

A *Pythium* causing stem rot of tobacco in Nicaragua and Indonesia.

By Charles Drechsler

(Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Plant Industry Station, Beltsville, Maryland, U.S.A.)

With Plate I—XIV.

In a brief abstract (Drechsler, 1934) 3 *Pythium* cultures isolated in 1927 from sugarcane (*Saccharum officinarum* L.) roots in Louisiana were held distinguished by their smaller reproductive structures from the robust cottony-rot fungus occurring widely in the United States, which on grounds set forth elsewhere (Drechsler, 1955) would seem best designated as *P. butleri* Subramaniam (1919). The 3 isolations from sugarcane, which bore the numbers 66, 96, and 126, respectively, were closely similar in morphology and development to 2 cultures received, also in 1927, from the Deli Proefstation at Medan, Sumatra, where they had been isolated from young tobacco (*Nicotiana tabacum* L.) plants affected with parasitic stemburn. Since the 2 Indonesian cultures besides bearing the numbers 2 and 3, respectively, came labelled as "*Pythium debraryanum*" it was presumed that they were representative of the *Pythium* species which Jochems (1927) in his comprehensive account of stem-burn mentioned as "probably de *Baryanum*". All 5 cultures failed unexpectedly to endure the conditions of storage that had proved generally suitable for other *Pythium* isolations, and thus were lost before they could be adequately studied. When subsequently Meurs (1934) in his treatment of 3 *Pythium* species he had found responsible for stem-burn of Deli tobacco explained that the one he described newly as *P. deliense* had in former years and in Jochems' paper been considered "probably *Pythium de Baryanum*", it appeared reasonable to infer that the new species had most likely been represented in the 5 isolations (Drechsler, 1952).

A culture closely resembling the 5 lost isolations and accordingly presumed to be conspecific with them was brought to me for identification by William Lautz soon after he had isolated it from browned tissue of a tobacco plant found affected with a destructive stem rot in a field near Managua, Nicaragua (Litzenberger and Stevenson, 1957), on August 23, 1954. In order to set forth the resemblances visibly, occasion is taken to supplement the illustrations pertaining to the Nicaraguan fungus (Pl. I—VI) with drawings

prepared from 2 Louisiana (Pl. VII) and both Indonesian cultures (Pl. VIII, IX) received in 1927. Similar drawings (Pl. X—XIV) prepared mainly in 1957 at the American Type Culture Collection, Washington, D. C., from material of the apparently authentic culture of *Pythium deliense* then listed as ATCC 12280, are included to show the differences whereby Meurs' species would seem distinct from the Nicaraguan parasite despite its similar host relationship and the inference suggested by the labels on the 2 cultures received from Sumatra. The culture ATCC 11433, though also ascribed to *P. deliense*, is omitted from comparison since its antheridia indicate closer relationship with the very familiar *P. ultimum* Trow than with *P. butleri*.

Opportunity has been lacking for any direct comparison of the Nicaraguan culture with *Pythium indicum* Balakrishnan (1948) as that species has not been available at the American Type Culture Collection. In view of protective restrictions on the importation of pathogenic materials it was not determined whether the type culture of *P. indicum* deposited in 1947 at the Agricultural Research Institute, Coimbatore, South India, is still living. Apparently the species has never been mentioned in any catalogue listing the micro-organisms maintained alive at the Centraalbureau voor Schimmelcultures, Baarn, Holland, or at the Commonwealth Mycological Institute, Kew, England.

Pythium indigoferae Butler (1907) likewise has not been propagated at the American Type Culture Collection and thus has not been available for direct comparison with the Nicaraguan fungus. Successive lists of the Centraalbureau voor Schimmelcultures, including those issued in 1931, 1932, 1933, and 1940, cite only a single culture of *P. indigoferae*. The citation in the list of 1931 acknowledges McRae as having supplied the isolate, whereas in the later lists Sideris is given such acknowledgement. This change probably implies no change in actual source, for Sideris (1931) mentioned that the organism was sent to him by McRae, who had obtained it from cucumber (*Cucumis sativus* L.) roots in India. Since Meurs, Saksena (1935, 1940), and Middleton (1943) each obtained from the Centraalbureau the culture of *P. indigoferae* used by him, the comparative studies of these authors, as also those of Sideris, would seem to have been carried out with an isolate derived from cucumber roots rather than from leaves of *Indigofera arrecta* Hochst., the plant parts on which Butler discovered the species growing epiphytically. Balakrishnan in carrying on similar studies used "a type culture of *P. indigoferae* obtained from the National Collection of Type Cultures, Delhi", without indicating the host plant or the material from which it originated. I have seen no report that identity of the isolate from cucumber roots with *P. indigoferae* was

established through side-by-side comparison of relevant cultures. It may not have been wholly fortuitous that although Butler's account of the epiphytic parasite seemingly evoked little suspicion of any close relationship to the widespread cottony-rot fungus, *Sideris* at once imputed such relationship to the isolate from cucumber roots by including that organism under the binomial *Nematosporangium indigoferae* (Butl.) Sid. in his section *Oligandra*, together with *N. aphanidermatum* (Eds.) Fitzp., *N. aphanidermatum* var. *hawaiiensis* Sid. and *N. butleri* (Subr.) Sid.

Because of its foreign origin the Nicaraguan fungus was tested for pathogenicity only on plant parts confined in covered glass dishes suitable for heat sterilization. Portions of agar well permeated with its young mycelium were thrust into small incisions made with a sterile scalpel in fruits of cucumber and of summer squash (*Cucurbita pepo* L.). In less than 3 days the cucumbers (Pl. I, A, B) became extensively softened by a watery decay. Most of the affected area was clothed with a moderately luxuriant layer of white aerial mycelium rather similar to the aerial layer usually enveloping cucumber fruits attacked by *Pythium ultimum* Trow var. *ultimum*. Possibly owing in part to their more delicate epidermis the summer-squash fruits, which also were rapidly invaded, permitted earlier and more copious development of aerial mycelium (Pl. I, C, D), thereby providing an appearance nearly like that resulting from attack by *P. butleri* (Drechsler, 1955, Pl. VII).

On various artificial substrata the Nicaraguan fungus similarly grows somewhat less rapidly and produces somewhat less abundant aerial mycelium than *Pythium butleri*. Thus in Petri plates of fairly soft maize-meal agar that were kept at 24° C. the mycelial forefront of the Nicaraguan fungus was found to advance an average distance of 29.2 mm in 24 hours, whereas under identical conditions *P. butleri* extended its mycelium radially an average distance of 34.1 mm during the same period. Examination of mycelium in the resulting cultures showed that the slenderest hyphal branches produced by either species measured 2 μ in width. Slightly less equality was observable between the coarsest axial hyphae of the two species, since in the Nicaraguan fungus these reached a width of 9 μ , and in *P. butleri* a width of 10 μ . Larger measurements have been reported notably in *P. indicum* to which its author ascribed hyphae "4 to 12 μ diameter, mostly 8 to 10 μ ", with the axial hyphae being further characterized as "very stout, often measuring over 10 μ in diameter". The appressoria formed singly (Pl. II, A—C) or in groups (Pl. II, D—F) on the glass floor under Petri plate cultures of the Nicaraguan fungus vary commonly from 10 to 14 μ in width, and accordingly would appear considerably smaller than the appressoria of *P. indicum*, which are stated often to exceed 20 μ in thickness. From the

scale of magnification indicated for them, however, the appressoria of *P. indicum* figured by Balakrishnan (1948, text-fig. 1, A, B) seem to measure only 8 to 12 μ in greatest width, and, similarly, none of the hyphae figured by him, except those obviously modified as young sporangia, seem more than 7.5 μ wide.

The Nicaraguan fungus gives rise to sporangia and zoospores less promptly and less abundantly than *Pythium butleri*. When slabs of Lima-bean agar permeated with young mycelium of *P. butleri* are transferred to a shallow layer of distilled water numerous stout, irregularly branched outgrowths are extended into the liquid in the course of 2 hours; and during the third hour many of these outgrowths, after undergoing division into several sporangia, become emptied one after another, with resultant development of zoospores in enormous numbers. With similar treatment the mycelium permeating slabs excised from a young Lima-bean-agar plate culture of the Nicaraguan fungus pushes out many submerge filamentous hypha, which during the first day, or the first two days, remain virtually undifferentiated, but afterwards give rise in scattered positions to noticeably distended elements, often somewhat branched or lobulated (Pl. III, A, a, b; B, a, b; C, a—m). If now the preparation is freshened by changing the distilled water several times many of the distended elements will put forth an evacuation tube with a hyaline apical cap (Pl. III, D—F:t). The protoplasm in the sporangium thus brought into being soon migrates into a vesicle (Pl. III, H—J:v) where it is fashioned into motile zoospores (Pl. III, G, v; K, v) after the manner usual in members of the genus. When the sporangium (Pl. III, F) is evacuated its membranous envelope (Pl. III, G) shrinks perceptibly in width.

On the whole the asexual reproductive units of the Nicaraguan fungus are smaller than those of *Pythium butleri*, though they display rather similar variety in their make-up. Filamentous parts, mostly 20 to 175 μ long, and distended lobules, usually 9 to 18 μ wide, enter into their composition. The evacuation tube (Pl. III, D—W:t), mostly between 2 and 6 μ in width, ranges commonly from 5 to 125 μ in length, so that the latter dimension may in some instances (Pl. III, M, t; P, t; U, t) be less than the width, and in other instances (Pl. III, I, t; V, t) may be greater than the length, of the concomitant sporangium. In a uniaxial terminal sporangium the evacuation tube most often originates at the tip and grows out either as a distal prolongation (Pl. III, H—J:t; L, t) or as a somewhat lateral branch (Pl. III, F, t; M, t; N, t; R, t), while in pluriaxial intercalary sporangia it may arise from an outwardly unmodified filamentous part (Pl. III, T, t; V, t) or from a distended part (Pl. III, U, t; W, t). On disintegration of the terminal vesicle 3 to 35 zoospores are released, which, after a variable period of motility, round up into globose cysts often 8 to 10 μ in

diameter (Pl. III, X, a—h) and then commonly germinate by putting forth germ tubes about $2\ \mu$ wide (Pl. III, Y, a—d). Ostensibly the Nicaraguan fungus, like *P. indigoferae*, is considerably less prolific than *P. indicum* whose sporangia are reported to produce 25 to 150 swarmers that after encysting measure 10 to $12\ \mu$ in diameter. In displaying decidedly short as well as relatively long evacuation tubes the Nicaraguan fungus might perhaps be held distinguished not only from *P. indicum*, in which the evacuation tube is described as “never very short”, but also from *P. indigoferae* in which the sporangia, according to Butler, open “laterally by short straight branches”.

In cultures of maize-meal agar the Nicaraguan fungus soon forms sexual reproductive apparatus (Pl. II, G—R; Pl. IV, A, C) abundantly with scarcely any sign of abnormal development. The oogonium (Pl. IV, A, a; B, a) and antheridium (Pl. IV, A, b; B, b) come into broad contact long before either organ is full-grown and ready to be delimited. A large proportion of the reproductive units are of monoclinal origin, their hyphal connections often being closely similar to the connections shown by Hesse (1874, Pl. II, Fig. 13, 14) in the widely familiar illustrations of sexual reproduction in his *Pythium debaryanum*, except for the important difference that the positions of the male and female components are here interchanged. The illustrations in question show an oogonium borne on an axial filament which some little distance away gives off a somewhat recurved branch destined to bear the attendant antheridium terminally, whereas in the Nicaraguan fungus the antheridium develops intercalarily (Pl. II, G; I; K; M, a; Pl. IV, A, C) or terminally (Pl. II, J; L, a, b; Pl. IV, B) on an axial filament which some little distance below — usually 15 to $35\ \mu$, but occasionally as much as $75\ \mu$ — gives off a recurved branch on which the oogonium is borne terminally. Less distinctive hyphal connections are shown in some monoclinal units where the oogonium and attendant antheridium are borne on separate branches originating from the same hypha (Pl. II, H; M, b). The production of several reproductive units highly varied with respect to their interrelations, on the branches of a single hyphal element is a conspicuous feature of the Nicaraguan fungus. In some instances (Pl. II, L, a, b; M, a, b; P, a, b) the plural units would seem to originate rather independently despite their close proximity to one another, but in other instances portions of an earlier unit, through renewal of growth or of differentiation, give rise to a later unit. The oogonial branch of an earlier unit (Pl. II, N, a) may, for example, give off 2 branches bearing the male and female components of a later unit (Pl. II, N, b); or the antheridial branch of an earlier unit (Pl. II, O, a) may put forth a branch bearing an oogonium (Pl. II, O, b) while the oogonial branch of the earlier unit, after some subapical elongation, bears an attendant antheridium; or the oogonial

branch of an earlier unit (Pl. II, P, b) may produce a subterminal antheridium to fertilize an oogonium (Pl. II, P, c) borne terminally on a branch it has given off proximally.

Although some displacement of adjoining hyphal parts must occur during the enlargement of the oogonium, the mature sexual units of the Nicaraguan fungus, whether they are monoclinal or diclinal, often still show by peculiar orientation of oogonium and oogonial branch that in the formative period the female component was brought together with the male component through appropriate elongation of the hyphal element directly supporting it. Strong curvature of the oogonium toward the antheridium was mentioned by Butler (1907) as a characteristic feature of *Pythium indigoferae*, while strong curvature of the filament bearing the oogonium was set forth by Meurs as being usual in the predominantly monoclinal sexual reproductive units of *P. deliense* and later was cited by Balakrishnan among the descriptive attributes of *P. indicum*. Meurs properly recognized the curved hyphal element bearing the oogonium as a branch given off by the relatively straight element bearing the antheridium. Balakrishnan used the term "stalk" impartially for the hyphae directly supporting the oogonium and the antheridium, yet in some of his illustrations (Balakrishnan, 1948, Fig. 1, L, R (upper); Fig. 2, E, F, G) a curved oogonial branch is clearly given off from a more nearly straight antheridial hypha, even if some other illustrations (Balakrishnan, 1948, Fig. 1, N, O) would seem ambiguous with respect to branching relationship. Similarly in two reproductive units shown in a figure given by Sideris (1931, Fig. 12, d, e) to illustrate *P. indigoferae* the oogonium seems borne on a strongly curved branch arising from the nearly straight hypha bearing the antheridium; whereas, in marked contrast, some of the few sexual reproductive units figured by Butler (1907, Pl. II, Fig. 3 [top], 5) in his original account of the species show the antheridium borne on a straight branch given off unmistakably by the distally curved hypha supporting the oogonium. *P. butleri* has not been mentioned among the few species that have been held distinguished by unusual orientation of the oogonium and its supporting hyphal element, yet the only monoclinal reproductive unit figured in relevant plates by Subramaniam (1919, Pl. V, Fig. 13) shows the oogonium borne terminally on a curved branch given off from a more nearly straight hypha bearing the attendant antheridium intercalarily. Except for the minor difference that the attendant antheridium is borne terminally, the same arrangement of parts is evident also in the one monoclinal reproductive unit shown in its entirety by Mitra and Subramaniam (1928, Pl. I, Fig. 12) to illustrate the morphology of a conspecific strain they assigned to *P. aphanidermatum* (Eds.) Fitzp. Again, an oogonium

borne terminally on a curved branch given off by a hypha producing the attendant antheridium distally is present in all of the several monoclinal units shown by Ramakrishna Ayyar (1928, Pl. II, Fig. 5, 6) in illustrations of another conspecific strain likewise referred to *P. aphanidermatum*. Conspicuous parallelism with the Nicaraguan fungus in respect to terminal development of the oogonium on a curved branch given off by the hypha bearing the attendant antheridium is readily recognizable also in most of the sexual reproductive units shown in figures provided by Butler (1913, Pl. V, Fig. 1, 3, 4) to illustrate his account of a *Pythium* he isolated from castor-bean (*Ricinus communis* L.) seeds and identified as *P. debaryanum*. The isolate, of course, appears to have been alien to the group under discussion as it produced globose rather than filamentous or lobulate sporangia.

The young oospore formed in maize-meal-agar plate cultures of the Nicaraguan fungus shows near its periphery somewhat massive protoplasmic blocks separated by radial clefts (Pl. II, H; L, a; N, b; P, a, c). It usually undergoes the internal changes incident to maturation less rapidly than oospores of the familiar *Pythium ultimum* or *P. debaryanum*. At full maturity the oospore has the unitary internal structure that De Bary (1881) observed in various members of the genus, always revealing a single largish reserve globule surrounded by a parietal layer of densely granular protoplasm in which is imbedded a single globose or oblate-ellipsoidal refringent body (Pl. II, G; I; J; K; M, a, b; O, a, b; P, b; Q; R). It is surrounded by a smooth wall, varying commonly from 1 to 1.6 μ in thickness and consisting of a thin colorless outer layer and a thicker, distinctly yellowish inner layer. It is loosely contained within the globose oogonial envelope, which often is 0.8 to 0.9 μ thick and like the similarly sturdy envelope especially of my *P. scleroteichum* and my *P. dissotocum* keeps its shape unchanged in agar cultures for many months. Two hundred oogonia from maize-meal-agar plate cultures in which the abundant sexual reproductive apparatus showed somewhat greater dimensional variations than are usual, gave measurements for diameter, expressed in the nearest integral number of microns, with a frequency distribution as follows: 11 μ , 1; 12 μ , 2; 14 μ , 1; 15 μ , 2; 16 μ , 2; 17 μ , 8; 18 μ , 9; 19 μ , 24; 20 μ , 29; 21 μ , 33; 22 μ , 44; 23 μ , 23; 24 μ , 17; 25 μ , 3; 26 μ , 1; 27 μ , 1. The 200 oospores contained within these oogonia gave measurements for diameter with the following distribution: 8 μ , 1; 10 μ , 2; 11 μ , 1; 12 μ , 2; 13 μ , 9; 14 μ , 10; 15 μ , 37; 16 μ , 47; 17 μ , 54; 18 μ , 30; 19 μ , 4; 20 μ , 2; 21 μ , 1. From the two series of measurements averages of 20.8 μ and 16.3 μ were computed for diameter of oogonium and of oospore, respectively.

In sexual reproductive apparatus formed by the Nicaraguan fungus on wet substratum, as, for example, pieces of infected squash

tissue shallowly irrigated with distilled water, the lateral dome-shaped part of the antheridium is usually less prominent and consequently somewhat smaller (Pl. IV, B, b; D—F: a) than in reproductive units formed in firm agar cultures. The oospores produced on wet or irrigated substratum often remain in a strongly vacuolated, immature condition (Pl. IV, D—F: a) for many days, though eventually, as a rule, most of them acquire the unitary internal organization characteristic of the resting state. On wet materials the fungus, much like *Pythium indigoferum* and *P. indicum*, often gives rise to sexual and asexual reproductive units close together. Distended lobulate or digitate sporangial components not only are formed on the antheridial hypha in positions above (Pl. IV, D—F: b) or below (Pl. IV, F, c) the antheridium but are developed also on the oogonial branch either intercalarily (Pl. IV, E, c) or laterally (Pl. IV, F, d).

Germination tests were made on oospores taken from maize-meal-agar plate cultures which after inoculation with the Nicaraguan fungus had for more than 130 days been protected against evaporation by being stored under an inverted battery jar. At the time the oospores were removed from the old cultures about 96 per cent of them retained the unitary organization of the resting state (Pl. V, A—C). The remaining 4 per cent revealed two refringent bodies imbedded in the parietal granular layer (Pl. V, D—F). During the ensuing 16 days the oospores were kept shallowly immersed in distilled water, which was changed once or twice each day. A room temperature near 22° C. was maintained throughout the period. Daily microscopic examinations showed that while some oospores incurred little or no visible change (Pl. V, A—C, G) even with rather prolonged irrigation, others after different intervals underwent increase in their refringent bodies first to 2, then to 4 (Pl. V, H—K), and finally to a definitive number varying often from 7 to 10 (Pl. V, L—O). Dark radial markings now appeared in the inner layer of the oospore wall, and the demarcation between this layer and the adjacent granular protoplasm became less distinct (Pl. V, M—O). After gradually assimilating the inner layer of the wall the protoplast pushed a protuberance into the oogonial chamber and through the oogonial envelope. On emerging the protuberance continued growth as a germ tube (Pl. V, P, R, T—V; Pl. VI, A, B). Some of the resulting hyphae continued to elongate vegetatively, each thus giving rise to a young mycelium (Pl. VI, C). Other germ hyphae yielded at the tip to allow the protoplasmic materials to migrate into an apical vesicle for their conversion into motile zoospores. The empty evacuation tube measured 5 to 100 μ in length. It was simple (Pl. VI, D, a—m: t) in some instances, but in other instances it was variously branched (Pl. VI, E, a—m: t), though provided, as a rule, with only one orifice. Disintegration of the vesicle released 4 to 14 swimmers

which after a period of motility came to rest and encysted (Pl. VI, F, a—g). When a portion of protoplasm failed to migrate into the vesicle one or more zoospores were fashioned within the oospore wall (Pl. VI, E, g) and often germinated in place.

Germination ensued more promptly in oospores of the Nicaraguan fungus that after their removal from a maize-meal-agar plate culture 174 days old were kept irrigated with distilled water at temperatures near 33° C. Within 24 hours fully nine-tenths of them had put forth germ-tubes (Pl. V, Q, S, W—Z). Many of the resulting hyphae soon ceased elongating and formed terminally an irregularly distended segment (Pl. V, X—Z) interpretable as a sporangium. Swarm-spores were not produced, however, until further irrigation was carried out at lower temperatures.

In their dimensions as well as in their moderate lobulation the sporangia (Pl. VII, A—F) produced on shallowly irrigated slabs of maize-meal-agar by isolate no. 66 obtained from a sugarcane root presented close parallelism with the Nicaraguan fungus. The emptied sporangium (Pl. VII, F) was provided with an evacuation tube (Pl. VII, F, t) commonly 10 to 15 μ long. Measurements of 31 mature sexual reproductive units showed variations in oogonial diameter from 14.7 to 26.8 μ , in oospore diameter from 12.7 to 20.7 μ , in thickness of oospore wall from 0.9 to 1.5 μ ; and averages of 22.7 μ , 18.0 μ , and 1.2 μ , respectively, were computed for the three dimensions.

In irrigated Lima-beam-agar preparations isolate no. 96 obtained from a sugarcane root gave rise to swarm-spores which soon after rounding up (Pl. VII, G, a—t) measured mostly 7.5 to 10 μ in diameter. When grown on maize-meal agar the isolate, like the Nicaraguan fungus, formed sexual reproductive apparatus in which the oogonium (Pl. VII, H, a, b) very often was borne terminally on a branch given off by the hypha bearing the antheridium (Pl. VII, H, c, d). One hundred sexual reproductive units from the under side of a maize-meal-agar plate culture in which unfortunately much degeneration was evident gave measurements for oogonial diameter, expressed in the nearest integral number of microns, that were distributable as follows: 16 μ , 1; 18 μ , 1; 19 μ , 13; 20 μ , 44; 21 μ , 25; 22 μ , 12; 23 μ , 4; and the oospores in the units, all of correct internal structure, gave measurements for diameter distributable thus: 12 μ , 1; 14 μ , 2; 15 μ , 34; 16 μ , 49; 17 μ , 13; 18 μ , 1. Averages of 20.3 μ and 15.7 μ were computed for diameter of oogonium and of oospore, respectively. Measurements for thickness of oospore wall varied from 0.8 to 1.6 μ , and yielded an average of 1.2 μ .

Twenty-five oogonia from a maize-meal-agar plate culture of isolate no. 126 obtained from an affected sugarcane root gave measurements for diameter varying from 20.4 to 25.1 μ . The

25 oospores contained in them — all of correct unitary internal structure — varied in diameter from 14.3 to 19.4 μ , and were surrounded by a wall 1.0 to 1.3 μ thick. From the three sets of measurements averages of 22.3 μ , 17.2 μ and 1.1 μ were computed for oogonial diameter, oospore diameter, and thickness of oospore wall, respectively.

When slabs excised from a young maize-meal-agar plate culture of the Indonesian *Pythium* isolate no. 2 were shallowly irrigated with distilled water, lobulate sporangial complexes (Pl. VIII, A—C) generally resembling those of the Nicaraguan fungus were produced. On unwet maize-meal-agar the fungus formed sexual reproductive apparatus rather abundantly, often, especially in the beginning, giving rise to monoclinal units in which the oogonium was borne terminally on a branch given off by a hyphal element bearing the antheridium subterminally (Pl. IX, A, a) or terminally (Pl. VIII, J, K; Pl. IX, B, a; C, a; D). The antheridial hyphae of such monoclinal units in many instances later furnished all the protoplasmic materials needed for the development of additional reproductive units nearby. Sometimes the oogonial branch of a first unit supplied both the male and the female component of a second unit (Pl. IX, A, b); sometimes the antheridial hypha of a first unit supplied a short-stalked oogonium while the oogonial branch supplied a subterminal antheridium to complete a second unit (Pl. IX, B, b); and sometimes, again, the antheridial hypha of a first unit extended a ramified branch bearing two terminal oogonia while the oogonial branch of that unit contributed a subterminal and an intercalary antheridium to complete a second (Pl. IX, C, b) and a third unit (Pl. IX, C, c), respectively. One hundred mature sexual reproductive units selected at random on the under side of a maize-meal-agar plate culture 20 days old gave measurements for oogonial diameter, expressed in the nearest integral number of microns, distributable as follows: 19 μ , 1; 20 μ , 10; 21 μ , 25; 22 μ , 25; 23 μ , 33; 24 μ , 6. The 100 oospores in these units gave measurements for diameter that were distributable thus; 15 μ , 7; 16 μ , 25; 17 μ , 47; 18 μ , 20; 19 μ , 1. The oospore wall varied from 1.0 to 1.5 μ in thickness. Averages of 21.9 μ , 16.7 μ and 1.2 μ were computed for oogonial diameter, oospore diameter, and thickness of oospore wall, respectively. Measurements of 25 reproductive units on the upper side of the same maize-meal-agar plate culture showed variations in oogonial diameter from 18.2 to 25.7 μ , in oospore diameter from 15.1 to 19.9 μ , and in thickness of oospore wall from 0.8 to 1.4 μ ; and gave averages of 22.4 μ , 17.3 μ and 1.0 μ , respectively, for the three dimensions.

The Indonesian *Pythium* isolate no. 3 gave rise on shallowly irrigated slabs of Lima-bean agar to swollen, branched, moderately lobulate complexes (Pl. VIII, D, E) generally similar to those formed

by the Nicaraguan fungus under like conditions. These complexes became converted into sporangial units (Pl. VIII, F, G) that ultimately produced swarmers which after a period of motility rounded up to form cystospores (Pl. VIII, H, a—z) mostly 7.5 to 10 μ in diameter. In germinating the cystospores emitted germ tubes 2 to 3 μ wide (Pl. VIII, I, a, b).

In maize-meal-agar plate cultures the Indonesian isolate no. 3 often initiated sexual reproduction by giving rise here and there to monoclinal units in which the oogonium was borne terminally on a branch given off by a hypha bearing the antheridium subterminally (Pl. VIII, L; Pl. IX, E, a) or terminally (Pl. VIII, M, a; Pl. IX, F, a; G; H, a; I; J, a). After completion of a first reproductive unit the antheridial hypha in many instances continued to bring protoplasmic materials, thereby permitting development of one or more additional units nearby. The antheridial hypha of the first unit in some instances supplied a stalked (Pl. VIII, M, b; Pl. IX, E, b; H, b) or a virtually sessile oogonium (Pl. IX, F, b; J, b) for a second unit, while the primary oogonial branch contributed the attendant antheridium in an intercalary (Pl. VIII, M, b; Pl. IX, H, b) or a subterminal position (Pl. IX, E, b; F, b; J, b). Sometimes both the stalked oogonium and the attendant intercalary antheridium of a third reproductive unit were supplied by the oogonial branch (Pl. IX, H, c) or the antheridial hypha (Pl. IX, J, c) of the first unit. One hundred oogonia selected at random on the lower side of a maize-meal-agar plate culture fully 20 days old gave measurements for diameter, expressed in the nearest integral number of microns, with a frequency distribution as follows: 17 μ , 1; 19 μ , 4; 20 μ , 12; 21 μ , 29; 22 μ , 34; 23 μ , 17; 24 μ , 3; and the mature oospores they contained, all with the unitary internal structure characteristic of the resting stage (Pl. VIII, L, M; Pl. IX, E—J), gave measurements distributable thus: 14 μ , 2; 15 μ , 13; 16 μ , 25; 17 μ , 42; 18 μ , 18. The oospore wall varied from 1.0 to 1.5 μ in thickness. Averages of 21.4 μ , 16.9 μ and 1.2 μ were computed for oogonial diameter, oospore diameter and thickness of oospore wall, respectively.

When small portions of maize-meal-agar well permeated with young mycelium of *Pythium deliense* (ATCC 12280) were thrust into small incisions made with a sterilized scalpel in fruits of summer squash, softening and water-soaking of neighboring tissue proceeded more slowly than after similar inoculation with the Nicaraguan fungus. Aerial mycelium developed more tardily, usually not becoming visible even as an arachnoid layer until the fourth day of incubation. After 5½ days of incubation in snugly covered glass dishes and at room temperatures near 24° C. the fungus had advanced lengthwise through the squash fruit a distance of approximately 40 mm in either direction. All of the invaded area except a marginal

border about 10 mm wide had then become clothed in a white cottony mass of mycelium.

On maize-meal agar and Lima-bean agar likewise *Pythium deliense* produced aerial mycelium less abundantly than the Nicaraguan fungus. Microscopical examination of a maize-meal-agar plate culture of *P. deliense* 6 days old revealed some scanty development of appressoria in contact with the glass floor of the Petri dish. The adhering organs were of the clavate type (Pl. X, A, a, b; B, a, b; C, a—c; D, a—e) familiar in many other members of the genus and measured usually 8 to 12 μ in width at the expanded tip.

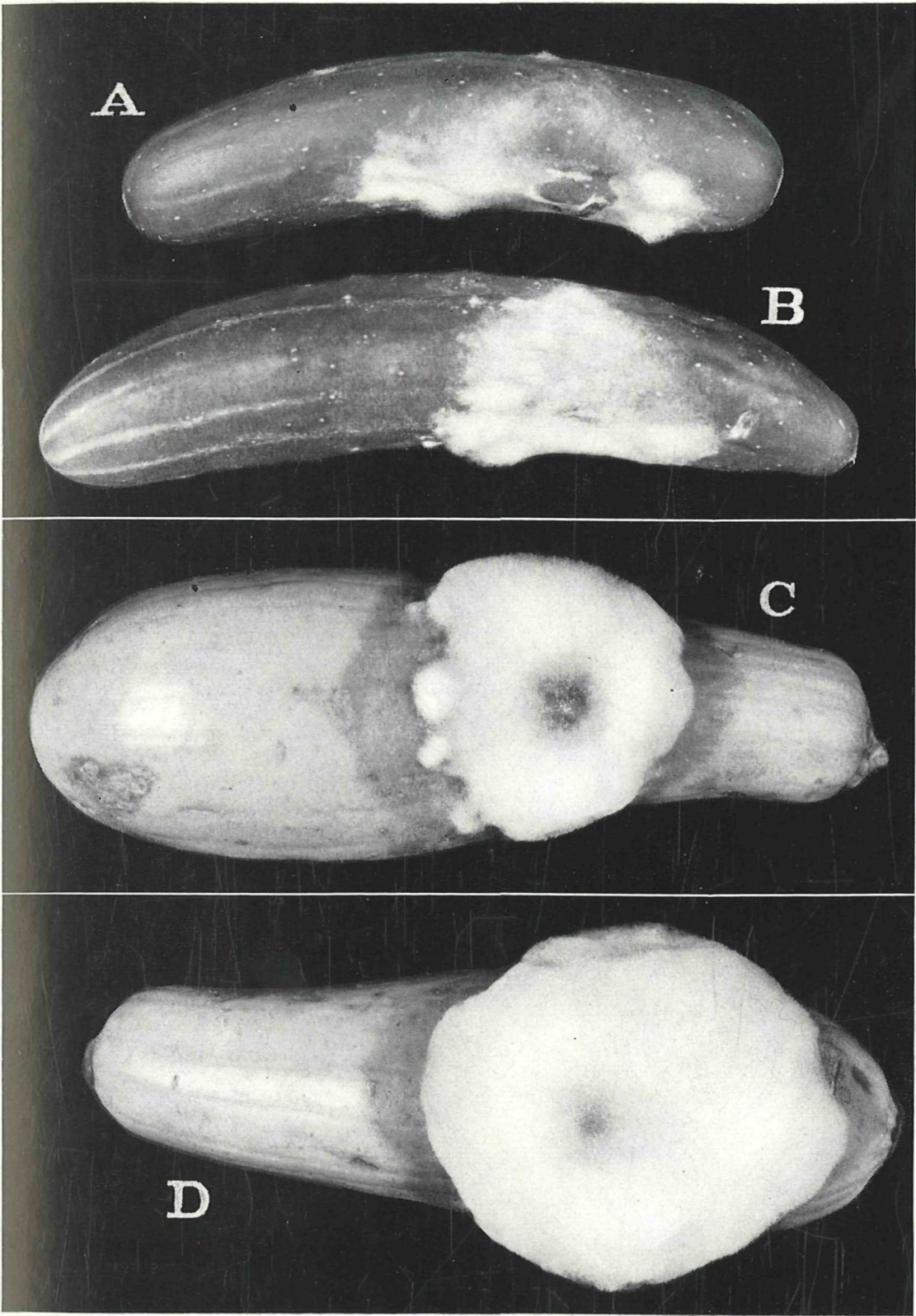
When slabs excised from a young maize-meal-agar plate culture of *Pythium deliense* were shallowly irrigated with distilled water, swollen cells (Pl. XI, A—L; M, a, b; N, a, b), often irregularly branched or lobulated, were formed in some quantity. These cells bore considerable resemblance to the sporangia of the Nicaraguan fungus, and, indeed, appeared interpretable as sporangia, though owing possibly to troublesome vibration resulting from heavy traffic on the busy street nearby they failed to produce zoospores.

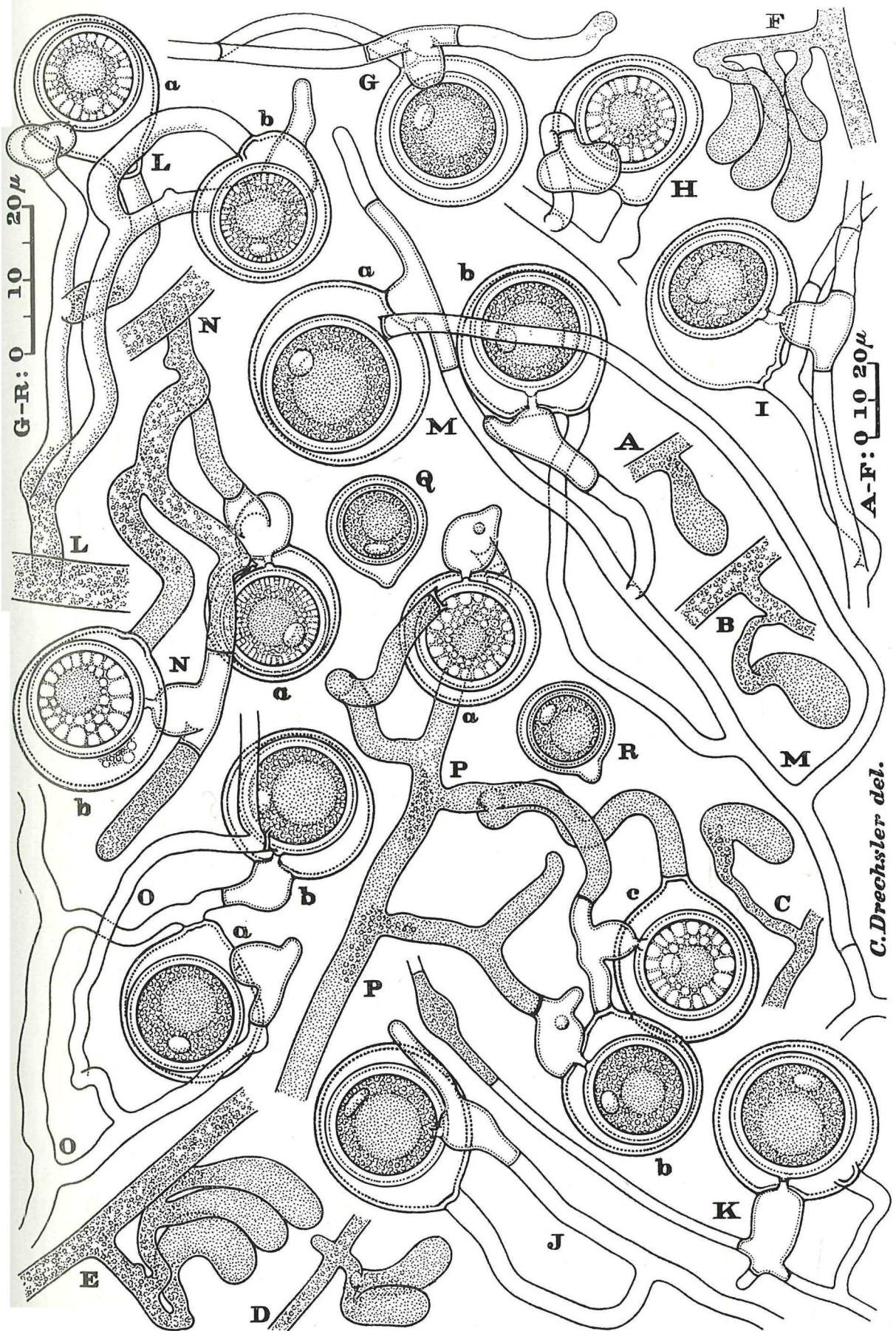
No unfavorable circumstance interfered noticeably with normal sexual reproduction of *Pythium deliense* in Petri dishes containing shallowly irrigated pieces of infected squash tissue (Pl. XI, O; Pl. XII, A—E) or shallowly irrigated slabs of Lima-bean agar well permeated with young mycelium (Pl. XIII, A—H). Obvious parallelism with the Nicaraguan fungus was shown here by many monoclinal reproductive units in which the the oogonium was borne terminally on a branch given off by a hypha bearing the attendant antheridium intercalarily (Pl. XI, O; Pl. XII, A—C; Pl. XIII, A—D), subterminally (Pl. XII, E; Pl. XIII, E, F), or terminally (Pl. XII, D). Even in the diclinous units (Pl. XIII, G, H) some intimate correspondence with the Nicaraguan fungus was recognizable in the frequently rather unusual orientation of the oogonium and its supporting hyphal branch — the feature regarded as especially noteworthy in related forms by several authors. While the sexual reproductive apparatus (Pl. XIV, A—M) produced on unwet maize-meal agar include a large proportion of diclinous units (Pl. XIV, A—C, E, F, I—M) the several Petri-plate cultures of *P. deliense* I prepared with this substratum formed both on the upper (Pl. XIV, D, a) and on the lower side (Pl. XIV, H) some monoclinal units in which the oogonium was borne terminally on a branch given off by the hypha bearing the antheridium. In very few instances the antheridial branch of such a monoclinal unit gave rise to a stalked oogonium while the primary oogonial branch supplied a subterminal antheridium to complete a second reproductive unit (Pl. XIV, D, b) after the manner conspicuously prevalent in the Nicaraguan fungus. Although now and then (Pl. XIV, L) the antheridium of *P. deliense*

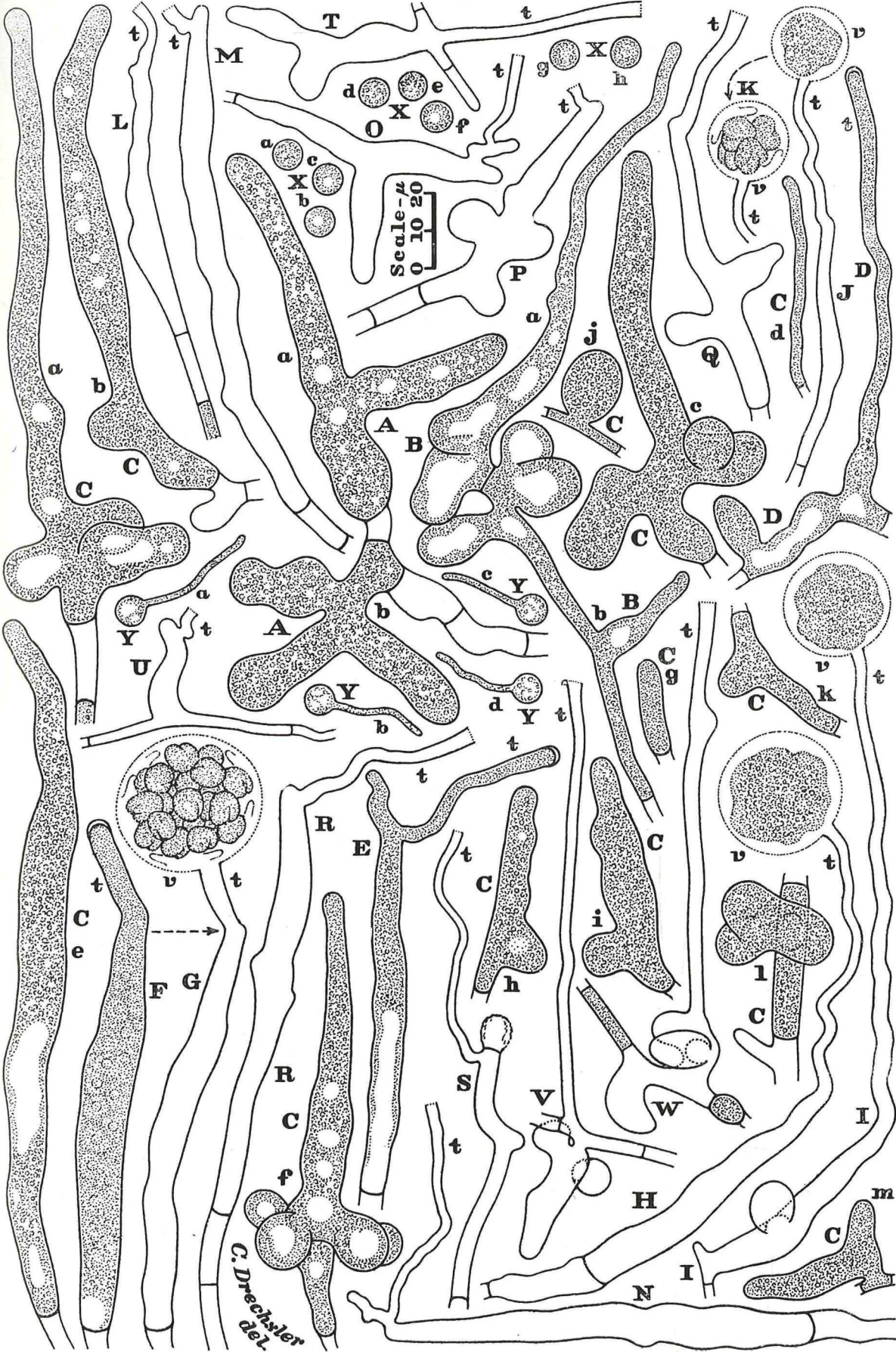
was found to consist wholly of a hyphal segment, it more usually was composed, like the homologous organ in closely related species, of a filamentous portion together with an orbicular or dolioform lateral protuberance apically applied to the oogonium. Since in *P. deliense* the protuberance measured commonly 3 to 18 μ in length and 7 to 13 μ in greatest width, while in the Nicaraguan fungus it varies from 3 to 10 μ in length and from 4.5 to 8 μ in greatest width, the antheridium of the former species appeared fully twice — or perhaps about $2\frac{1}{2}$ times — as large as that of the latter. In its considerably larger antheridia as well as in its somewhat larger oogonia and oospores *P. deliense* shows better agreement than the Nicaraguan fungus with the original description of *P. aphanidermatum*. Indeed, until the fungus represented in Meurs' isolate is known not to occur in the United States either outdoors or in greenhouses it deserves consideration in questions relating to the application of the specific name invented by Edson.

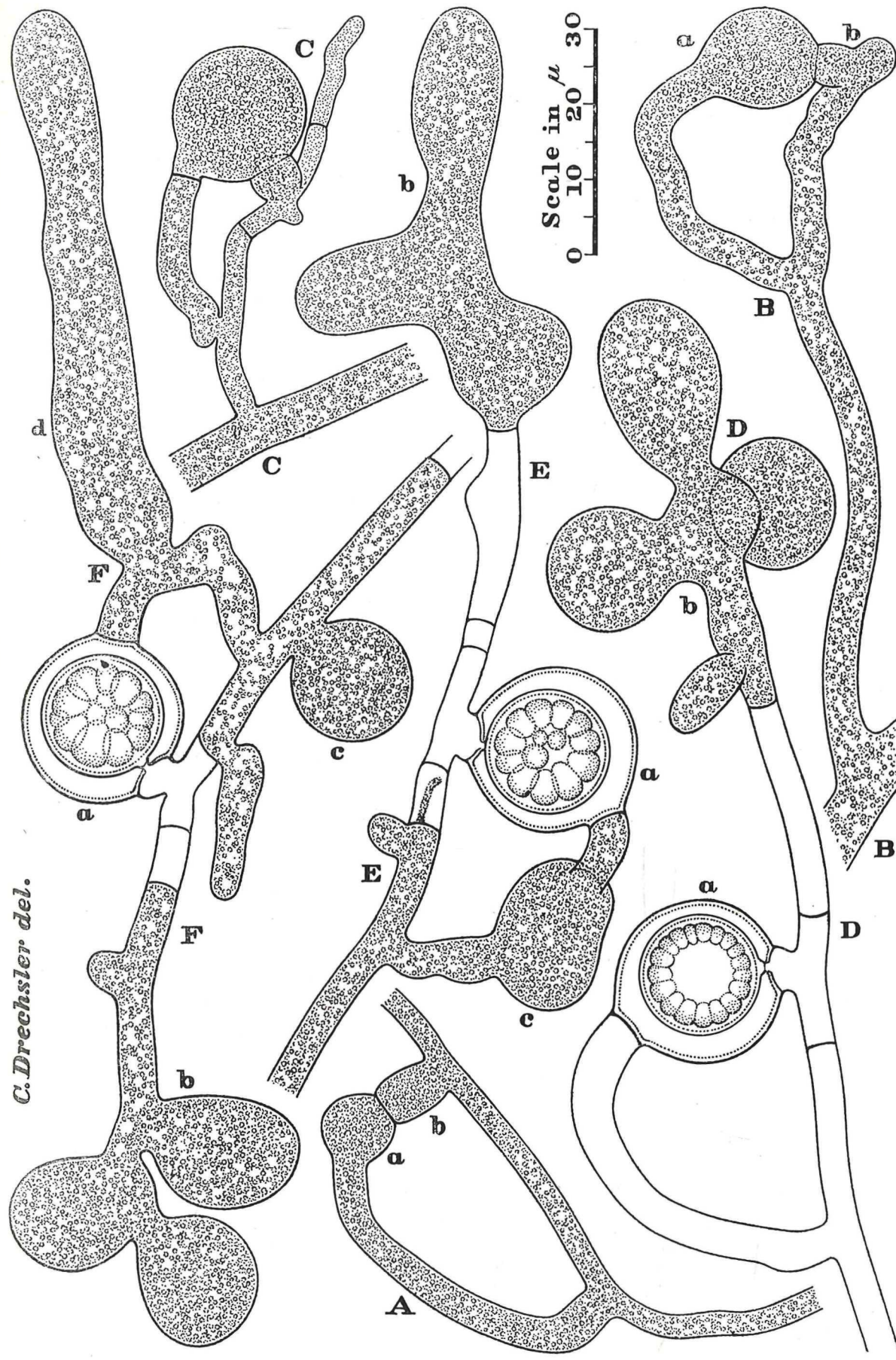
In the hyphal relations of its monoclinal sexual reproductive units the Nicaraguan fungus differs markedly from Butler's figures of *Pythium indigoferae*, though agreeing with Sideris' figures of the isolate from cucumber roots which was referred to that species. While the possibility can not be excluded that the opposite types of hyphal relationship displayed in *P. debaryanum* and *P. deliense*, respectively, may occur together in *P. indigoferae* much as in *P. butleri*, there would seem to be at least an equal likelihood that the cucumber-root isolate is alien to *P. indigoferae* and conspecific with the Nicaraguan fungus. None of the antheridia shown in Butler's figures are intercalary, and according to Balakrishnan intercalary antheridia like those found in *P. indicum* — and assuredly found also in the Nicaraguan fungus — are not seen in *P. indigoferae*. Butler characterized germination of the oospores in *P. indigoferae* as "rapid, by a hypha, not by zoospores", whereas under suitable though by no means unusual or exacting conditions the oospores of the Nicaraguan fungus germinate readily by producing swarmers.

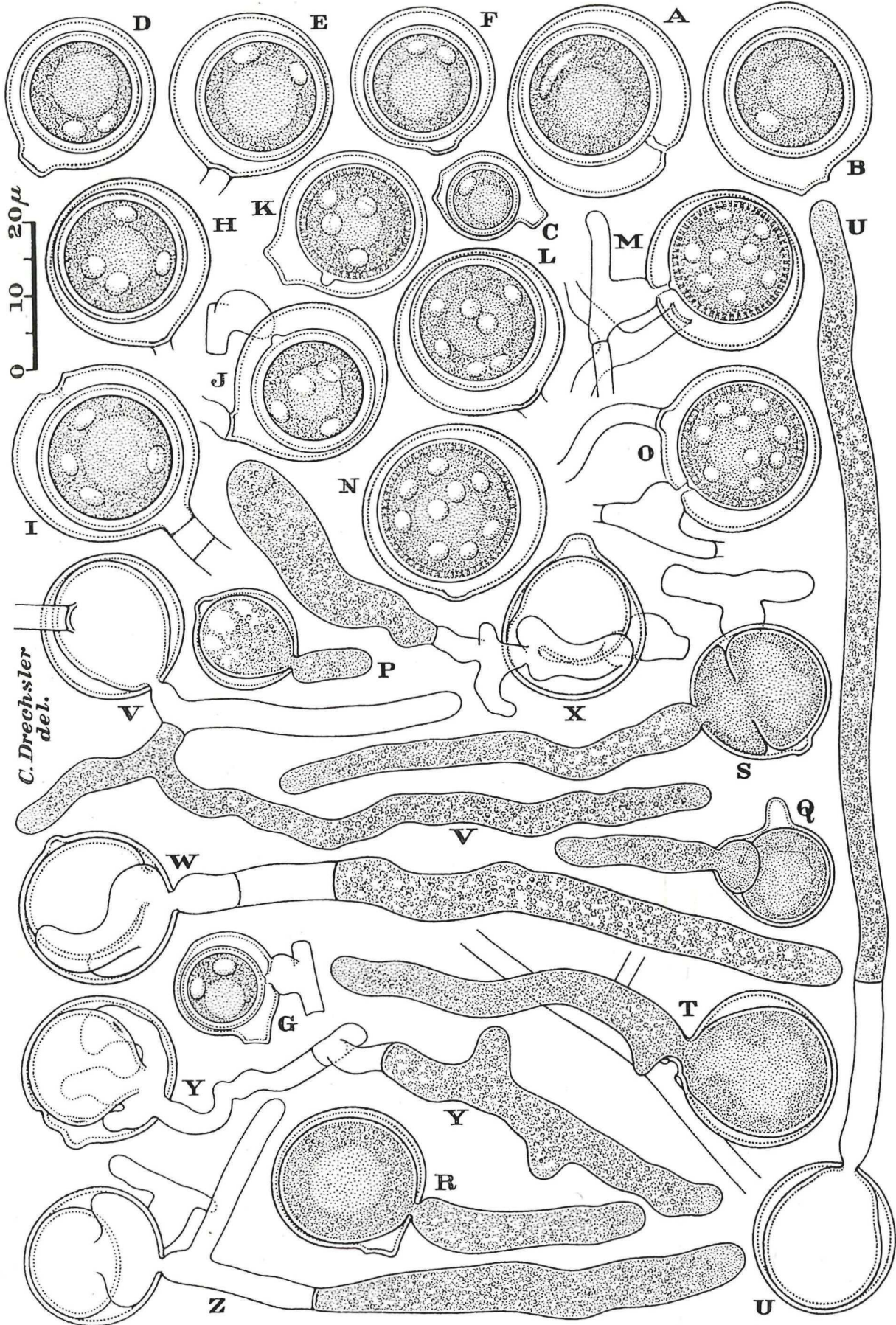
Difference in the same phase of development might be held likewise to distinguish the Nicaraguan fungus from *Pythium indicum* as only germination by a germ tube was reported for the oospores of that species. Differences from *P. indicum* have been noted previously in the less prolific sporangia, the smaller cystospores and the narrower appressoria of the Nicaraguan fungus. Somewhat slower mycelial growth may provide a supporting difference. In 24 hours at 24° C. the Nicaraguan fungus was found to extend its mycelium radially about 5 mm less than *P. butleri*, the familiar cottony-rot parasite, whereas *P. indicum* was set forth as growing faster at 26–27° C. than *P. aphanidermatum*, representing presumably the same parasite. Further, while the oogonia of the Nicaraguan fungus

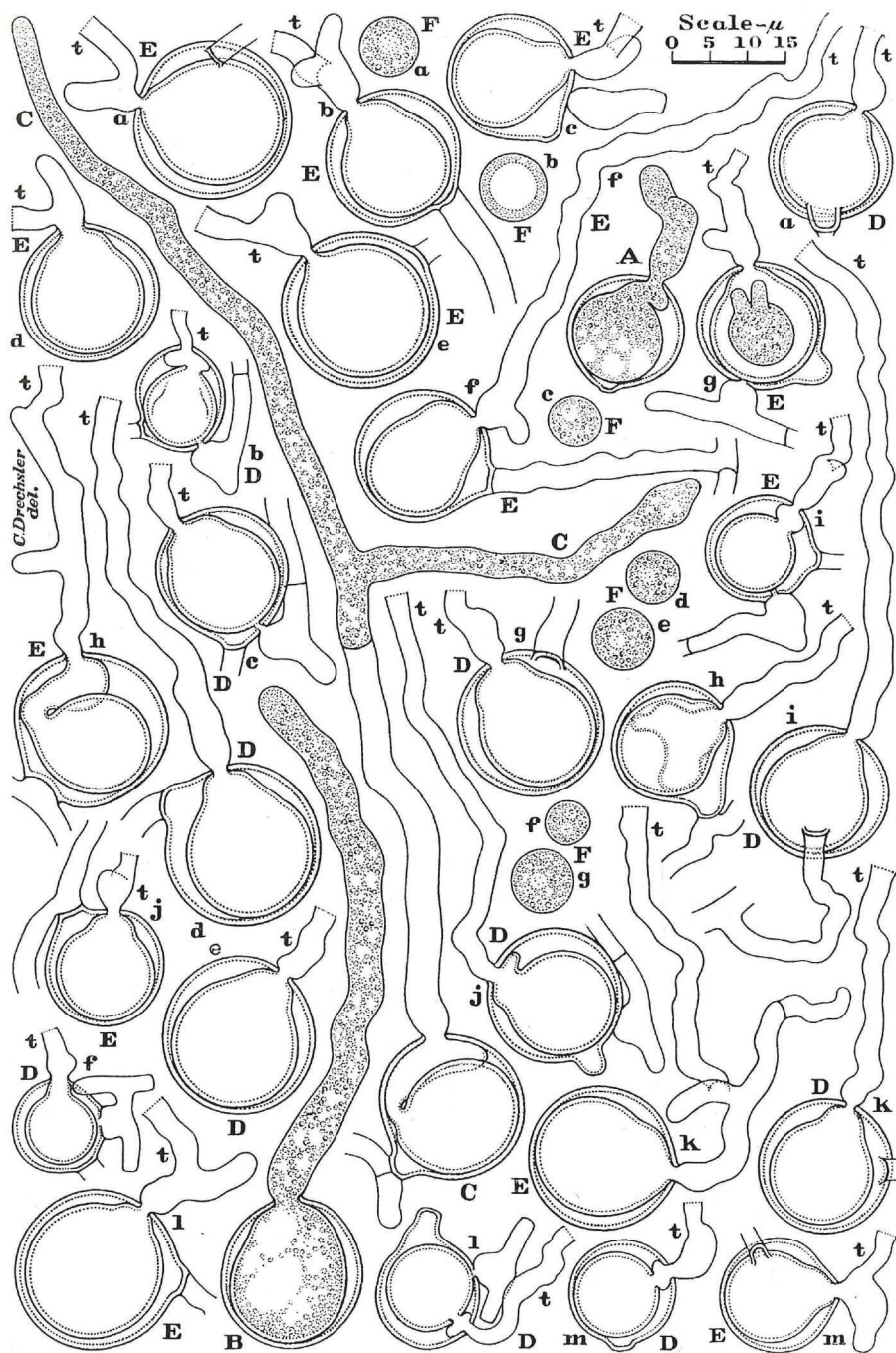


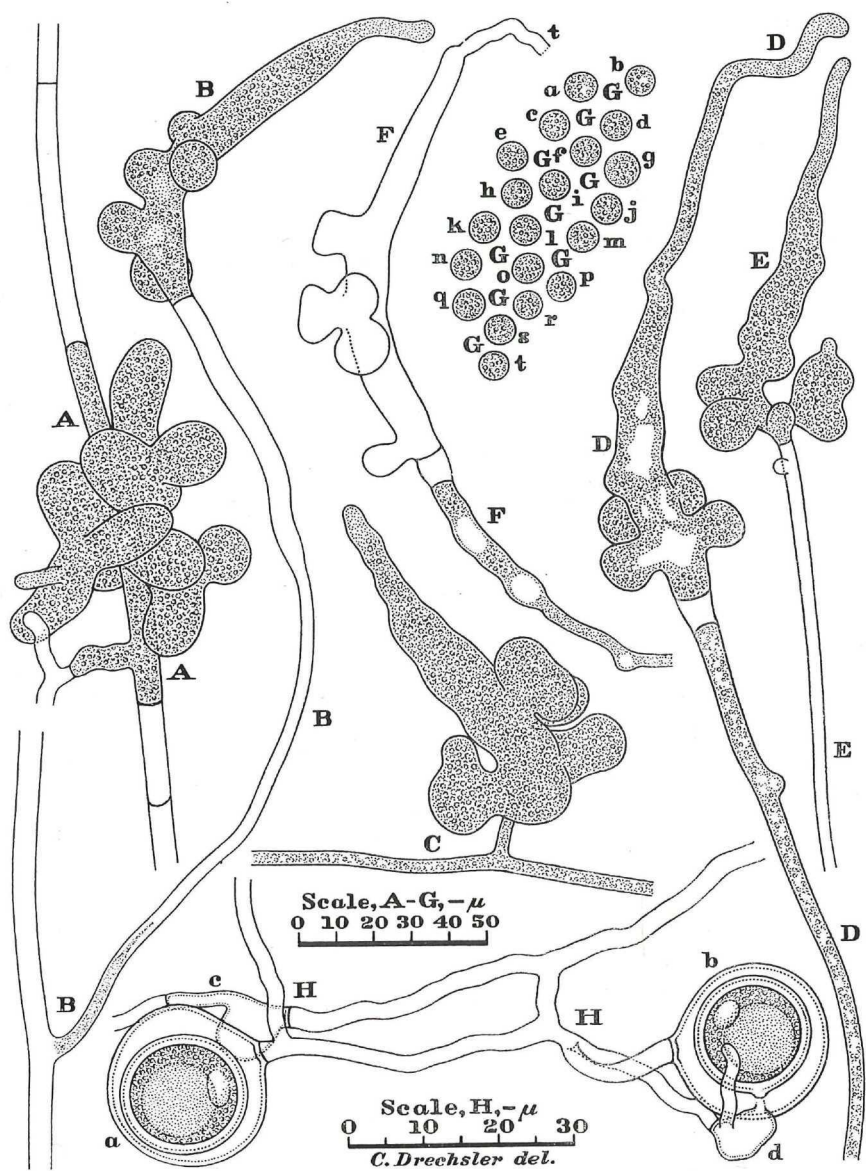


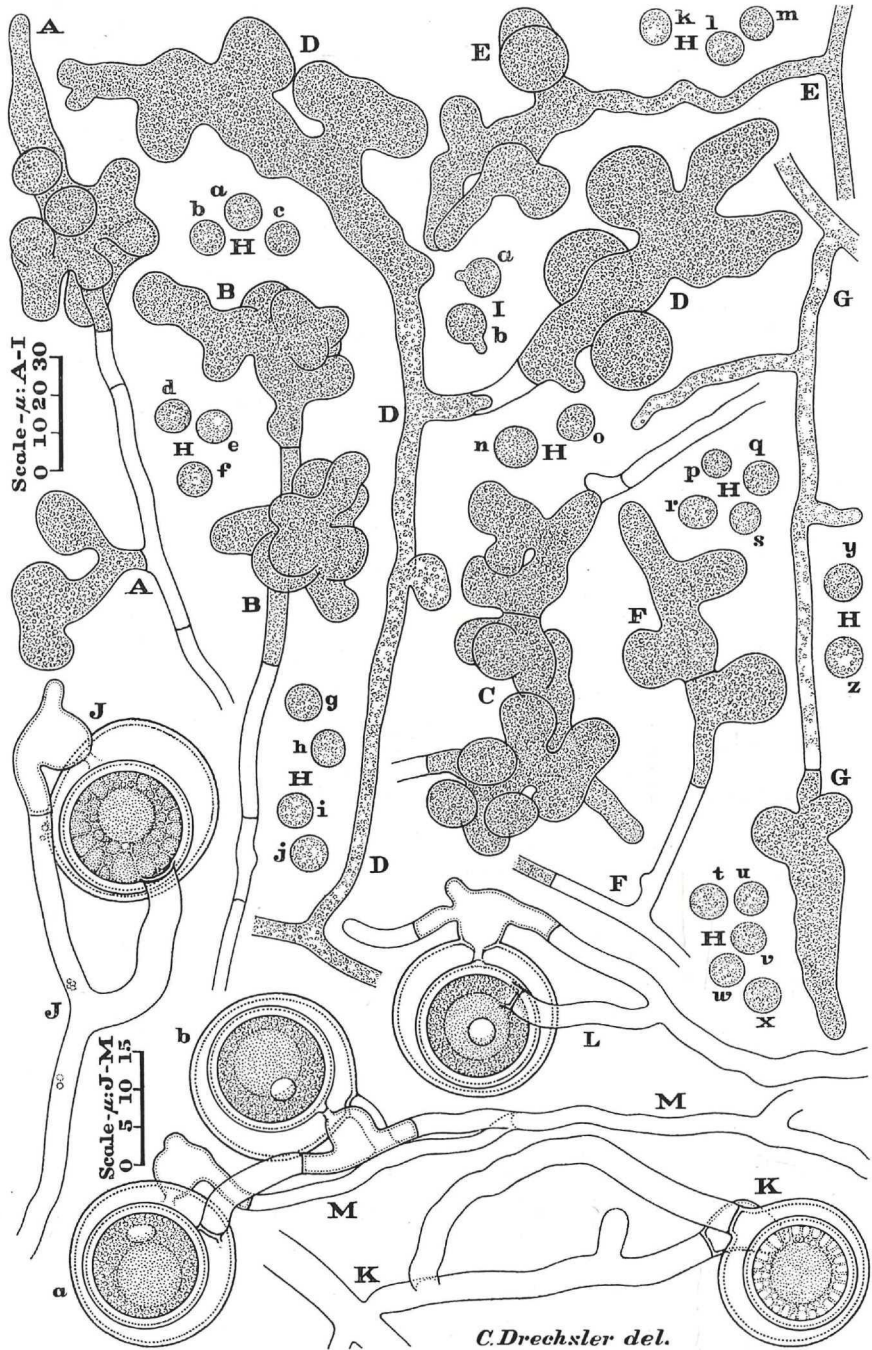


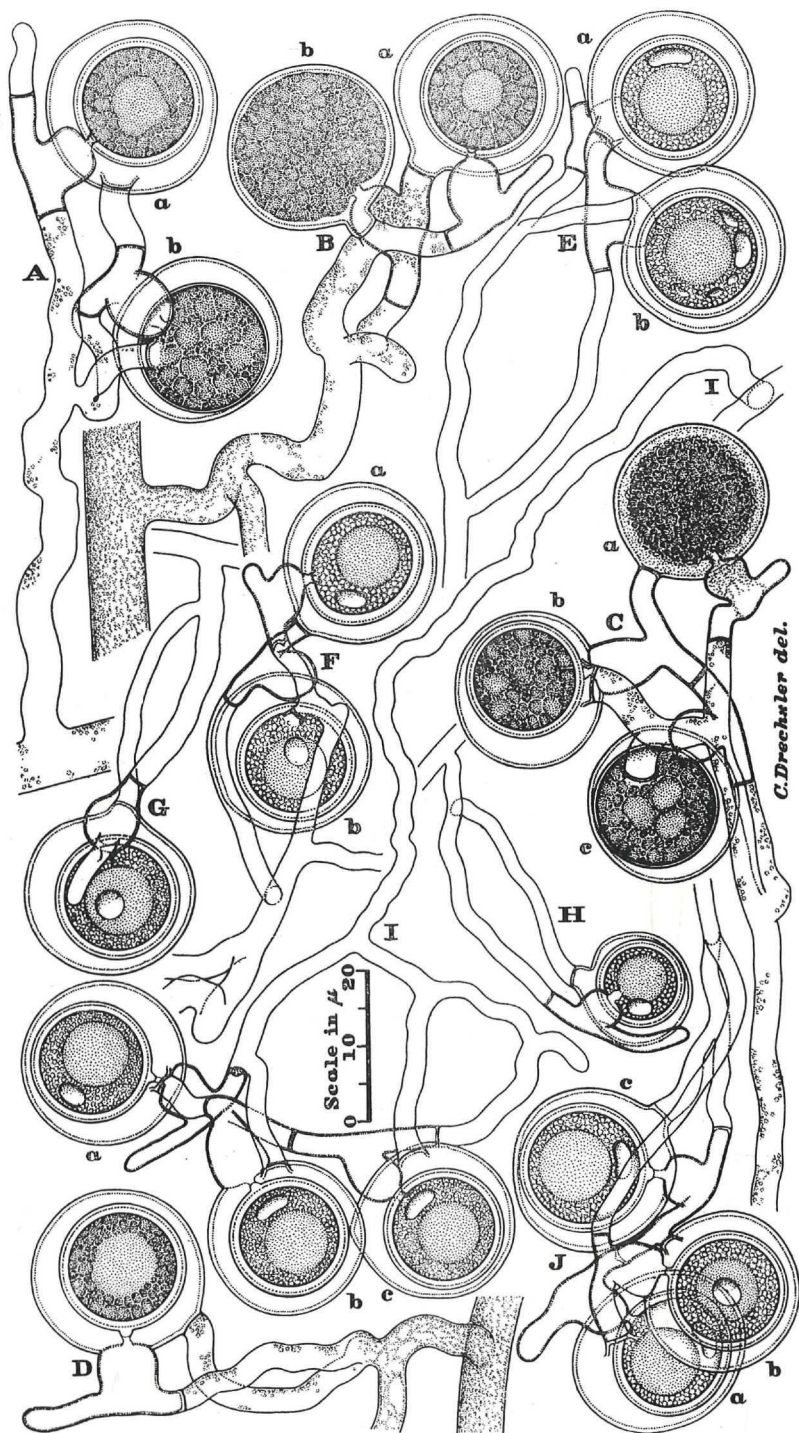


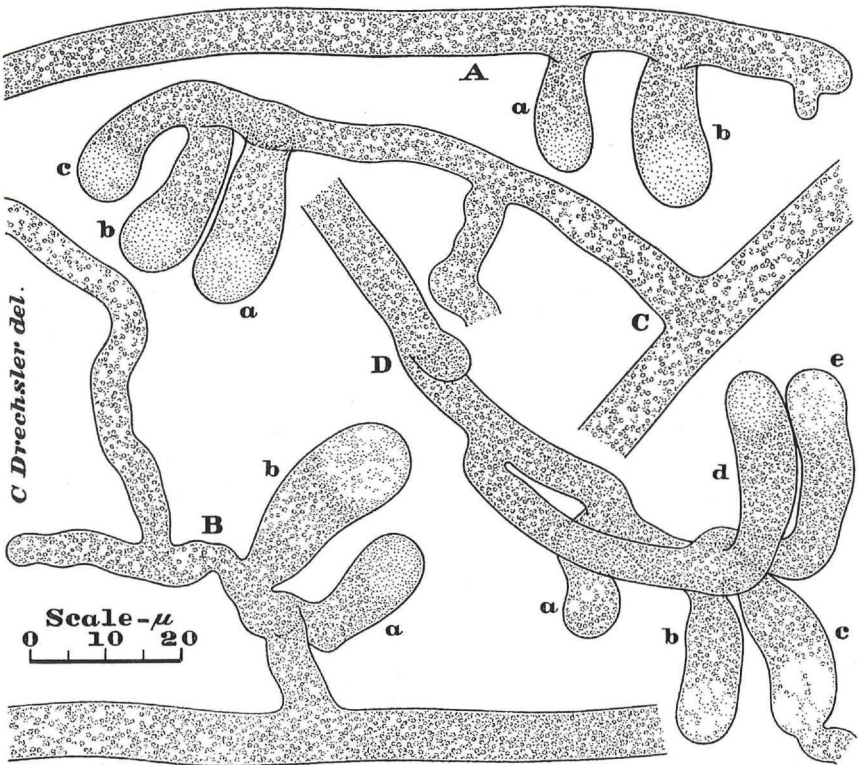


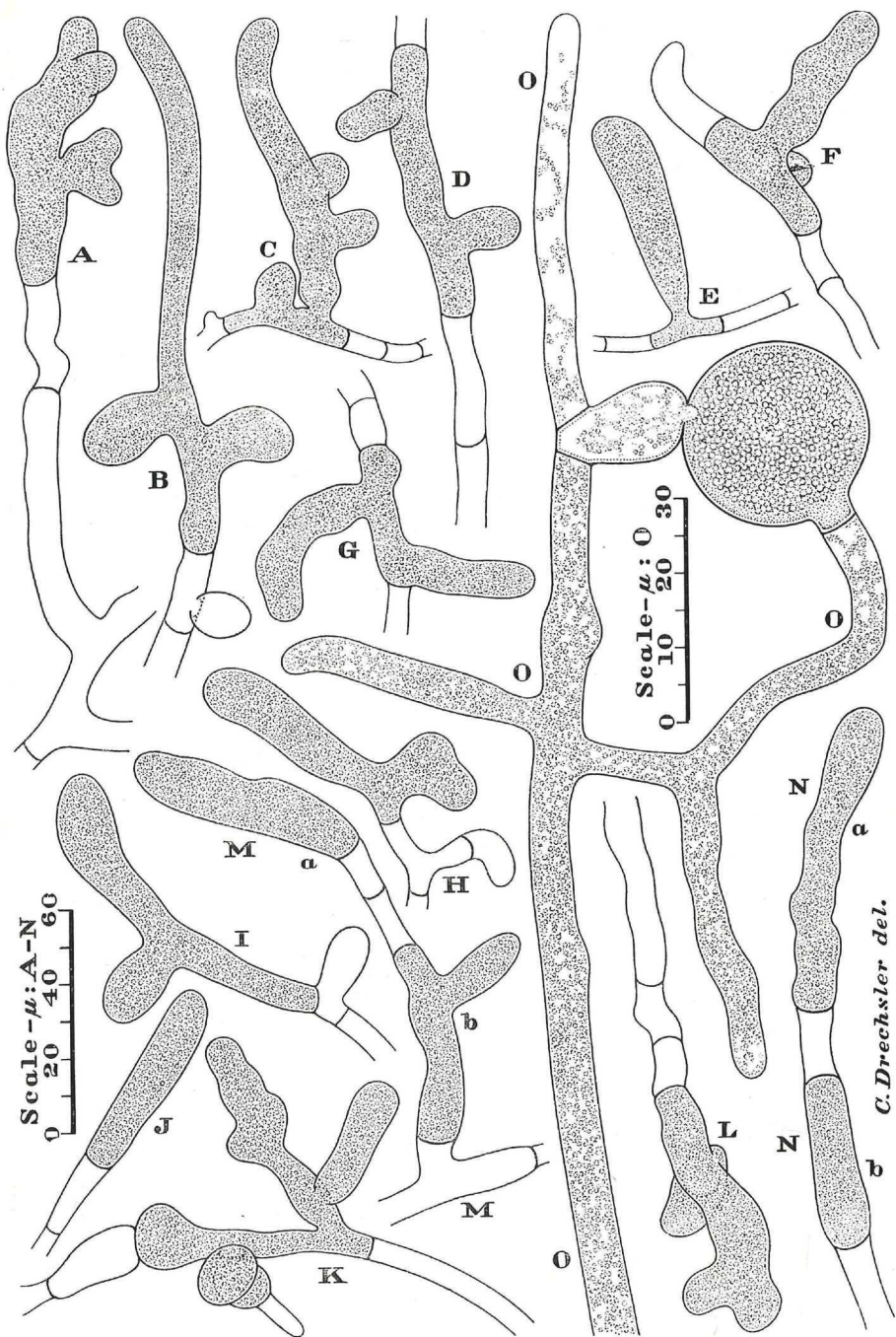


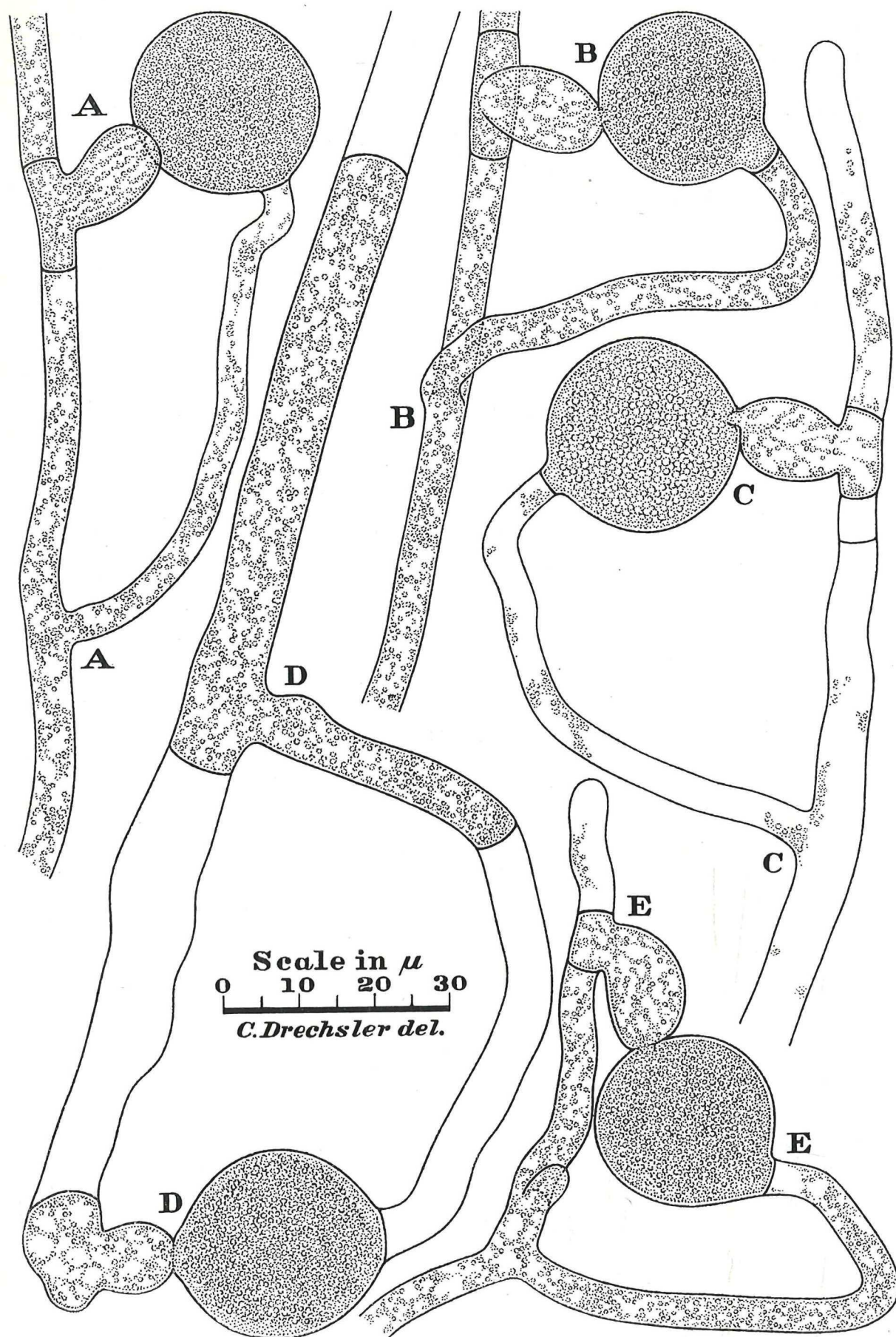


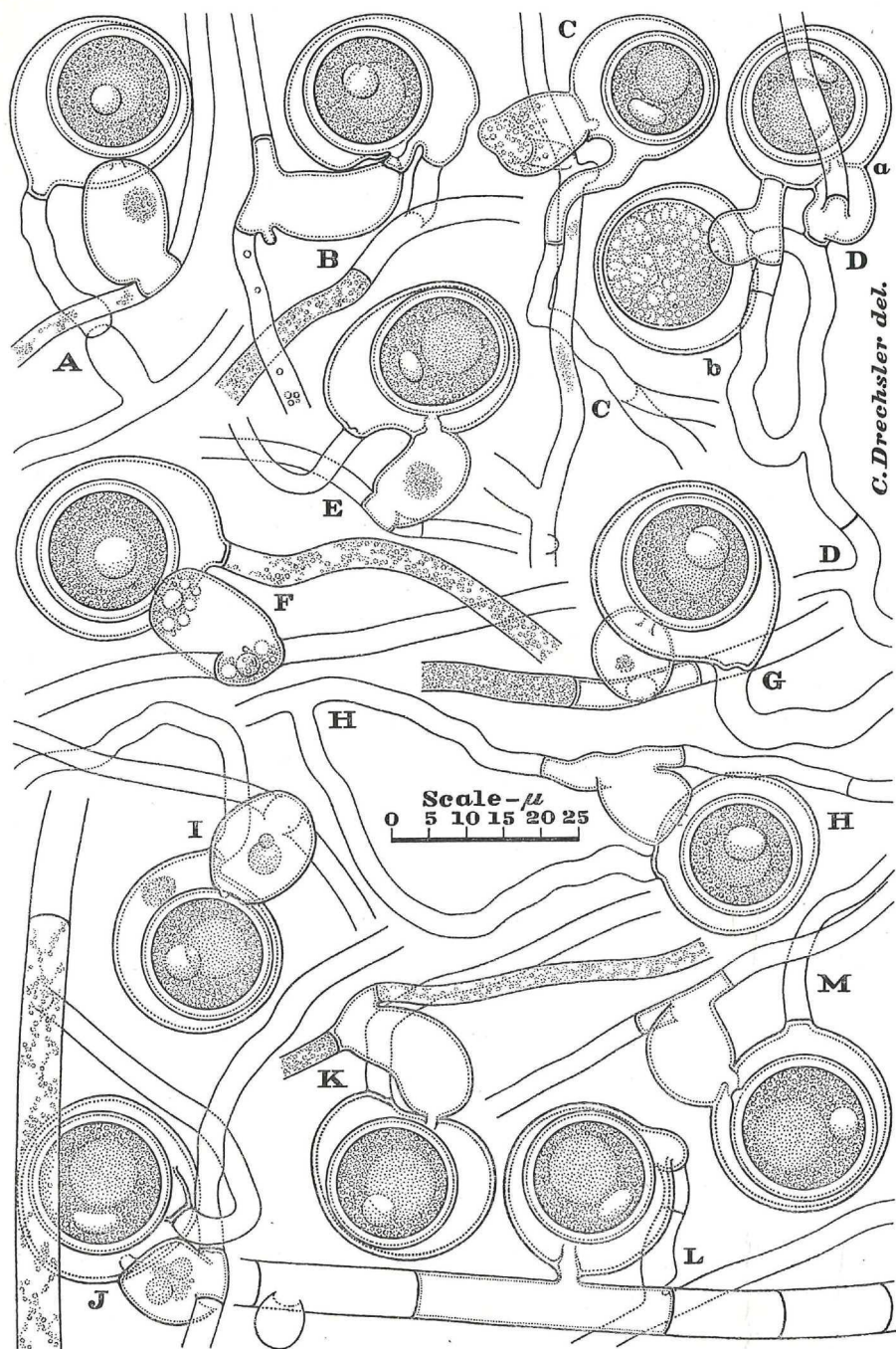


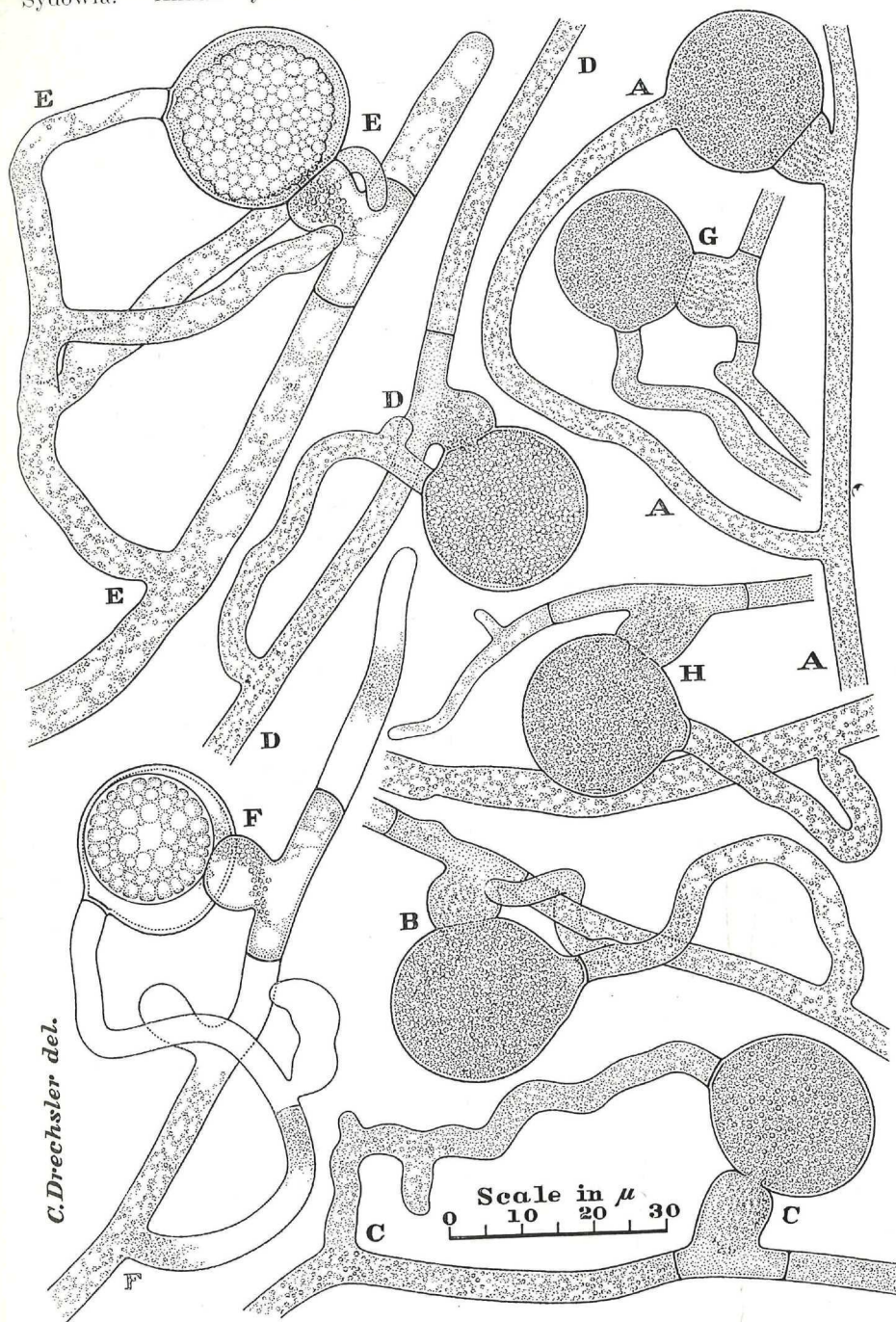












appear always to be formed terminally, those of *P. indicum* are stated to be frequently intercalary. On the other hand the Nicaraguan fungus agrees rather closely with *P. indicum* in the dimensions of its oogonia and oospores as well as in the shape and relative size of its generally single antheridia. Especially noteworthy is the striking resemblance it bears to *P. indicum* in the development of several sexual reproductive units in clustered arrangement on a branched hyphal element.

Owing to the uncertainty attending any evaluation of the various similarities and differences when cultures of the several intimately related species are not available for comparison, the Nicaraguan fungus is here only provisionally designated as *Pythium* sp. (cf. *P. indicum* Balak.). Dried material of it has been deposited under the number 71633 in the National Fungus Collection, Plant Industry Station, Beltsville, Maryland; and a living culture of it has been placed under the number 13763 in the American Type Culture Collection, Washington, D. C.

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Explanation of Plates I—XV.

Plate I. A, B, Cucumber fruits which after being inoculated with the Nicaraguan *Pythium* were kept covered in a glass dish for 68 hours at temperatures near 24° C.; $\times \frac{1}{2}$. C, D, Summer-squash fruits which after being inoculated with the Nicaraguan *Pythium* were kept covered in a glass dish for 44 hours at temperatures near 24°C.; $\times \frac{2}{3}$.

Plate II. Appressoria and sexual reproductive apparatus produced in maize-meal-agar plate cultures by Nicaraguan *Pythium*. A—F, Appressoria formed singly or in groups on floor of Petri dish; $\times 500$. G—K, Portions of hyphae on each of which is borne a monoclinal sexual reproductive unit; $\times 1000$. L—O, Portions of hyphae bearing 2 monoclinal sexual reproductive units, a and b; $\times 1000$. P, Portion of hypha branched distally and bearing 3 interconnected sexual reproductive units, a—c; $\times 1000$. Q, R, Relatively small oogonia, each containing a fully mature oospore; $\times 1000$.

Plate III. Asexual reproductive apparatus of Nicaraguan *Pythium* obtained by irrigating with distilled water some slabs of maize-meal agar well permeated by the fungus; $\times 500$. A, B, Portions of hyphae, each having 2 swollen cells, a and b, capable of developing into sporangia. C, Hyphal segments, a—m, variously distended or lobulated, each capable of developing into a sporangium. D—F, Sporangia with hyaline cap on tip of evacuation tube, t, indicating readiness to discharge contents, G. Same sporangium as in F, but drawn 20 minutes later; showing the empty envelope of sporangium and evacuation tube, t, surmounted by a vesicle, v, containing 18 zoospores in active motion. H—J, Newly emptied sporangia, each with its evacuation tube, t, surmounted by a vesicle, v, containing undifferentiated protoplasm. K, Distal portion of same sporangium as in J, but drawn 15 minutes later; showing evacuation tube, t, with vesicle, v, containing 4 active zoospores ready for liberation. L—S, Empty envelopes of terminal sporangia, each showing its evacuation tube, t, open at the tip. T—W, Empty intercalary sporangial envelopes, each with open evacuation tube, t. X, Encysted zoospores, a—h. Y, Germinating zoospores, a—d.

Plate IV. Reproductive apparatus of Nicaraguan *Pythium*: parts A and C produced in maize-meal-agar plate culture 4 days old; parts B, D, E, and F produced on pieces of infected squash fruit shallowly irrigated with distilled water; $\times 1000$. A, Very young intercalary sexual reproduc-

tive unit showing broad contact between oogonium, a, and antheridium, b. B, Young terminal sexual reproductive unit in which neither the oogonium, a, nor the antheridium, b, has yet been delimited by a septum. C, Subterminal sexual reproductive unit in which both oogonium and antheridium have been delimited. D—F, Distal portions of hyphae, in each of which a sexual reproductive unit, a, has been formed a short distance (7—30 μ) below a terminal lobulated asexual unit, b; in E, besides, a lobule, c, has developed intercalarily on the oogonial branch; and in F two sporangial elements, c and d, have been produced, respectively, near and from the oogonial branch.

Plate V. Oospores, A—Z, of Nicaraguan *Pythium* taken from two maize-meal agar cultures 134 days (A—P, R, T—V) and 174 days (Q, S, W—Z) old, respectively; they were then drawn either immediately (D—F) or after shallow irrigation for 1 day (Q, S, W—Z), for 3 days (H, J, L, R), for 6 days (P, T), for 8 days (M, O), for 10 days (A—C, G) or for 16 days (I, K, N, U, V); $\times 1000$. A—C, Oospores still in a resting state despite prolonged aging and prolonged irrigation. D—F, Oospores, which though not irrigated, show incipient after-ripening in having 2 refringent bodies. G, Oospore showing only 2 refringent bodies after rather prolonged irrigation. H—J, Oospores showing more advanced after-ripening in having 4 refringent bodies. K, Oospore showing 4 refringent bodies and striated or darkened wall. L, Oospore with refringent bodies but with wall not markedly darkened or striated. M—O, Oospores with 9 or 10 refringent bodies and markedly darkened wall. P—T, Oospores that are each extending a germ hypha externally. U—W, Oospores that have each yielded up its contents to produce a vegetative germ hypha. X—Z, Oospores that have each contributed its contents to produce a young sporangium.

Plate VI. Oospores of the Nicaraguan *Pythium* that are germinating or have germinated after they were shallowly irrigated with distilled water following their removal from a maize-meal-agar plate culture 134 days old; $\times 1000$. A, B, Oospores that are each extending a germ hypha after being irrigated for 3 days. C, Empty oospore which yielded up its contents to form a branched germ hypha. D, Empty envelopes, a—m, of oospores that germinated by developing into sporangia; each envelope provided with an empty unbranched evacuation tube, t, open at the tip; drawn after irrigation for 6 (i, k), 8 (a, b, j), 9 (c, h), 10 (f), 13 (d, e, l, m) or 16 (g) days. E, Empty envelopes, a—m, of oospores that germinated by developing into sporangia; each envelope provided with an empty branched evacuation tube, t, open at the tip; drawn after irrigation for 3 (d), 6 (b, i), 8 (e, m), 9 (g, h), 10 (f, j, k, l), 13 (a) or 16 (c) days. F, Encysted zoospores, a—g, resulting from germination of oospores.

Plate VII. Reproductive apparatus of two conspecific *Pythium* isolates from diseased roots of Louisiana sugarcane; parts A—F being from isolate no. 66, and parts G and H from isolate no. 96; magnification in parts A—F is $\times 500$, but in parts G and H is $\times 1000$. A—E, Young lobulate sporangia obtained by irrigating with distilled water some slabs of maize-meal agar well permeated with mycelium of the Louisiana fungus. F, Empty sporangium with evacuation tube, t, open at the tip; from same irrigated material as parts A—E. G, Encysted zoospores obtained by irrigating with distilled water some slabs excised from a young maize-meal-agar plate culture. H, Two interconnected monoclinous sexual reproductive units found in a maize-meal-agar plate culture 19 days old; the 2 oogonia, a and b, are borne terminally on branches given off by the hyphae bearing the antheridia, c and d.

Plate VIII. Reproductive apparatus of two conspecific *Pythium* isolates (nos. 2, 3) originating from diseased stems of young tobacco plants in Sumatra: parts A—C produced on slabs which after being excised from a young maize-meal-agar plate culture of isolate no. 2 was shallowly irrigated with distilled water, $\times 500$; parts D—I produced on similarly irrigated slabs of Lima-bean-agar well permeated with mycelium of isolate no. 3, $\times 500$; parts J and K produced in maize-meal-agar plate culture of isolate no. 2, $\times 1000$; parts L and M produced in maize-meal-agar plate culture of isolate no. 3, $\times 1000$. A—G, Portions of hyphae with variously lobulated sporangia. H, Encysted zoospores, a—z. I, Zoospores, a and b, in early stage of germination. J, K, Separate monoclinal sexual reproductive units, each with oospore somewhat immature. L, Separate monoclinal sexual reproductive unit with the oospore in mature resting state. M, Hypha bearing 2 interconnected sexual reproductive units, a and b, each with fully mature oospore.

Plate IX. Sexual reproductive apparatus formed in maize-meal-agar plate cultures by two conspecific *Pythium* isolates (no. 2, 3) originating from diseased stems of young tobacco plants in Sumatra; $\times 1000$ throughout. A—D, Hyphae in a 5-day-old culture of isolate no. 2, variously branched and terminating in one or more somewhat immature monoclinal reproductive units: A and B each bearing 2 such units, a and b; c terminating in 3 such units, a—c; and D bearing one such unit. E—J, Branched hyphae in a 20-day-old culture of isolate no. 3 which terminate in one or more fully mature monoclinal reproductive units: E and F each bear 2 such units, a and b; G and I each bear one such unit; H and J each bear 3 such units, a—c.

Plate X. Mycelial hyphae with appressoria formed on floor of maize-meal-agar plate culture of *Pythium deliense* (ATCC 12280), $\times 1000$. A, B, Portions of hyphae, each with 2 appressoria, a and b. C, Branched hypha with 3 appressoria, a—c. D, Branched hypha with 5 appressoria, a—e.

Plate XI. Reproductive apparatus of *Pythium deliense* (ATCC 12280): parts A—N formed on slabs which after removal from a young maize-meal-agar plate culture were shallowly irrigated with distilled water; part O produced in 3 days on piece of infected squash fruit shallowly immersed in distilled water. A—L, Portions of hyphae, each containing a distended cell presumably capable of developing into a sporangium; $\times 500$. M, N, Portions of hyphae, each containing 2 distended cells, a and b, presumably capable of developing into sporangia; $\times 500$. O, Portion of mycelium showing a monoclinal sexual reproductive unit at the time of fertilization; $\times 1000$.

Plate XII. Five monoclinal units of sexual reproductive apparatus, A—E, produced after 3 days when pieces of squash fruit infected with *Pythium deliense* (ATCC 12280) were shallowly irrigated with distilled water; $\times 1000$. In E the antheridial hypha presumable was at first nearly straight but was bent markedly through the growth of the oogonium and the antheridium.

Plate XIII. Eight units of sexual reproductive apparatus, A—H, formed in 2 days when slabs excised from a young Lima-bean-agar plate culture of *Pythium deliense* (ATCC 12280) were shallowly irrigated with distilled water.

Plate XIV. Fourteen sexual reproductive units (A—C; D, a, b; E—M) of *Pythium deliense* (ATCC 12280) from a maize-meal-agar plate culture 23 days old, eleven of them (A—C, E, F, H—M) being taken from the bottom side and three (D, a, b; G) from the upper side of the culture.

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