in such cultures was deemed appropriate since both fungi are widely operative as causal agents of damping-off, root rot, and stem rot in many phanerogamic crop plants and consequently are most often encountered by plant pathologists, by whom pathogenic microorganisms have long been studied on agar media. As the discussion and illustrations previously devoted to *P. debaryanum* covered its morphology only in part, some additional aspects of development disclosed by that species on wet substratum are given attention herein. Besides encouraging zoospore formation free liquid water makes for longer antheridial branches and narrower antheridia, so that sexual reproductive apparatus developed under wet conditions has a characteristic rangy appearance. To investigators of the aquatic microflora, who mostly have continued to study oomycetes in submerged or irrigated material, the rangier reproductive habit may even nowadays be more familiar than the somewhat compact habit usual in unwet substrata. The modifications resulting from aquatic conditions often appear here and there in Petri plate cultures, owing to condensation of water within the covered dishes in quantities sufficient to form shallow pools or wet areas on the agar surface. Sometimes, indeed, when Petri plate cultures are stored during periods of hot weather in a refrigerator equipped with an efficient temperature-control device the agar becomes wetted so extensively that indications of zoospore formation may be found almost everywhere.

The same isolation that was used in preparing the illustrations of *Pythium debaryanum* in my earlier paper (Drechsler, 1952, figs. 7, 8) was used also in illustrating development of the species on irrigated slabs of maizemeal agar (Figs. 1–3). Another isolation, one obtained in May, 1922, from a softened pea (*Pisum sativum* L.) root taken near Madison, Wisconsin, was employed in illustrating development on irrigated slabs of Lima-bean agar (Figs. 4, 5). When they were tried out at different times in the greenhouse the two isolations showed moderate capabilities for the destruction of seedlings. In wound-inoculation experiments with plant products like cucumber (*Cucumis sativus* L.), squash (*Cucurbita pepo* L.) and watermelon (*Citrullus vulgaris* Schrad.) fruits they likewise showed effectiveness as agents of rapid decay in more mature fleshy vegetable tissue.

A mycelium of *Pythium debaryanum* actively growing in a Petri plate of maizemeal agar often displays a branching habit generally similar to that of my *P. anandrum* (Drechsler, 1939, fig. 12), in that its long axial hyphae are rather markedly distinguished from the relatively short, somewhat closely ramified lateral branches borne on them. The same branching habit is frequently recognizable also in *P. mamillatum* Meurs (1928) and *P. spinosum* Sawada (1927), whereas in *P. ultimum* growth is more promiscuous, with the lateral branches more often elongating indeterminately much like the hyphae from which they originated. Later, when mycelium of *P. debaryanum* is being emptied of contents to provide material for the production of sporangia (Fig. 1, A, t) and oogonia, successive stages in progressive evacuation of all hyphal components are marked by deposition, usually at comparatively short intervals, of successive convex retaining walls (Fig. 1, A, a-s). The tubular membrane of the empty filaments often evanesce more rapidly than the cross-walls, with the result that the matted empty hyphae on the surface of agar plate cultures 10 to 15 days old are frequently visible under the microscope only by their innumerable curved septa. Aging Petri plate cultures of *P. mamillatum* and *P. spinosum* similarly may show many crescentic cross-walls within tubular membranes that have largely vanished from sight. In *P. ultimum* the matted aerial mycelium affords only a rather meager display of curved septa.

Hesse, working with water cultures, described the zoosporangia of *Pythium debaryanum* as being formed terminally both on the lateral branches and on the main hyphae; and, indeed, terminal zoosporangia are produced abundantly on mycelium that has grown out from irrigated pieces of natural or artificial substratum. In agar plate cultures, however, zoosporangia of globose or prolate ellipsoidal shape are more often formed intercalarily (Fig. 2, A-E; Fig. 3, A-M; Fig. 4, E, a). Some sporangia develop close together on the same hypha (Fig. 2, F, a, b; Fig. 4, Y, a-c). Here and there remnants of protoplasm are gathered into cylindrical cells (Fig. 2, G, a, b; Fig. 3, N-P) which despite their unmodified shape are capable of serving as zoosporangia. When slabs excised from agar plate cultures 10 to 15 days old are placed in a shallow layer of water under cool conditions—I have used temperatures near 10° and 17° C.—some of the sporangia present will often in less than 1 or 2 hours put forth an evacuation tube (Fig. 2, H, t; Fig. 3, Q, t; Fig. 4, A, t) that soon forms a cap of dehiscense at its tip. This cap suddenly yields, and after the manner usual in the genus becomes inflated into a vesicle (Fig. 2, I, v) as it receives the protoplasmic materials discharged through the evacuation tube. Conversion of the protoplasm into laterally biciliate motile zoospores (Fig. 3, R, v Fig. 4, B, v) ensues in about 20 minutes. Normally the zoospores escape and swim away when the vesicle wall collapses under their collective battering, but where the supply of water is inadequate the whole zoospore progeny (Fig. 2, J, a-r) may encyst without moving from its place of origin.

The evacuation tube in *Pythium debaryanum* may arise from any position on the sporangium (Fig. 2, H-S:t; Fig. 3, Q-X: t; Fig. 4, A-Y: t). Its observed length in my cultures has varied from 2μ (Fig. 4, V, t) to 135μ (Fig. 3, U, t). Instances in which the tube is shorter than 5μ or longer than 50μ are, however, decidedly infrequent. Examples more than 100μ long (Fig. 3, T, t; U, t) were discovered only by prolonged search in material displaying many thousands of empty sporangia in positions favorable for unambiguous observation. Hesse's statement that the evacuation tube is longer than the radius and shorter than the diameter of the sporangium bearing it sets forth a dimensional relation that assuredly is frequent in the species.

Although zoospore development often begins soon after a slab excised from an agar plate culture has been transferred to water, only a small proportion of all the sporangia present will commonly participate in such development at the start. Swarm-spore formation usually continues for several days as more and more of the remaining sporangia, in apparently haphazard sequence, produce evacuation tubes and discharge their contents. After 2 or 3 days considerable numbers of zoospores, some in encysted condition (Fig. 3, Y, a-e; Fig. 4, Z, a-g), others in an actively germinating state (Fig. 3, Y, f), may be found strewn about. Here and there imperfect cleavage of discharged masses of protoplasm results in grouped encysted bodies (Fig. 3, Z, a-d) frequently larger than normal zoospores and often of irregular shape.

The sexual reproductive apparatus of *Pythium debaryanum* formed on wet or submerged substratum, much like that formed on unwet agar, may be of either monoclinous (Fig. 1, B-L; Fig. 5, A-L) or diclinous (Fig. 1, M; Fig. 5, M-O) origin. Often in monoclinous reproductive units a solitary male branch arising at some little distance from the oogonium supplies a single terminal antheridium (Fig. 1, B-K; Fig. 5, A-F), though occasionally it supplies 2 antheridia (Fig. 5, G, a, b). Frequently 2 male branches arise from the oogonial hypha, each supplying an antheridium (Fig. 5, H, a, b). In some instances, however, where 2 antheridia are present, only one is borne on a branch (Fig. 5, I, a), the other being composed of a hyphal segment immediately adjacent to the oogonium (Fig. 5, I, b). Sometimes where 3 antheridia are present 2 of them (Fig. 5, J, a, b; Fig. 5, K, a, b) may be borne on separate branches arising from the oogonial hypha either on the same side (Fig. 5, J) or on opposite sides (Fig. 5, K) of an intercalary oogonium, while the third may consist of a cylindrical hyphal segment (Fig. 5, J, c; K, c) adjacent to the oogonium. Further variety in positional relationships of the male cells is provided now and then in instances where an oogonium is supplied with 4 antheridia, 2 of them (Fig. 5, L, a, b) perchance borne terminally on separate branches, a third consisting of a sessile pouchlike outgrowth (Fig. 5, L, c) arising in immediate proximity to the oogonium, and a fourth composed of a hyphal segment (Fig. 5, L. d) adjacent to the oogonium. Fertilization of an oogonium solely by an antheridium composed of an adjacent hyphal segment is observable only somewhat rarely in *P. debaryanum* (Fig. 1, L),

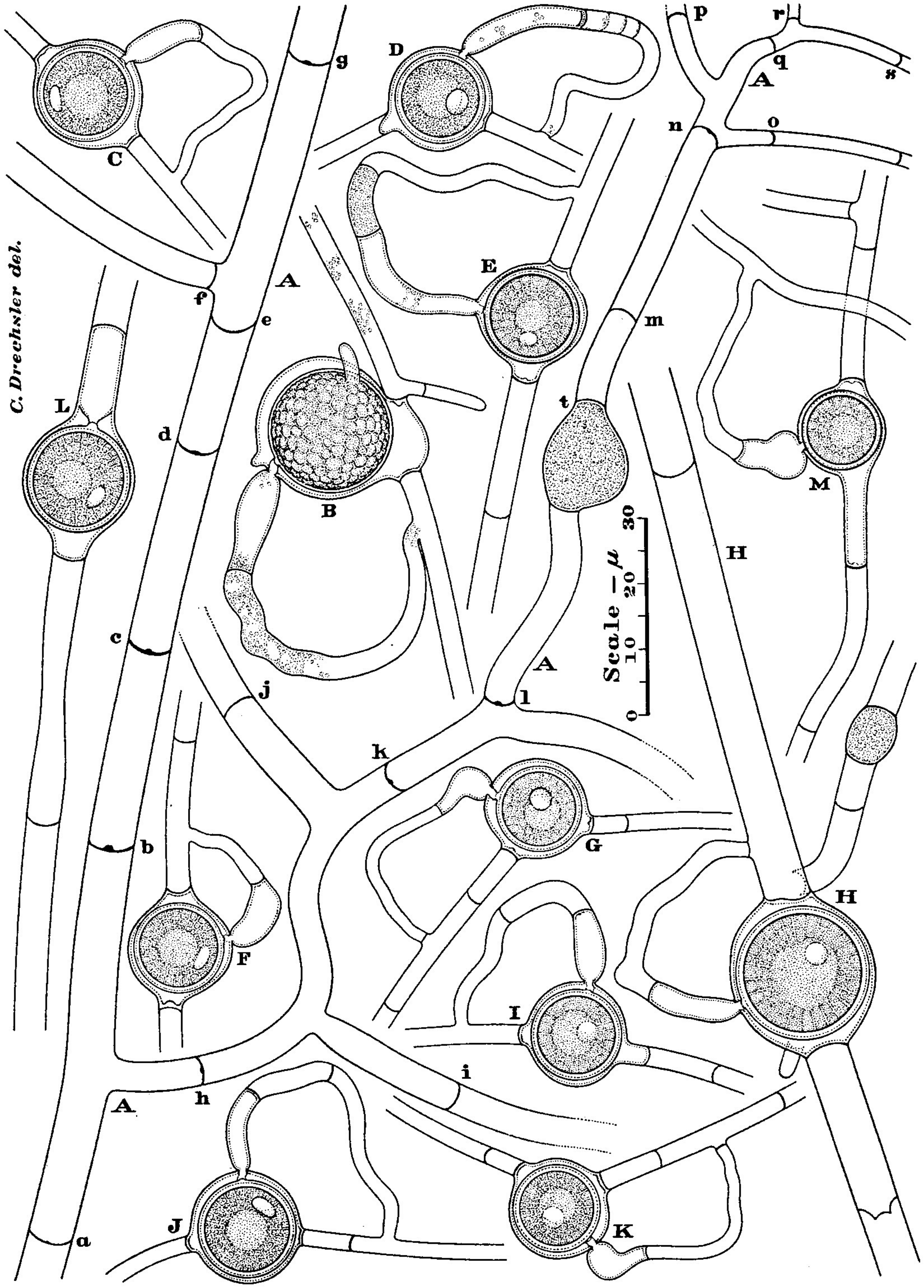

FIG. 1.—*Pythium debaryanum*, on wet maizemeal agar; × 1000. A, Empty mycelium with curved cross-walls, a-s, and a conidium, t. B-K, Units of monoclinous sexual apparatus, each with a single antheridium borne terminally on a branch. L, Monoclinous reproductive unit with antheridium consisting of a hyphal segment adjacent to oogonium. M, Diclinous reproductive unit. (Oospore young in B, nearly but not wholly mature in C, E-M.)

but is exceedingly frequent in *P. ultimum.* Indeed, such cylindrical antheridia as well as pouch-like sessile antheridia borne laterally in immediate proximity to oogonia are generally infrequent in *P. debaryanum,* and in many cultures are found only after some search.

Since for the most part the longer antheridial branches in monoclinous reproductive apparatus of *Pythium debaryanum* formed on wet substratum arise at no greater distance from the oogonium than the male branches in monoclinous apparatus formed in unwet agar cultures they necessarily follow more circuitous courses. In diclinous as in monoclinous reproductive apparatus the antheridia borne terminally on branches are commonly longer and narrower on wet substratum than in unwet agar cultures. A reciprocal relation between diclinous reproductive units comes to light in instances where two neighboring mycelial hypha (Fig. 5, O, a, b) each bear an oogonium (Fig. 5, O, c, d) that is fertilized by an antheridium (Fig. 5, O, e, f) borne on a branch given off by the other.

Hesse (1874, p. 25, line 2) described the oogonial envelope of *Pythium debaryanum* as being smooth and in his several illustrations pertaining to sexual reproduction the oogonium is shown without protuberances. It seems possible that only 4 individual oogonia are represented in these illustrations since 5 of his figures (Hesse, 1874, figs. 15–19) show such resemblances that they might well have been prepared at successively later stages from the same unit of sexual apparatus as his 2 figures (Hesse, 1874, figs. 13, 14) of earlier stages. In the fungus best conforming to Hesse's description smooth oogonia, as a rule, predominate strongly, yet if a considerable number of them are examined closely, scattered examples are usually found that bear one, two, or three protuberances (Fig. 1, B, H; Fig. 5, D, G, K). Mostly these protuberances are of cylindrical shape, and are broadly rounded at the tip like the fingers of a glove. Frequently they are only 2 or 3μ long, yet often, again, they measure 10 to 20μ in length. The shorter protuberances usually remain continuous with the oogonium, though the longer ones often become delimited by a basal septum.

This meager ornamentation might be disregarded but for the circumstance that isolations are often obtained which while closely resembling those with mostly smooth oogonia will rather commonly show digitations in sufficient numbers to be noticed even under cursory examination. The oogonia produced when such isolations are grown on Petri plates of maizemeal agar often vary markedly with respect to degree of ornamentation, some being devoid of protuberances, and others bearing 1 to 7 digitations in their upper and equatorial aspects. Throughout a culture the average number of protuberances exposed to view hardly ever seems much in excess of 3. In some cultures the same isolations appear very nearly smooth, with the oogonia there displaying, on the average, considerably less than one digitation apiece. Nevertheless the tendency toward development of protuberances in readily noticeable quantity apparently persists in these isolations over many years as an inherent character.

It is difficult to hold very strong convictions as to whether the isolations with this character represent merely a variant of *Pythium debaryanum* or a separate species. In the monographs of Matthews (1931) and Middleton (1943) *P. irregulare* Buisman (1927) is treated as a separate species with a circumscription that would evidently admit the noticeably digitate isolations under discussion and would exclude the nearly smooth isolations herein considered referable to *P. debaryanum.* This circumscription may not be strictly in agreement with the morphology of Buisman's fungus. Through the Centraal Bureau voor Schimmelcultures a culture of *P. irregulare* given by Buisman was supplied to me a few years after the species had been described. When the culture was transferred to maizemeal agar and Lima-bean agar the resulting sexual reproductive apparatus (Fig. 6, A-I) displayed few oogonial protuberances (Fig. 6, C, D), the degree of ornamentation observed not exceeding that usual in the relatively smooth isolations assigned to *P. debaryanum.* In the very numerous monoclinous reproductive units the antheridium was commonly borne on a branch arising some distance from the oogonium (Fig. 6, A-F). Where 2 antheridia were present in monoclinous units each was most frequently borne on a separate branch (Fig. 6, G, a, b), though in occasional instances one was borne on a branch (Fig. 6, H, a) while the other developed from a segment (Fig. 6, H, b) immediately adjacent to the oogonium. Buisman stated in her account that she never had seen hypogynal antheridia, yet after some little search in subcultures of her fungus a monoclinous reproductive unit was found in which the oogo-

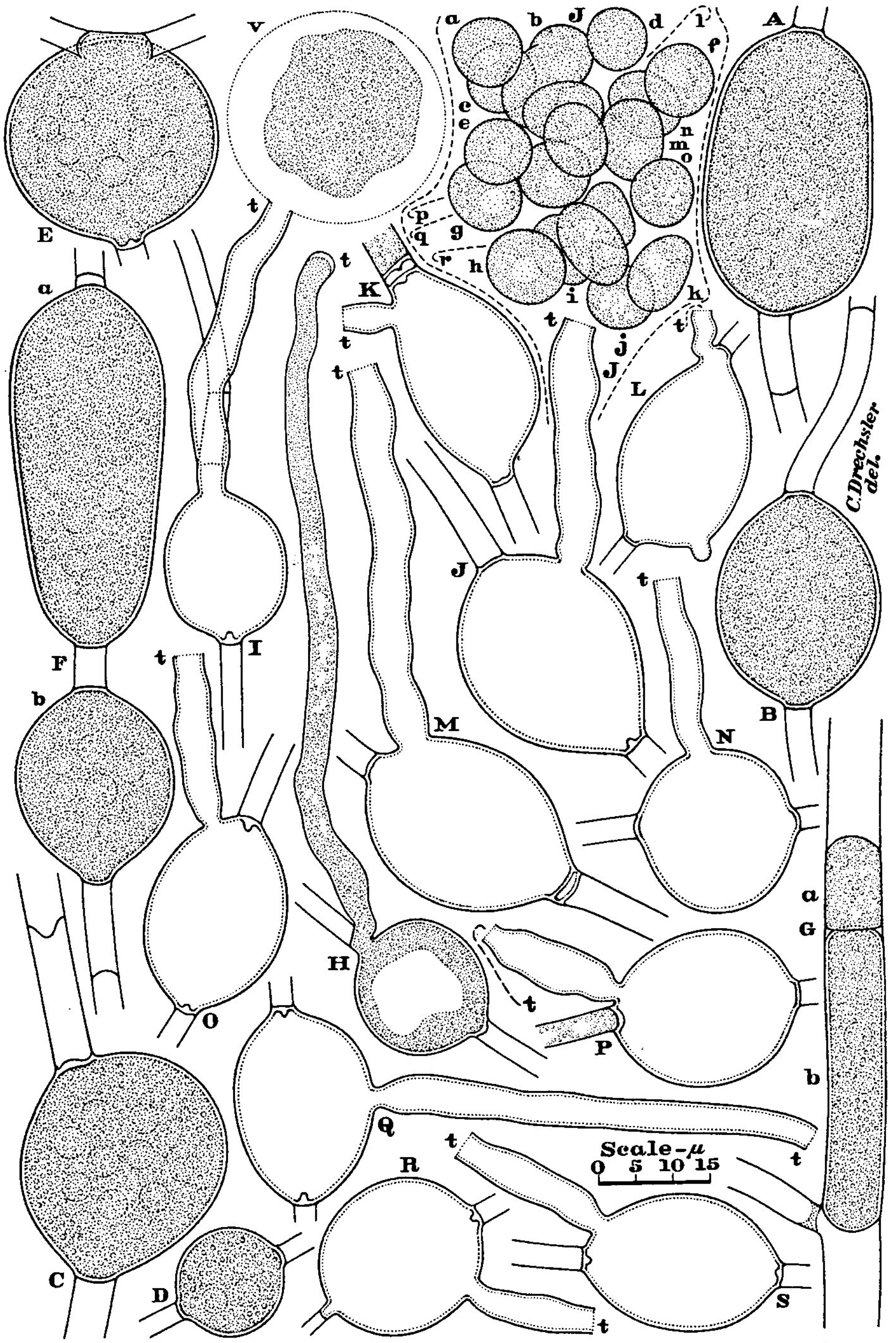

FIG. 2.—Asexual reproductive apparatus of *Pythium debaryanum* as found produced on wet maizemeal agar, drawn with the aid of a camera lucida at a uniform magnification; × 1000 throughout. A-E Intercalary zoosporangia. F, Two zoosporangia, a and b, formed near together. G, Two zoosporangia, a and b, formed in a wide hypha from remnants of protoplasm. H, Zoosporangium with unusually long evacuation tube, t, surmounted by hyaline cap. I, Newly discharged zoosporangium showing evacuation tube, t, with vesicle, v, at its tip. J, Discharged zoosporangium with its progeny of zoospores, a-r, encysted near mouth of its evacuation tube, t. K-S, Empty zoosporangia, each with its evacuation tube, t.

nium had been fertilized by 2 antheridia formed in immediate proximity to it, one consisting of an adjacent hyphal segment (Fig. 6, I, a), while the other, of pouch-like shape (Fig. 6, I, b), was sessile on the oogonial hypha.

Although Buisman distinguished *Pythium irregulare* from *P. debaryanum* mainly by the protuberances found on its oogonia, the 8 oogonia figured by her (Buisman, 1927, figs. 9, 10) show in all scarcely a dozen protrusions, and of these only 5 would seem of the digitate type. In some of her specimens the departures from a globose or ellipsoidal shape appear as if they might be attributable to collapse of portions of the oogonial envelope. Such collapse is considerably more pronounced in *P. debaryanum* than in *P. ultimum* since the oogonial envelope is noticeably thinner in the former species than in the latter, and consequently is more easily deformed either through mechanical disturbance or through loss of water. On the surface of agar cultures, where both *P. debaryanum* and *P. ultimum* often give rise to sexual apparatus most abundantly, the oogonial envelopes in mature reproductive units of *P. debaryanum* commonly show very marked deformation from collapse. As Buisman (1927, p. 10) apparently used no really efficient method for removing bacteria from her cultures it seems probable, besides, that at times the oogonia of her *P. irregulare* may have developed some of the promiscuous malformations often resulting from excessive bacterial contamination.

Buisman gave no descriptive details concerning either the three isolations she dealt with under the binomial *Pythium debaryanum* or the one isolation she identified as *P. debaryanum* var. *pelargonii* Braun. A statement given in her discussion of *P. irregulare* and reading "The antheridia are usually of the same type as those of *P. debaryanum*, but I never saw hypogynal antheridia" implies in its use of the adversative "but" that she recognized hypogynal antheridia as occurring in *P. debaryanum*, and thereby suggests that perhaps she applied Hesse's binomial to the species herein treated as *P. ultimum*. Such application, approximately, was urged some years later by Van Luijk (1934) in his proposal to adopt for *P. debaryanum* the circumscription set forth in De Bary's (1881a, 1881b) publications. Van Luijk believed this circumscription, which he intimated was supported by Butler, had governed usage until 1927 with complete satisfaction. He held that several faults in Hesse's paper discredited the original account of *P. debaryanum* so seriously that by itself it could not be regarded as providing a valid description. According to Van Luijk the faults in Hesse's account were rectified by De Bary in a characterization accurately portraying a fungus that can be found any day. In view of the imputed corrections the authorship of the species was broadened, so that the familiar binomial appeared as *P. debaryanum* Hesse em. De Bary.

Van Luijk regarded parasitism and the scope of asexual reproduction with respect to the presence or absence of zoospore development as being of no moment in distinguishing species; and he held it doubtful whether the differences in the antheridia of *Pythium debaryanum* and *P. ultimum* were sufficient to separate these two fungi. These views are largely contrary to my observations on the parasites under discussion. In my collection of cultures sameness or otherness has as a rule been indicated reliably by the capability of newly formed globose asexual reproductive bodies to produce zoospores and by the positional relation of the antheridium or antheridial branch in monoclinous sexual apparatus. The two features are most helpful, besides, in interpreting some of the early literature on the species sharing the particular kind of parasitism here in question, including the pertinent descriptive writings of Hesse, De Bary, and Trow.

These writings were all based on fungi obtained from seedlings that had succumbed to damping-off. Although this serious disease affects seedlings of many phanerogamic crop plants and is caused in rather considerable measure by species of *Pythium* the number of species effective enough in its causation to invite notice are few. In our middle and northern latitudes, where conditions of temperature and moisture during spring and early summer should not differ greatly from those in Alsace and Great Britain, the two species herein treated as *P. ultimum* and *P. debaryanum*, together with the somewhat digitate form treated by Matthews and Middleton as *P. irregulare*, are virtually the only damping-off parasites found which like the fungi described by Hesse, De Bary, and Trow give rise to relatively smooth oogonia and subglobose zoosporangia or conidia. Through this fortunate circumstance the few species mentioned are indicated rather definitely as the ones with which the writings of Hesse, De Bary, and Trow were

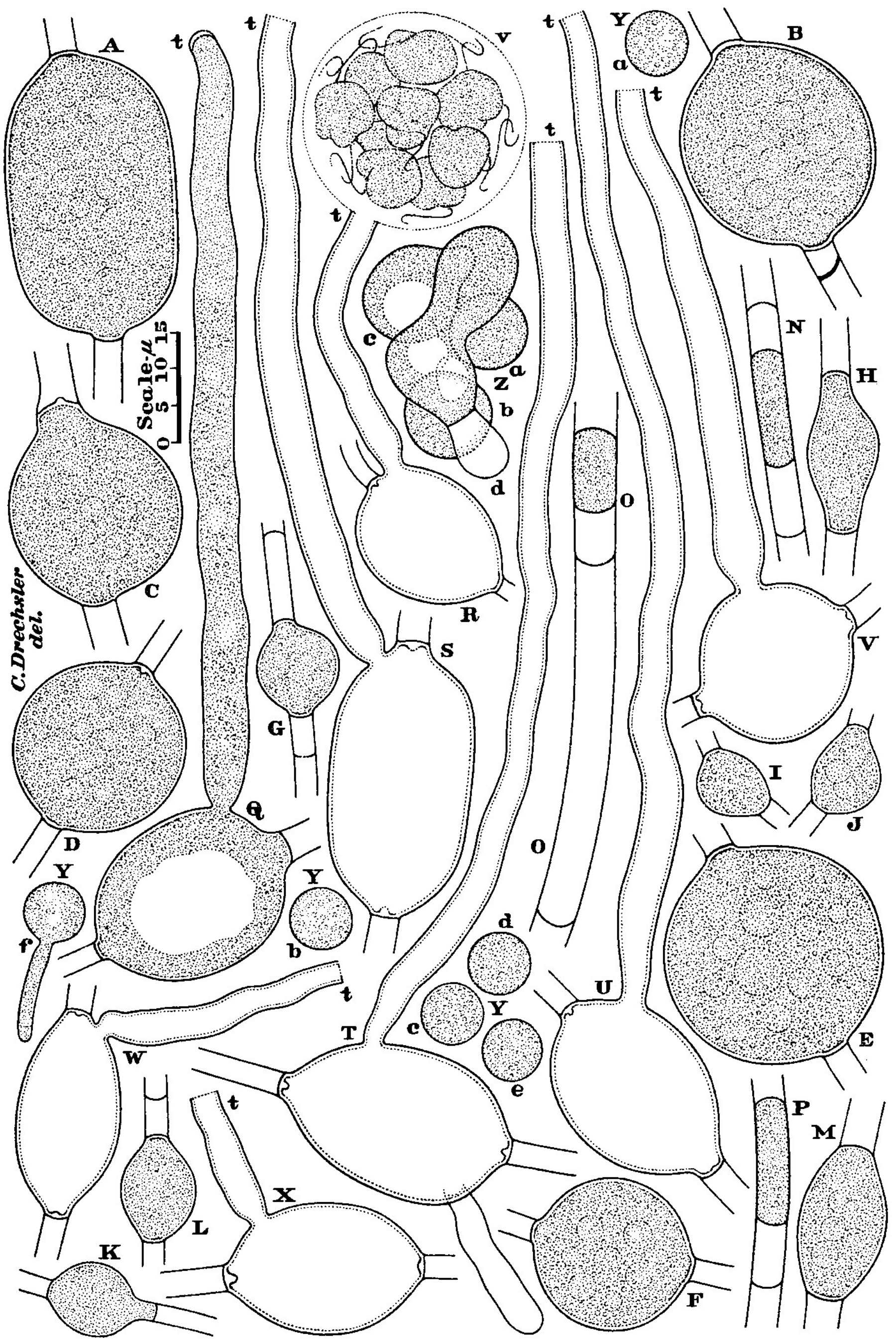

FIG. 3.—Asexual reproductive apparatus of *Pythium debaryanum* as found produced on wet maizemeal agar, drawn with the aid of a camera lucida at a uniform magnification; × 1000 throughout. A-M, Intercalary zoosporangia of various sizes and of globose or ellipsoidal shape. N-P, Small cylindrical zoosporangia formed in hyphae from remnants of protoplasm. Q, Zoosporangium with unusually long evacuation tube, t, surmounted by hyaline cap. R, Zoosporangium showing an evacuation tube, t, with a vesicle containing zoospores. S-V, Empty zoosporangia, each with an exceptionally long evacuation tube, t. W, X, Empty zoosporangia, each with an evacuation tube, t, of ordinary length. Y, Six encysted zoospores, a-f, of which one, f, is germinating. Z, Group of 4 encysted masses of protoplasm, a-d, resulting from frustrated development in discharged sporangial contents.

for the most part concerned. Thus De Bary's firsthand account of the damping-off fungus he considered to be *P. debaryanum* can be safely understood as relating unambiguously to a species found operating very widely and destructively as a causal agent of damping-off—to the species herein termed *P. ultimum*—though the morphology it sets forth would apply almost equally well to a closely related congeneric species, *P. paroecandrum* Drechsler (1940), which so far has come to light only as a cause of root rot and rootlet-tip discoloration. In Hesse's original and earlier description of *P. debaryanum* is set forth recognizably another parasitic species scarcely less widespread and efficient in the causation of damping-off than the one which De Bary presented under the same binomial. The differences whereby present-day isolations can be separated are detectable in the writings of the two pioneer investigators. Isolations in which very frequently the relatively smooth oogonium is fertilized by an antheridium borne terminally on a branch arising from the oogonial hypha at a distance from the oogonium about equal to the oogonial diameter—the relation of parts depicted by Hesse—will readily produce zoospores from zoosporangia of mycelial origin. On the other hand, damping-off isolations in which the oogonium is commonly fertilized by an antheridium consisting of an adjacent hyphal segment or of a lateral pouch-like cell arising in immediate proximity to the oogonium—the positional relationships most frequent in De Bary's illustrations—are decidedly reluctant to produce zoospores from conidia of mycelial origin, and yet will form them rather consistently from germinating after-ripened oospores or from sporangia borne on germ hyphae extended by after-ripened oospores. It is hardly surprising that De Bary, though a master of unsurpassed proficiency, found zoospores to be produced sparingly, whereas Hesse with only the limited experience of a student would seem to have obtained zoospores abundantly during the first few weeks he worked with his fungus. Van Luijk's success in obtaining zoospores from the isolations he referred to *P. debaryanum* Hesse em. De Bary may have been due primarily to his use of material from an old dried-up pure culture, for in such a culture the oospores present would ordinarily have had ample time to undergo the changes of after-ripening. If, as seems probable, the isolations were referable to *P. ultimum* they need not have been of exceptional reproductive behavior like the cultures that Ark and Middleton (1949) observed giving rise to zoospores from ordinary conidia.

In his text Hesse gave no measurements for the hyphae and reproductive bodies of *Pythium debaryanum*. Apparently he relied entirely on his illustrations to convey metric information. Unfortunately, at the magnifications given in the legends his figures show excessive approximate values for all dimensions: 13μ for width of a main mycelial filament; 45μ for diameter of zoosporangium; 20 to 23μ for diameter of encysted zoospores; 75μ and 50μ for length and width, respectively, of an intercalary conidium; 45 to 48μ for diameter of oogonium; 37μ and 10μ for length and width, respectively, of an antheridium; and 37μ for diameter of oospore. Since in any of the damping-off species—and the damping-off species are among the coarsest members of the genus—13μ is about twice the usual width of an elongating axial hypha a few hundred microns from its tip, the actual magnification would seem about twice that given in the legend. Indeed, if the magnifications given in all the legends were doubled the smaller dimensional values then derived from the figures would be in tolerably good agreement with the usual measurements for either of the damping-off fungi under discussion, which except for differences in thickness of oospore wall and in length of monoclinous antheridial branches are, in general, of nearly equal size. How the error might have come about is conjectural. It may perhaps be no mere coincidence that on the two folded plates of the published dissertation Hesse's figures occupy rectangular areas about twice the width of the printed pages. Should the procedure for making lithographs 80 years ago have readily permitted reproduction of illustrations at reduced sizes the magnifications appearing in the legends might have been given in the expectation that the plates would be adjusted to the width of the page. In any case, the error, though most unfortunate in the original account of a species which with respect to its binomial has become one of the most familiar of all fungi, is so very obvious that it should not occasion any serious misunderstanding as to what damping-off parasite was the main subject of Hesse's description.

In sexual apparatus of *Pythium debaryanum* produced on wet or unwet agar substratum the antheridia borne terminally on branches are

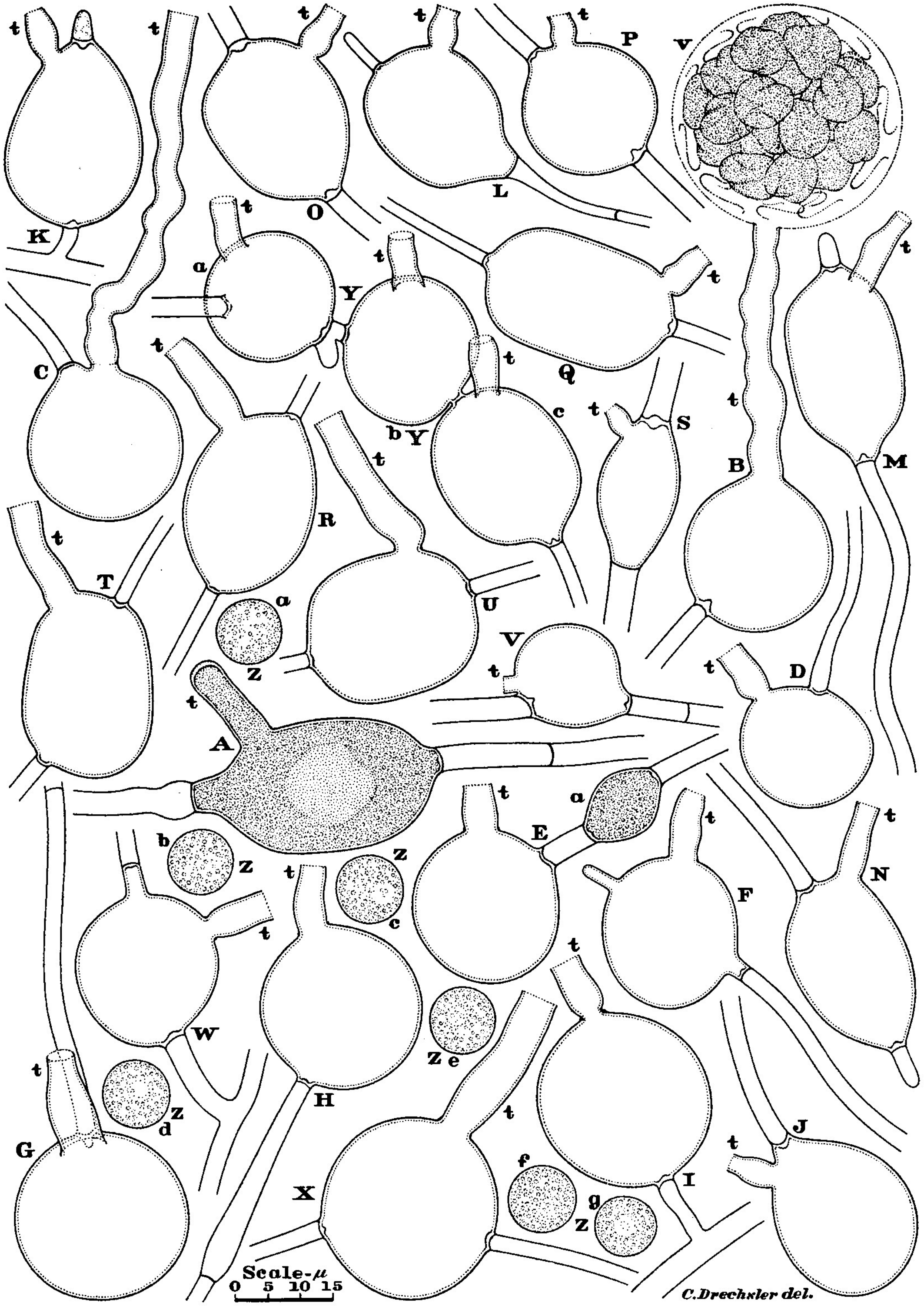

FIG. 4.—*Pythium debaryanum*, on moist Lima-bean agar; × 1000. A, Sporangium shortly before discharge. B, Sporangium with active zoospores in vesicle, v. C-J, Empty terminal sporangia. E, a, K-X, Empty intercalary sporangia. Y Three empty sporangia, a-c, close together. Z, Encysted zoospores, a-g. (A-Y: t, evacuation tube.)

rather consistently applied to the oogonium by their tips. However in sexual apparatus formed on extramatrical submerged mycelium, terminally borne male cells are often observable in more varied postures. Postural relations infrequent in agar cultures are shown in the illustrations not only of early workers like Hesse (1874) and Sadebeck (1875), who had only submersed material available for study, but also of later workers who have studied such material from preference. Thus, several of the figures of *P. irregulare* given by Matthews (1931, pl. 24, fig. 4; pl. 25, figs. 1, 3, 9) show antheridia applied laterally, and in at least two instances the male cell seems intimately in contact with the oogonium throughout its length (Matthews 1931, pl. 25, fig. 3, upper; fig. 9). In the few figures of sexual reproductive apparatus given by Hesse the antheridium is shown applied laterally, though the area of contact is rather well forward and of either small or only moderate extent. Hesse's text does not mention what portion of the antheridium makes contact with the oogonium, his most directly relevant phrases (sich dieser innig anschmiegt; das Anlegen des Pollinodiums; lagert sich an die Membran; dem Momente ihres Anlegens; durch Anlegen an die Membran des Oogoniums) not specifying directly either lateral or apical application, even if the word "anschmiegt" might connote rather extensive contact.

Sadebeck (1875, p. 124) in discussing sexual development in his *Pythium equiseti* stated that the antheridium is most often applied by its tip, that sometimes it becomes attached laterally to the oogonium, that very often it enwraps the oogonium, and that therefore the fertilization tube is extended either apically or laterally. In one of the figures given by Sadebeck (1875, pl. 4, fig. 3) a longitudinally applied antheridium is shown extending a lateral fertilization tube into the oogonium. In another figure of Sadebeck's, in which are shown two connected monoclinous reproductive units, the antheridium in one unit (Sadebeck, 1875, pl. 3, fig. 8, right) is narrowly applied to the oogonium with its tip, while the antheridium in the other unit (Sadebeck, 1875, pl. 3, fig. 8, left), is applied lengthwise, so that it enwraps the oogonium closely and rather extensively. The circuitous male branch in the former unit arises from the oogonial stalk at a distance from the oogonium about equal to the width of that organ. The male branch in the latter unit arises from the same hypha as the oogonial stalk, its origin being separated from the oogonium by hyphal elements having a combined length approximately equal to twice the oogonial diameter. None of Sadebeck's illustrations show any antheridium formed in immediate proximity to the oogonium, and in his text the antheridia in monoclinous units are, with regard to their hyphal relations, discussed only as being borne terminally on branches. As might be expected from the arrangement of its monoclinous sexual apparatus the fungus several times gave rise to zoospores following transfer of infected *Equisetum* prothallia to water. Since infected prothallia always disintegrated completely without leaving any visible residue, the host substratum used by Sadebeck for swarm-spore production must have been rather newly invaded, and thus presumably should not have contained any after-ripened oospores.

Because of its ready parasitism on many host plants De Bary considered it likely that his fungus might have been observed by other workers besides Hesse. He stated that the description of *Pythium equiseti* left scarcely any doubt that Sadebeck's fungus was identical with the one he and Hesse had studied. All doubt as to the presumed identity was dispelled for him when some healthy *Equisetum* prothallia, after being inoculated with his fungus, were destroyed "murderously" in a few days. Without the advantages provided in collections of pure cultures—advantages accruing from opportunity not only to examine isolations wholly free of alien organisms but also to compare with respect to morphology, development, and pathogenicity isolations obtained from different host plants, in different regions, and at different times—it is not surprising that De Bary failed to uncover the correlated differences in antheridial morphology and zoospore production whereby the damping-off parasite figured by him is distinguished from the fungus corresponding better to the descriptions and illustrations of Hesse and Sadebeck. The latter fungus may well have been present in some of De Bary's material, since he mentioned (De Bary, 1881 b, p. 526) that in some cultures zoosporangia were formed in relatively large numbers, intermixed with resting conidia produced at the same time and under apparently similar conditions. Besides, unless he had seen Hesse's species now and then in his own material it appears unlikely that he would so readily have noted in the description and figures of *P. equiseti*

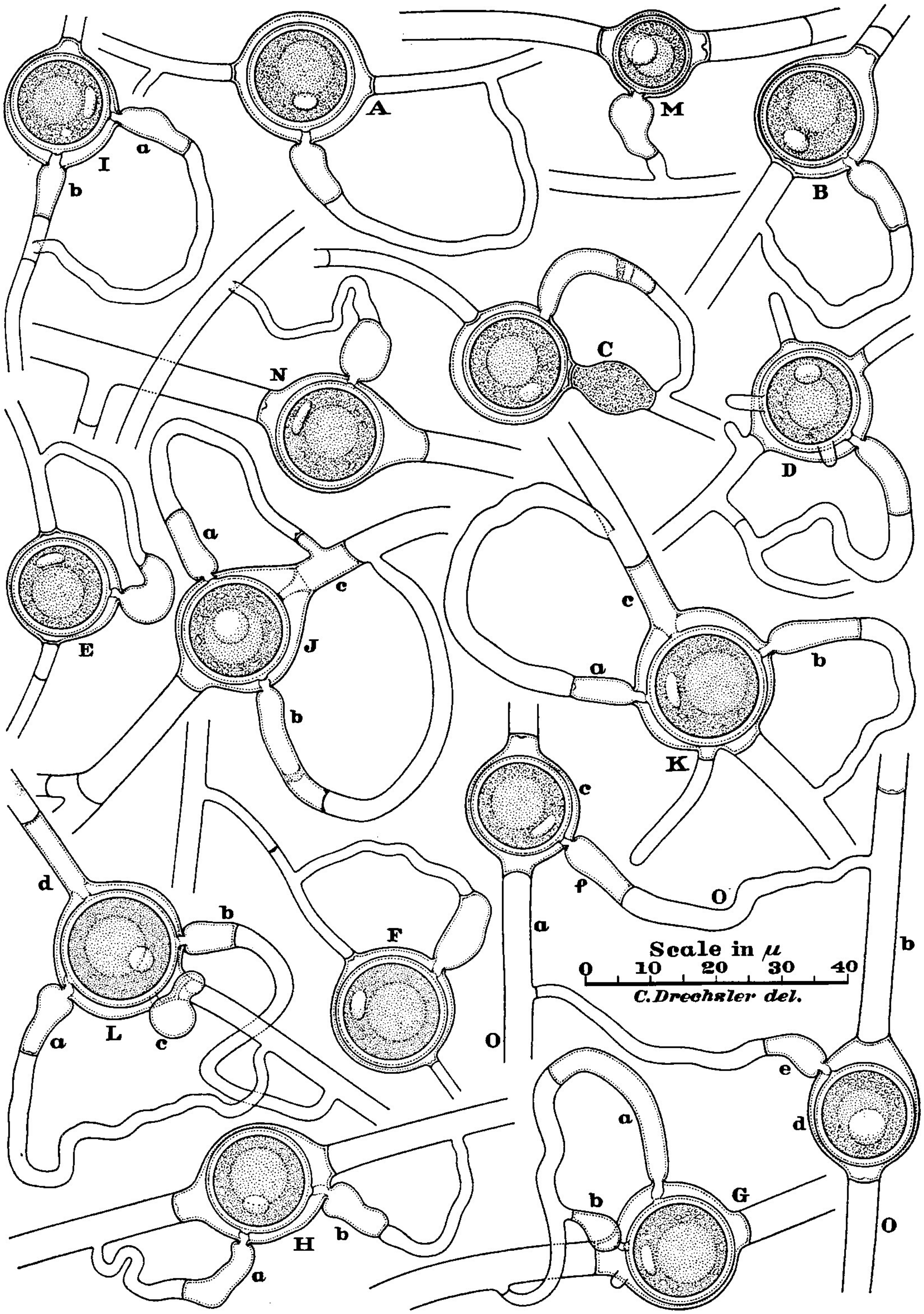

FIG. 5.—Mature sexual reproductive apparatus of *Pythium debaryanum* formed on moist Lima-bean agar; × 1000. A-F, Monoclinous units with single antheridia. G-I, Monoclinous units with 2 antheridia, a and b. J, K, Monoclinous units with 3 antheridia, a-c. L, Monoclinous unit with 4 antheridia, a-d. M, N, Diclinous units. O, Two connected diclinous units: a, b, parent hyphae; c, d, oogonia; e, f, antheridia.

any very persuasive similarity to the damping-off parasite depicted in his illustrations. His ready acceptance of Sadebeck's account would seem to imply that lateral fertilization tubes and laterally applied antheridia—even clasping antheridia—were not regarded by him as features incongruous with the morphology of *P. debaryanum*. It appears probable therefore that De Bary considered the treatment of antheridial morphology in Hesse's dissertation less as being wrong and requiring correction than as being insufficient and requiring amplification. Unhappily the amplification he provided would seem to have been derived for the most part, if not wholly, from a closely related species with similar parasitic tendencies.

Corroboration of Hesse's illustrations showing laterally applied antheridia and lateral fertilization tubes has come mainly from studies carried out on submerged material in water cultures. The positional instability usual in such cultures and the ease with which the more delicate submerged parts may be pushed out of place or pressed out of shape are not to be disregarded.

In Butler's (1907) monograph the treatment of the sexual reproductive stage in *Pythium debaryanum* conforms closely to De Bary's account. Butler's relevant firsthand observations then were limited to material obtained by causing cress seedlings to damp off at Freiburg im Breisgau, Germany. Although he found conidia and oospores produced abundantly, he saw no sporangia. His failure to obtain zoospores, together with his description of antheridia in the species as "from the same or another hypha as the oogonium, often formed close below the latter, and not seldom hypogynal," suggests strongly that the Freiburg fungus was the same as the one figured by De Bary.

A later paper published by Butler (1913) on *Pythium debaryanum* presents a firsthand account of a conspicuously different fungus he found at Pusa, India, in the caruncle of castor (*Ricinus communis* L.) seed that after being sown in pots of unsterilized soil had failed to germinate. In this fungus Butler did not observe any antheridia that consisted individually of a cylindrical hyphal segment adjacent to the oogonium. Often the antheridium was borne terminally on a branch arising from the oogonial hypha at some distance from the oogonium, the distance in six monoclinous reproductive units pictured (Butler 1913, figs. 1–4) ranging from about 10μ to 60μ. Above the junction of the two sexual stalks the female stalk was often longer than the male stalk and consequently was often curved. The resulting postural relation manifestly was the reverse of that shown in the sexual reproductive unit figured by Hesse, where the

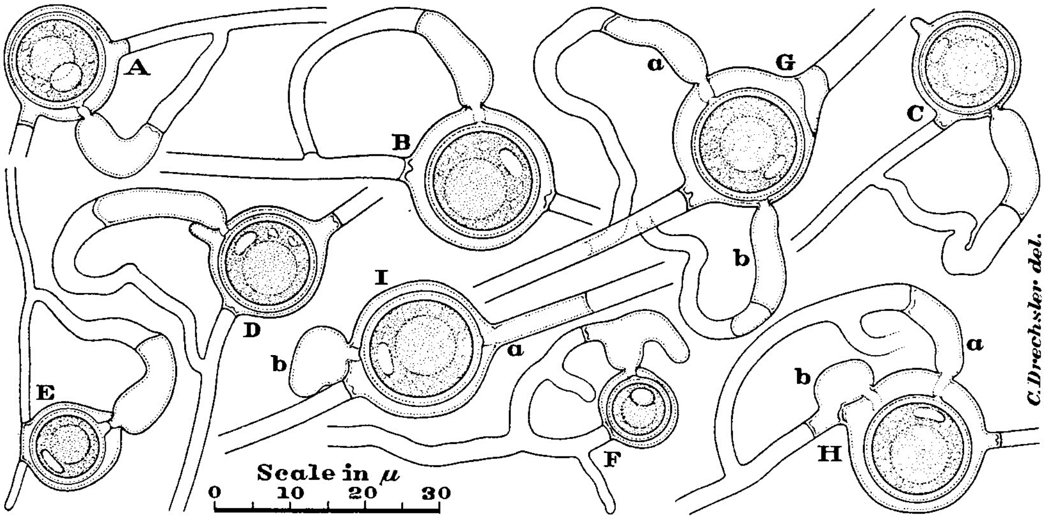

FIG. 6.—Mature sexual reproductive apparatus formed on moist Lima-bean agar inoculated with Buisman's isolation of *Pythium irregulare*, × 1000. A-E, Monoclinous reproductive units, each with a single antheridium. F, Monoclinous reproductive unit with a terminal antheridium bearing a lateral protuberance by which it is attached to the oogonium. G, Monoclinous reproductive unit with 2 antheridia, each borne terminally on a branch. H, Monoclinous reproductive unit with one antheridium, a, borne terminally on a branch, and another, b, formed in immediate proximity to the oogonium. I, Monoclinous reproductive unit with 2 antheridia, of which one, a, consists of a hyphal segment adjacent to the oogonium, while the other, b, is laterally sessile in immediate proximity to the oogonium.

straight oogonial stalk is shorter than the longer curving antheridial branch; and assuredly was the reverse of the postural relation most usual in monoclinous reproductive apparatus of the damping-off parasite herein treated under Hesse's binomial. Similar curvature of the oogonial stalk is found rather often in cultures of *P. butleri* Subr. and *P. aphanidermatum* (Eds.) Fitzp. (= *P. deliense* Meurs), and is conspicuous in Butler's (1907, pl. 2, figs. 3, 5, 6) illustrations of his *P. indigoferae.* Although the text contains no comment on the shape of the antheridium, all eight of the antheridia figured show a distinctive outward form in that they consist of a somewhat enlarged clavate terminal part together with a lateral protrusion by which they are united to the oogonium. Antheridia of approximately such design are found somewhat infrequently in *P. debaryanum* (Fig. 6, F) but are very numerous in *P. butleri* and *P. aphanidermatum.* It is not known whether the curious resemblance between the Indian fungus and *P. aphanidermatum* with respect to the make-up of their sexual apparatus may have had some influence in leading Jochems (1927) to identify provisionally as *P. debaryanum* the stem-burn parasite that Meurs (1934) later described under the binomial *P. deliense.* Apart from its production of zoospores from globose rather than from lobulate zoosporangia the Indian fungus with its relatively delicate mycelium appears clearly alien to Hesse's damping-off parasite, as well as to the other widely distributed damping-off parasite herein discussed, which after being set forth firsthand by De Bary was not described under a separate binomial of unambiguous application until Trow (1901) presented it somewhat mistakenly (Drechsler, 1935) as a new saprophytic species, *P. ultimum.* No species corresponding to the firsthand description and illustrations given by Butler has ever come to light among the cultures I have isolated from diseased plants and other materials. Since the Indian fungus was found in a locality where as high a temperature prevails as is ordinarily found in the tropics, its distribution may well be restricted to regions considerably warmer than central Maryland.

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