

SEVERAL SPECIES OF PYTHIUM CAUSING BLOSSOM-END ROT OF WATERMELONS

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A field decay of watermelon, *Citrullus vulgaris* Schrad., fruits resulting from invasion by a parasitic species of *Pythium* was observed by Nelson¹ in Michigan during the summer of 1916. According to brief reports by Coons (8) and by Coons and Nelson (9) affected melons, whether ripe or immature, showed blackened withered spots at the blossom end. While in some instances only a blemish about 75 mm. in diameter resulted from an infection, in other instances the fruit was wholly involved. On artificial inoculation the causal organism obtained in pure culture and held to be probably identical with *P. debaryanum* Hesse, was found to produce decay in healthy watermelons, and to bring about a leaky condition in both muskmelon, *Cucumis melo* L., and cucumber, *C. sativus* L., fruits, though not in potato, *Solanum tuberosum* L., tubers.

Some years later in a brief abstract (13) I recorded a destructive outbreak of two types of blossom-end decay that had occurred in a watermelon field on Arlington Experiment Farm, Arlington, Virginia, during the summer of 1922. Of these types of decay, one, manifested externally by chocolate-brown or bluish-brown discoloration, was held referable to a new species of *Pythium* whose spiny oogonia were commonly found fertilized by 1 to 4 branch antheridia; the other, manifested externally by lighter brownish discoloration, being attributed to *P. debaryanum*. Subsequently, in a consideration mainly of the cottony leak of cucumbers, 2 species of *Pythium* were mentioned (14, p. 1037, 1041) as being responsible for chocolate-brown blossom-end rot of watermelons: one of the species producing mulberry-like zoosporangia; the other giving rise to zoosporangia most often consisting individually of a subspherical part together with an adjacent portion of one or both hyphal elements between which it is intercalated. The lighter colored or buff blossom-end decay received mention (14, p. 1038) as a field trouble apparently widely distributed in the Middle Atlantic States, and there caused for the most part by the same fungus as that most frequently causing cottony leak of cucumbers,—the widely familiar fungus, which, though often cited under the binomial *P. aphanidermatum* (Eds.) Fitzp., would seem, on grounds elsewhere (18) set forth, more correctly designable as *P. butleri* Subr.

The two spiny parasites causing dark-brown blossom-end rot that were provisionally distinguished in 1925 by their very different zoosporangia I described in 1930, along with 13 congeneric forms, as species new to science (17); the fungus with moriform zoosporangia being presented under the

¹ Nelson, R. A field rot of watermelon caused by *Pythium*. Program 11th Ann. Meet. Bot. Soc. of Amer. held in New York, Dec., 1916, Pub. 66. Short statement of content of this paper, given in footnote by Coons and Nelson (9).

binomial *Pythium periplocum*, that with the modified subspherical zoosporangia under the binomial *P. acanthicum*. Apart from these two species, both made known only in their pathogenic connection with diseased watermelons, I attributed decay of watermelon fruits in Florida and Georgia to *P. myriotylum*, also a species then newly described, which, however, like *P. butleri*, was recognized besides as a parasite on various other host plants.

Walker and Weber (30), in an account of watermelon diseases of Florida published in 1931, cited *Pythium debaryanum* and *P. aphanidermatum* among the fungi associated with a group of abnormalities they discussed as blossom-end rot. Kheswalla (22), 5 years later, referred a malodorous rot of watermelons in Baluchistan to *P. aphanidermatum*. To the same species was ascribed in 1937 a watery soft rot found affecting watermelons in the Salt River Valley of Arizona (5). Ramsey, Wiant, and Link (26) in a recent treatise on market diseases of fruits and vegetables, enumerate *P. acanthicum*, *P. aphanidermatum*, *P. artotrogus* de Bary, *P. myriotylum* and *P. periplocum* among the organisms causing decay of watermelon fruits.

Although several species of *Pythium* have thus been recorded in the literature as agents causing blossom-end rot of watermelons, these do not comprise all the members of the genus occurring in such parasitic relationship. In some hundreds of cultures that, as opportunity offered during the last 17 seasons, I have isolated from affected melons in different regions, at least 4 other species are to be recognized: *P. ultimum* Trow, *P. irregulare* Buism., *P. mammillatum* Meurs, and *P. helicoides* Drechsl. The first 3 of these additional species have become more or less widely known from their association with damping-off and rootlet decay of various crop plants; and may, therefore, more appropriately be discussed later in connection with a few other forms of similar pathogenic tendencies. For that matter, the less familiar *P. helicoides* also is known to occur on subterranean parts of a crop plant, its original description (17) having been based on cultures isolated from affected roots of the common bean, *Phaseolus vulgaris* L. Indeed, this rather rare fungus has been obtained almost as frequently from diseased roots as from decaying watermelons; but, whereas the few instances of discoloration in the small underground structures have supplied only meager evidence of harmful parasitism, the instances of extensive decay in the massive fruits have, despite their small number, provided an impressive display of destructiveness. Thus, similarity in pathogenic behavior rather than taxonomic kinship brings *P. helicoides* together with the two distinctive blossom-end parasites *P. acanthicum* and *P. periplocum* for such descriptive treatment as may be necessary properly to supplement the earlier diagnoses.

The usual inception of *Pythium* decay in watermelons as a well-marked blossom-end rot finds an explanation mainly in the anatomical structure of the fruit itself. The unbroken epicarp of the berry appears highly efficient in excluding invasion by any species of *Pythium*. Such invasion does not ordinarily ensue, even when the epicarp, together with the chlorophyll-bearing hypoderm immediately underneath (1), is removed over considerable areas

through mechanical scraping, or through the feeding of superficially gnawing insects; the very firm stone-cell layer then exposed offering a barrier apparently not easily transgressed by many fungi, notwithstanding the conspicuous freedom with which it is penetrated by the widespread anthracnose organism, *Colletotrichum lagenarium* (Pass.) Ell. and Hals. Deeper wounds, whereby the stone-cell layer is interrupted, usually permit entrance by species of *Pythium*, as also of other fungi, including, for example, *Corticium vagum* B. and C., often encountered in blossom-end rot, and the one or possibly several species of *Diplodia* more familiarly associated with stem-end rot.

Owing apparently to imperfect scarring of the epicarp and incomplete closure of the stone-cell layer at the attachment of the withered floral remnants, the berry is inadequately protected against fungus invasion over a minute apical region. Under normal conditions species of *Pythium* are unable to gain entrance into uninjured melons except by the narrow avenue of insufficiently indurated tissue directly underneath the floral scar. The means by which the parasite gains its initial foothold can only be surmised. In instances where a melon decaying from the distal end is found with its tip directed downward and resting on the ground, it is not difficult to presume that infection took place by growth of hyphae from the soil into the floral remnants and thence through the vulnerable gap in the stone-cell layer. Most infected fruits, however, are found in approximately horizontal positions, or are otherwise disposed in a manner little suggestive of earlier contact between blossom end and ground. In these more numerous instances of infection, it may be conjectured that conidia or oospores of the invading parasite reached the floral scar mainly in particles of soil lifted by winds or splashed upward by rains; germination of the adhering reproductive bodies presumptively having taken place later on when a small quantity of water had remained deposited for a sufficiently long time during periods of dewy, foggy, or rainy weather.

Manifestly, in dry seasons and to a considerable degree in seasons of moderate, well distributed precipitation, the circumstances attending such infection might reasonably be expected to favor organisms with spores not only highly resistant to the rather severe desiccation to which the surface soil of watermelon fields is ordinarily exposed, but also given to especially prompt germination when water becomes available, even in small amounts, for a relatively short period. And, indeed, the one species of *Pythium* generally predominant in the causation of blossom-end rot, *P. acanthicum*, is conspicuous for the unusually prompt germination of its adequately resistant oospores. Under somewhat unusual weather conditions, other species may come to predominate locally; as notably, for example, when *P. butleri* gains a destructive ascendancy in Maryland and Virginia during or immediately following spells of excessively hot, moist weather. High temperatures combined with ample moisture appear very important also for the development of *P. myriotylum*; this parasite evidently being limited in the United States to a southern distribution because of its exacting thermal requirements.

Once having gained a foothold the progress of *Pythium acanthicum*, *P. periplocum*, or *P. helicoides* through an invaded watermelon fruit is marked externally in an advance of chocolate-brown, dark-brown, or bluish-brown discoloration beginning at the blossom end and proceeding toward the stem end. On cutting open invaded specimens, affected parts are easily distinguished from healthy parts by their brownish discoloration and their softened watery texture. The outer rind often presents a water-soaked appearance as if pickled; yet, owing to its sturdy cellular structure, it usually retains some noticeable firmness, while the pulp or inner mesocarp becomes exceedingly flaccid, and on slight pressure gives off its dilute sepia juice in copious quantity. Under a microscope the large-celled tissues of the inner mesocarp are seen to be abundantly permeated by mycelial hyphae that course haphazardly in all directions with little evident regard for the presence of the thin cell membranes. The thickened cell walls of the middle mesocarp and outer mesocarp for the most part constrain passage of the hyphal filaments through their pits; resistance to mycelial extension, therefore, becoming apparent especially in the harder small-celled portions of the outer mesocarp.

At moderate temperatures and in the absence of too severe bacterial contamination, progress of an invading *Pythium* mycelium usually continues until the affected watermelon has been wholly traversed. The somewhat dry, blackish, superficial lesions that ordinarily do not develop further than blossom-end blemishes have in my experience consistently failed to yield any cultures of *Pythium*; and must accordingly be held referable to other causes, despite some writings tending to assimilate them to *Pythium* decay. Nor, again, has it been possible to ascertain any connection between malformation of watermelon fruit and susceptibility to blossom-end rot of any kind attributable either to species of *Pythium* or to the biologically similar if taxonomically remote *Corticium vagum*.

Like other cucurbitaceous fruits, watermelons, when invaded by species of *Pythium*, including those herein considered, give off a distinctive marshy odor due directly to the action of the mycelium rather than to activity of the bacteria following its advance through the succulent host tissues. Multiplication of these bacteria, however, soon brings about unmistakable putrefaction and gradual disintegration, first in the portion of the melon earliest affected, then throughout the massive berry. Worms, insects and other forms of animal life usually participate diligently in the final uninviting stages of decomposition; although when very young fruits without succulent pulp are invaded, especially in cooler weather, by any of the fungi causing dark brown blossom-end rot, saprophytic molds may intervene more conspicuously, to help destroy the somewhat mummified structures in a slower and less unsavory manner.

As might be expected, all the various species of *Pythium* isolated from tissues affected with blossom-end rot readily produce decay on being inoculated into healthy watermelons by incisions through the stone-cell layer. Whether such experimental results can properly be interpreted as proof of

pathogenicity, except in a limited sense, appears doubtful, since, through similar procedure, decay of watermelons is likewise induced by numerous species of *Pythium* that hitherto have never been found affecting this fruit under natural conditions. Of these numerous forms one is given comparative treatment herein, which, along with *P. acanthicum* and *P. periplocum*, was referred to in an earlier paper (14, p. 1041, 1042) as a species incapable of causing decay in cucumber fruit, even after being introduced into incisions through the epicarp.

In most seasons and in most of our watermelon-growing sections, the losses due to blossom-end rot caused by species of *Pythium* would seem not serious enough to justify special control measures. Yet, the rather substantial losses prone to occur during wet years in some parts of the Middle Atlantic States, especially in fields intended to be harvested late in August or in September, suggest that preventive measures might at times be profitably undertaken here. While no experimental work has been done on means of control, it may reasonably be presumed that the fungicidal paste recommended by Orton and Meier (25) for prevention of stem-end rot could be employed advantageously also as a prophylactic against blossom-end decay. One application of the adhesive preparation over the minute area covered by the flower scar—an operation requiring little labor or material—should ordinarily suffice to forestall invasion by any species of *Pythium* or by *Corticium vagum*. On the occasion of such treatment, melons found with their apical ends in contact with the ground could conveniently be shifted into positions less favorable for attack by parasitic fungi.

PYTHIUM ACANTHICUM

In the course of the present studies *Pythium acanthicum* has been encountered more frequently as the cause of blossom-end rot of watermelons (Fig. 1, A, B, C) than all its congeners taken together. This general predominance came to light no less clearly where blossom-end rot occurred only in negligible quantity as, for example, in fields near Kennett, Missouri, and Decker, Indiana, that on inspection late in August, 1924, showed only 1 or 2 affected specimens to an acre, than where the disease was highly destructive, as in some fields near Annapolis, Maryland, that late in September, 1924, showed more than a fourth of the crop being ruined by the decay. The fungus has always been found in greater or smaller quantity whenever watermelon fields with maturing fruit have been surveyed for the presence of blossom-end rot. It has been identified in numerous cultures isolated from material collected near Williamsburg, Maryland, Diamond Springs and Smithfield, Virginia, early in August, 1923; near Gainesville, Leesburg, and Bradenton, Florida, and Thomasville, Georgia, early in June, 1925; near La Fayette, Indiana, late in August, 1928; near Salisbury, Maryland, late in August, 1938; and near Beltsville, Maryland, late in September, 1938. Moreover, a few cultures of the parasite were obtained from rather dry, shallow, cankered lesions that appeared on living watermelon vines at Arlington Ex-

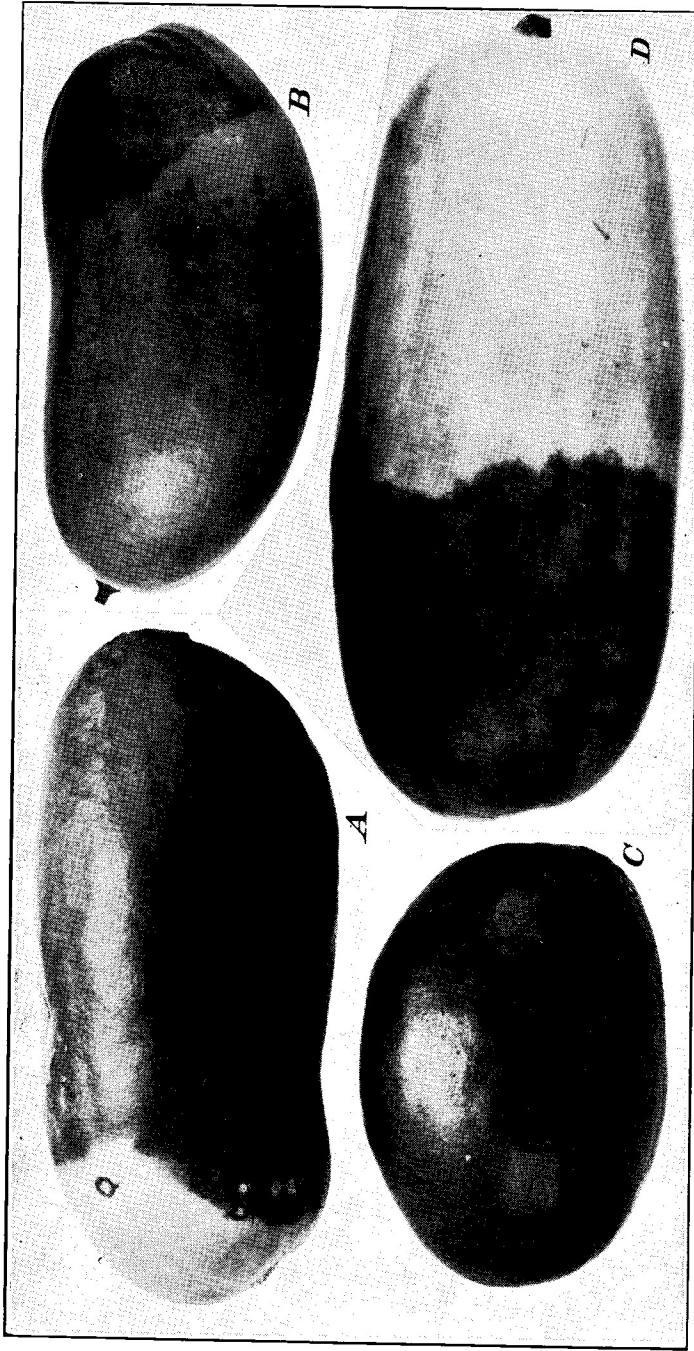


FIG. 1. Watermelon fruits affected with blossom-end rot due to *Pythium acanthicum*; approximately $\times 1$. A. Specimen of the variety Irish Gray, in large part decayed as the result of spontaneous infection in the field; from the affected tissues pure cultures of the fungus were obtained. B, C. Specimens of the variety Tom Watson, naturally infected in the field; from both specimens pure cultures of the parasite were obtained. D. Specimen of the variety Irish Gray 10 days after artificial inoculation at the blossom end with a pure culture of the fungus that had been isolated from a longitudinal canker near the base of a watermelon stem.

periment Farm in September, 1922, usually extending several centimeters along the basal portions of the stems. When inoculated into sound watermelons these cultures caused dark-brown or bluish-brown decay (Fig. 1, D), indistinguishable from the decay following inoculation with cultures derived from naturally infected fruits.

In pure culture on an agar medium rich in nutrient materials, as, for example, Lima-bean-decoction agar, *Pythium acanthicum* gives rise to a submerged lustrous radiating mycelium that often displays the kind of regional variegation in density manifested to the naked eye in a cumulous appearance. On media less rich in nutrients, such as maize-meal agar, the wholly submerged mycelial growth is often so nearly indiscernible to the naked eye that the presence of a growing fungus would, on ordinary scrutiny, not be surmised. The parasite is slower with respect to rate of mycelial extension than the more widely known congeneric forms causing damping-off of seedlings; besides being easily distinguishable from the latter in its more delicate mycelial habit and its very meager emission of extramatrical hyphae.

Development of zoosporengia with production of zoospores can be induced conveniently by excising small pieces of newly invaded tissue from an affected melon, and keeping them bathed in a shallow layer of fresh water. A number of asexual reproductive units obtained through such irrigation that are shown in figure 2, A-I, and figure 3, I-K, present a rather usual range of variations in the dimensions of the 1 or more rarely 2 subspherical parts making up most of the individual sporangium, together with concomitant variations in the length and position of the hyphal part or parts making up the remainder of the sporangial body. Comparable variations are evident, too, with respect to place of origin, length and orientation of the evacuation tube (designated throughout by the letter *t*). In the assortment of reproductive units shown are represented 10 different strains of the fungus, each derived from a separate lesion. Of these 10 strains one (Fig. 2, A, a-k) was isolated from a cankered watermelon stem in 1922; another (Fig. 2, H, a, b) was isolated from a watermelon fruit with blossom-end rot, selected at Arlington Experiment Farm in 1922; 6 (Fig. 2, B, a-e; Fig. 2, D, a-e; Fig. 2, E; Fig. 2, F, a-e; Fig. 2, I, a, b; Fig. 3, G-I) came from separate watermelon fruits affected with blossom-end rot collected near Williamsburg, Maryland, in 1923; and 2 (Fig. 2, C, a-d; Fig. 2, G) came from a pair of decaying watermelons collected near Diamond Springs, Virginia, in 1923. Strain differences in morphology of sporangial apparatus, if recognizable at all, are assuredly little pronounced.

Asexual reproduction ensues very satisfactorily also when pieces of substratum permeated with well-nourished young mycelium are removed from plate cultures of Lima-bean agar and irrigated by successive changes of fresh water. On that medium, as in watermelon tissue, *Pythium acanthicum* gives rise readily to an abundance of biciliate zoospores (Fig. 3, L) that, after a period of motility, become quiescent and round up (Fig. 3, M) to germinate later by the production of germ tubes (Fig. 3, N, a-c). Though liberation

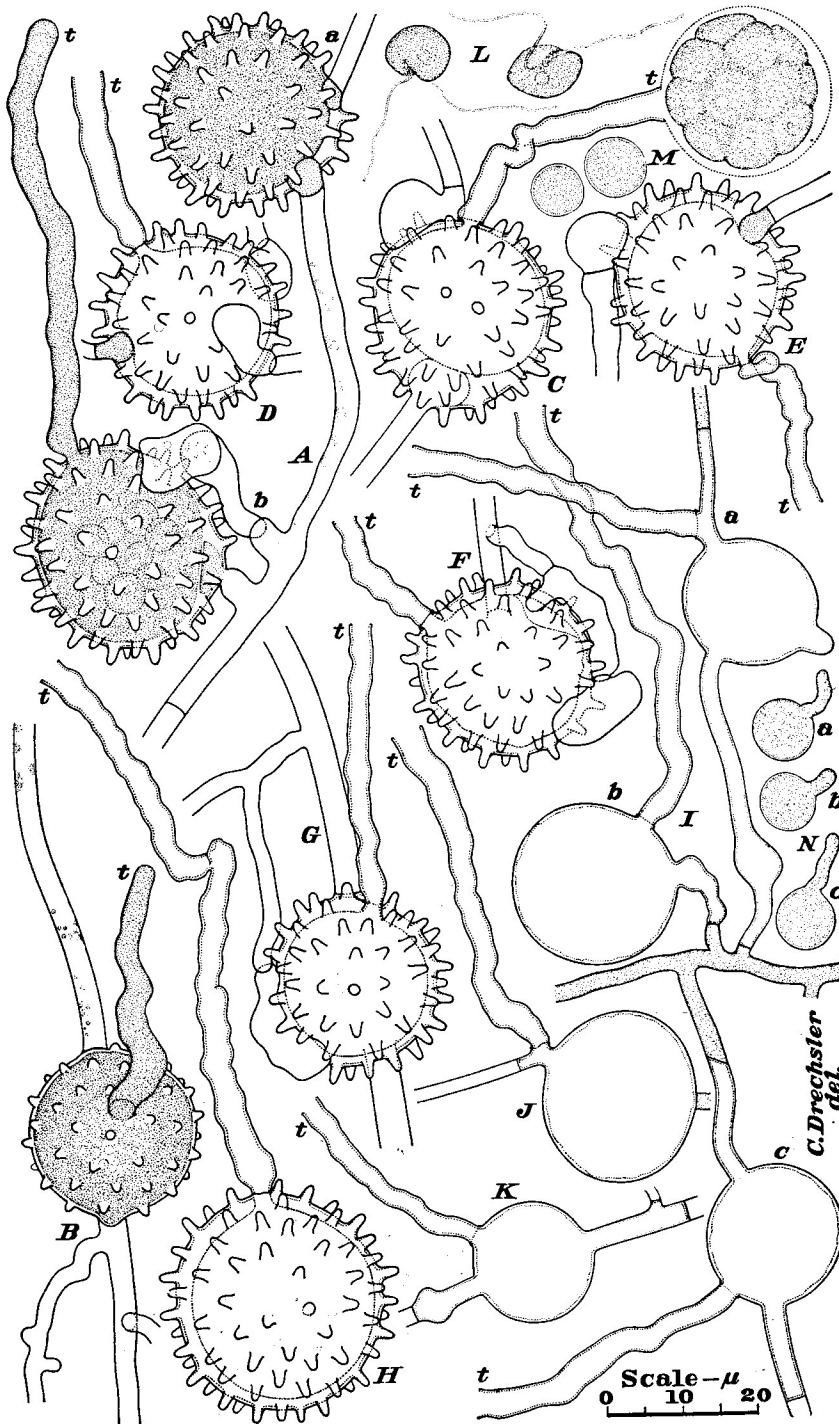


FIG. 3. *Pythium acanthicum*. A-H. Germination of oospores by production of zoospores. I-K. Emptied asexual sporangia. L-N. Zoospores in successive phases. $\times 1000$ throughout.

of zoospores may be regarded as the normal function of all asexual reproductive units that are formed in irrigated material, many globose structures, whether because of increasing bacterial contamination or because of the presence of excessive soluble food materials, germinate vegetatively by putting forth germ tubes, often in considerable numbers (Fig. 2, A, j, k).

It appears unlikely that under natural conditions *Pythium acanthicum* in watermelon fruits affected with blossom-end rot can give rise to zoosporangia. However, sexual apparatus is normally developed very abundantly in invaded watermelon tissue. The enormous numbers of mature oospores resulting from such development usually reveal the organization of contents that here, as in most oomycetes, is associated with longevity in these bodies,—the single central reserve globule being surrounded by a parietal layer of granular protoplasm wherein is imbedded a single oblate ellipsoidal refringent body. Accordingly, when their succulent matrix undergoes foul putrefaction previous to disintegration, the oospores show little degeneration and correspondingly little reduction in power of germination.

As the manipulations necessary for mounting the flaccid host tissues on glass slides usually entail some disturbance of parts, the morphology of the sexual apparatus of *Pythium acanthicum* can be studied more satisfactorily in thin slices of a moderately hard transparent gel. Maize-meal-decoction agar containing in suspension a moderate amount of fine maize-meal sediment, well distributed by agitation shortly before the medium is ready to solidify, has been utilized to advantage; its chemical composition encourages copious development of sexual apparatus with production of correctly organized oospores in immense numbers, and its physical consistency permits the cutting of paper-thin slices suitable for microscopic examination under objectives of high magnification. In figure 4, A–P, are shown 16 units of sexual apparatus drawn undisturbed from a preparation of such maize-meal agar made from a culture originally derived from a decaying watermelon fruit collected near Decker, Indiana, in 1924. The assortment of sexual units illustrates the usually intercalary position of the oogonium (Fig. 4, B–P); the usually rather even distribution of the slightly tapering, bluntly rounded oogonial protuberances (Fig. 4, A–N, P); the occasional paucity of such protuberances, especially on oogonia of small size (Fig. 4, O); the origin of the usually single (Fig. 4, A–I) antheridium from the oogonial hypha a short distance from the oogonium; the delimitation of the female organ mainly by massive plugs; the frequently, though not invariably, broad application of the antheridium; and the pluralism of reserve globules in the oospore during a protracted period of early maturity (Fig. 4, A–E, G, H) preceding their coalescence at full maturity (Fig. 4, F, I–P).

With respect to dimensions of oogonium and oospore, different strains of *Pythium acanthicum*, in comparison to one another, display a moderate range of variability, such as would seem to be usual among the more stable species of oomycetes, rather than such a wider range as was found characteristic of the graminicolous parasite *P. arrhenomanes* Drechsl. by Rands and Dopp

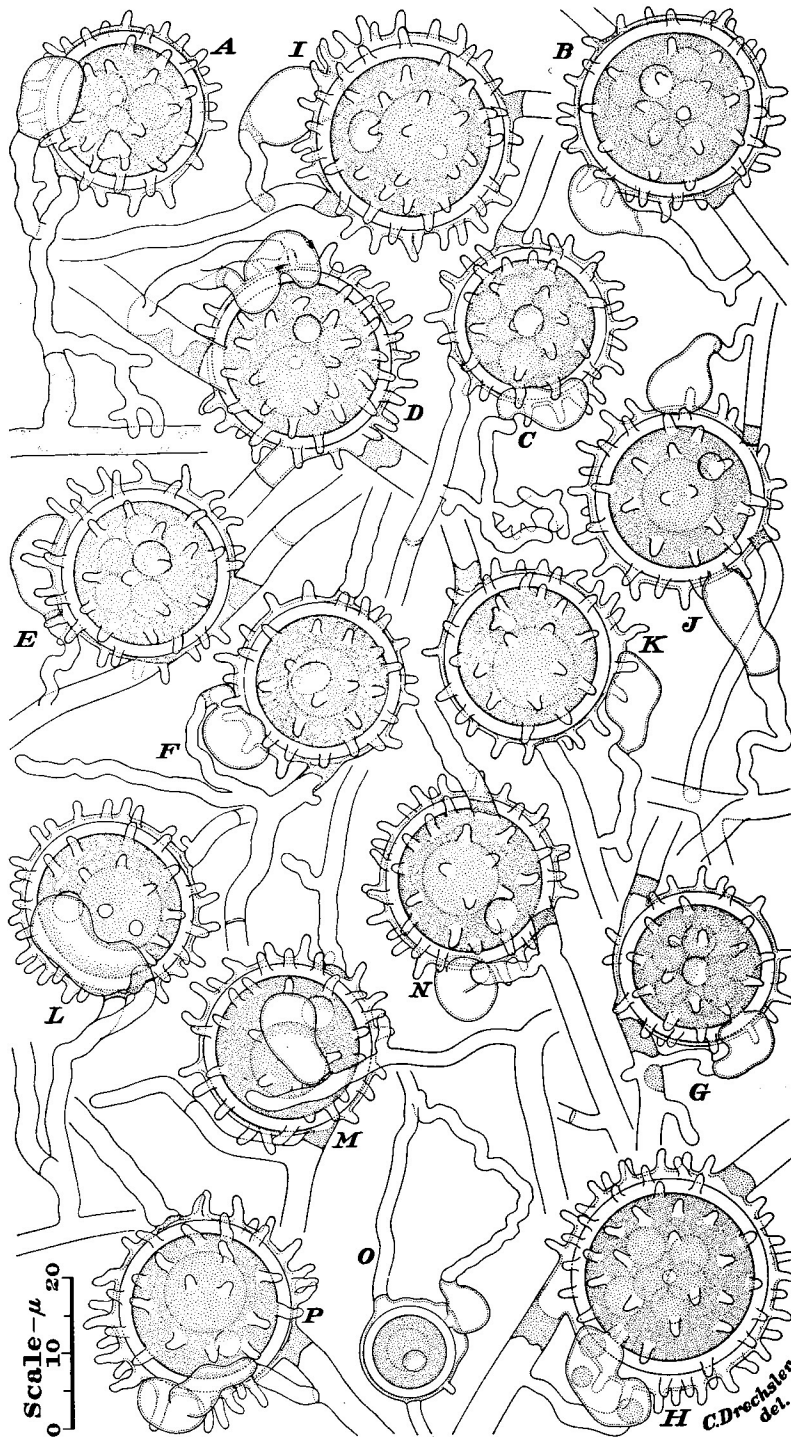


FIG. 4. A-P. Sexual apparatus of *Pythium acanthicum*; $\times 1000$ throughout.

(28). The relevant metrical data submitted in the diagnosis of the species were based on measurements of the same strain from Decker, Indiana, that was used in preparing the illustrations shown in figure 4. The 200 oogonia chosen at random in their maize-meal-agar matrix gave the following distribution of values for diameter expressed to the nearest micron: 13 μ , 1; 18 μ , 2; 19 μ , 4; 20 μ , 5; 21 μ , 11; 22 μ , 25; 23 μ , 40; 24 μ , 52; 25 μ , 30; 26 μ , 15; 27 μ , 7; 28 μ , 5; 29 μ , 2; 30 μ , 1. Measurements of the 200 oospores contained within these oogonia gave a distribution of values for diameter, as follows: 12 μ , 1; 15 μ , 1; 17 μ , 3; 18 μ , 5; 19 μ , 7; 20 μ , 29; 21 μ , 41; 22 μ , 49; 23 μ , 39; 24 μ , 10; 25 μ , 8; 26 μ , 5; 27 μ , 2.

Oospores of the fungus germinate freely, without any rest period, at any time after attaining approximate maturity. In pure water free of nutrient substances, germination begins with dispersion of the large reserve globule and disappearance of the thick oospore wall (Fig. 3, A, a). The protoplast expands to fill the oogonial chamber, except for the narrow protuberances (Fig. 3, A, b; B), and pushes through the oogonial wall a narrow process that, after some widening and considerable elongation, becomes recognizable as an evacuation tube (Fig. 2, A, t; B, t). With inflation of the gelatinous apex of this tube (Fig. 3, C, t) the granular contents are delivered into a terminal vesicle, there to be fashioned into zoospores in exactly the same manner as in vesicles resulting from dehiscence of asexual sporangia. The oogonium, thus emptied, now reveals in its interior a delicate subspherical membrane, which, from its continuity with the evacuation tube (Fig. 3, C-H, t) presumably represents an envelope that must have continued to surround the oosporic protoplast after the thick oospore wall had disappeared.

In fresh water containing nutrient substances and, of course, on unstaled solid artificial media, oospores of *Pythium acanthicum* germinate in the more commonplace manner, by the production of vegetative hyphae. Kept in the stale substrata in which they were developed, they retain their normal internal structure, and with it their vitality, rather longer than oospores of various other members of the genus. When protected from excessive evaporation cultures of the fungus in tubes of maize-meal agar have always been found to yield new growth promptly on being transferred to fresh substratum after 3 years of storage. The enduring vitality and ready germination of its oospores evidently serve the fungus well in meeting the peculiar difficulties that must ordinarily be associated with blossom-end infection by soil-borne parasites.

PYTHIUM PERILOCUM

The decay of watermelons caused by *Pythium periplocum*, whether following spontaneous infection at the floral scar (Fig. 5, A) or following artificial inoculation by incision through the stone-cell layer (Fig. 5, B), is closely similar in outward appearance to that caused by *P. acanthicum*. Externally, it is manifested by the advance of chocolate-brown, dark-brown or bluish-brown discoloration; internally it is accompanied by pronounced softening,

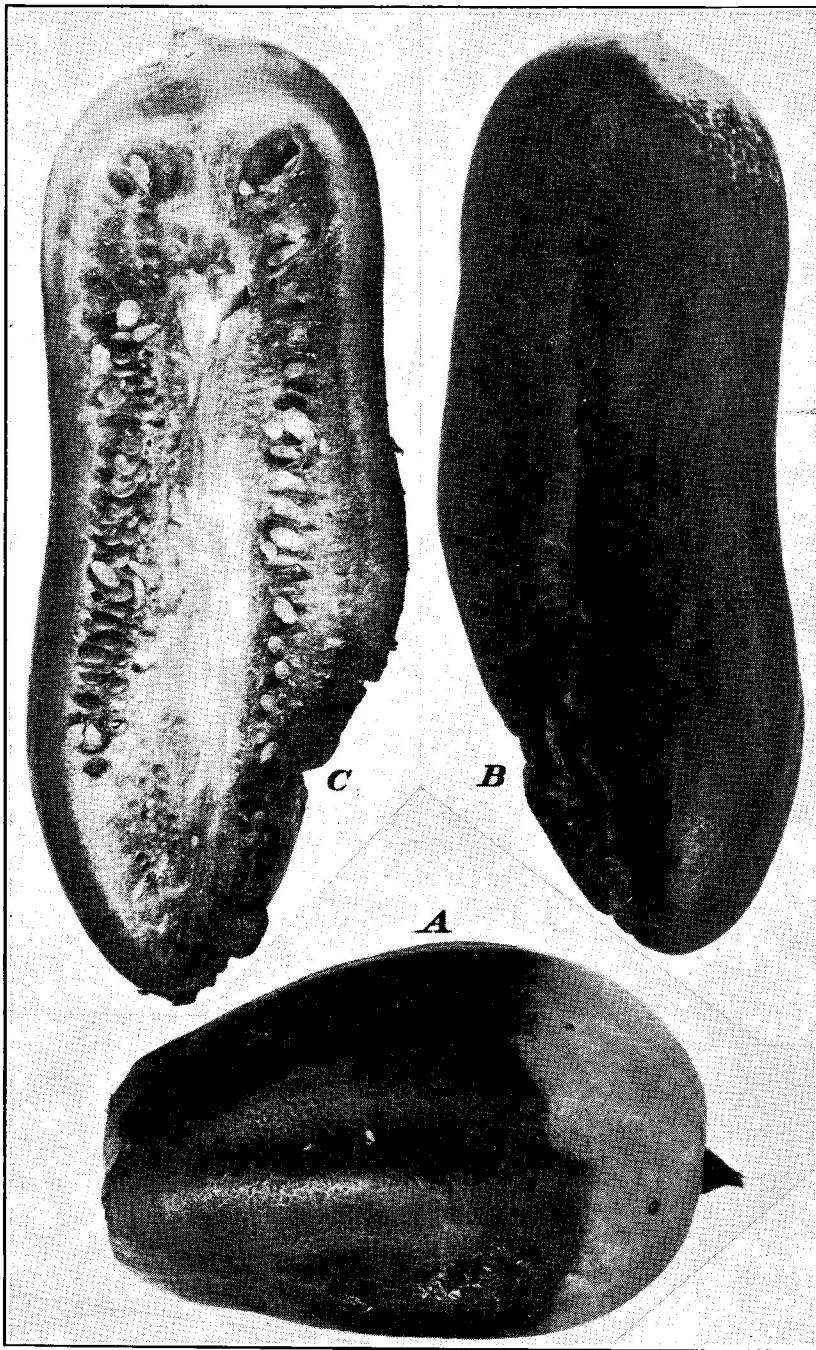


FIG. 5. Watermelon fruits affected with blossom-end rot due to *Pythium periplocum*; approximately $\times \frac{1}{4}$. A. Specimen of the variety Irish Gray in process of decaying following spontaneous infection in the field; from the softened invaded tissues cultures of the parasite were readily obtained. B. Specimen of the variety Irish Gray 15 days after inoculation with a pure culture of *P. periplocum*,—the inoculation having been made by incision at the apical end of the fruit, which was kept attached to the living vine until photographed. C. Longitudinal section of the specimen shown in B.

especially of the large-celled tissues, destruction of the red pigment normal in the pulp of ripening fruit, and diffusion of a dilute sepia stain (Fig. 5, C). Yet, however strongly the 2 congeneric parasites may resemble one another in pathological effect, *P. periplocum* is encountered far less frequently than the other, and may, indeed, be regarded as a somewhat rare fungus. It was isolated from 2 watermelons affected with blossom-end rot at Arlington Experiment Farm in September, 1932; from 1 affected melon found near Williamsburg, Maryland, in August, 1923; and from 5 affected melons collected near Diamond Springs, Virginia, in August, 1923. Apart from diseased watermelon fruits, it came to light, together with *P. irregulare*, in a maize-meal-agar plate culture planted with pieces excised from cuttings of the sand pear, *Pyrus serotina* Rehd., that, on attempted propagation in March, 1923, in a nursery at Ludowici, Georgia, had failed to put forth roots.

On artificial media *Pythium periplocum* extends its mycelium with a rapidity that may be considered moderate for members of the genus. The resulting cultures present somewhat the same appearance to the naked eye as parallel cultures of *P. acanthicum*, though a difference is usually recognizable in the presence of some aerial mycelium. Under a microscope the submerged mycelium is distinguished more especially by a conspicuous development of abundantly and intricately ramifying branches attached at intervals laterally to the fairly straightforward axial hyphae (Fig. 6, A).

When well-nourished vegetative mycelium, either in newly invaded watermelon tissue, or in a rich agar medium, as, for example, Lima-bean agar, is irrigated by shallow immersion in fresh or possibly sterile water, some of the closely ramifying branches become increasingly and irregularly distended (Fig. 6, B, C) to give rise, alone or in union with similar structures, to massive lobulate zoosporangial complexes (Fig. 6, D, E). The individual complex, together often with a portion of axial hypha, simple or branched, is delimited through the deposition of a septum or of plural septa, and an evacuation tube is put forth (Fig. 6, D, t) that after attaining some length comes to bear a refringent cap at its expanded tip (Fig. 6, E, t). On receiving the discharged protoplasmic contents of the lobulate structure, this cap is inflated into a thin vesicular membrane (Fig. 6, F, t), which persists as a protective envelope during the period of 15 to 30 minutes when the granular material is being fashioned into zoospores after the manner familiar in species of *Pythium* (Fig. 6, G, t). While a single evacuation tube (Fig. 6, H, t) ordinarily suffices for the dehiscence of a sporangial complex of moderate volume, 2 or even 3 evacuation tubes (Fig. 6, K, t, t, t) often serve in the discharge of unusually massive complexes. Rupture of a vesicle often liberates from 100 to 125 actively motile zoospores that, except for occasional instances of incomplete cleavage (Fig. 6, J, b), consist of a somewhat reniform protoplast bearing 2 lateral cilia (Fig. 6, J, a). The organs of locomotion are made more distinctly visible in preparations exposed to osmic acid fumes and stained with gentian violet (Fig. 6, K). After a variable period

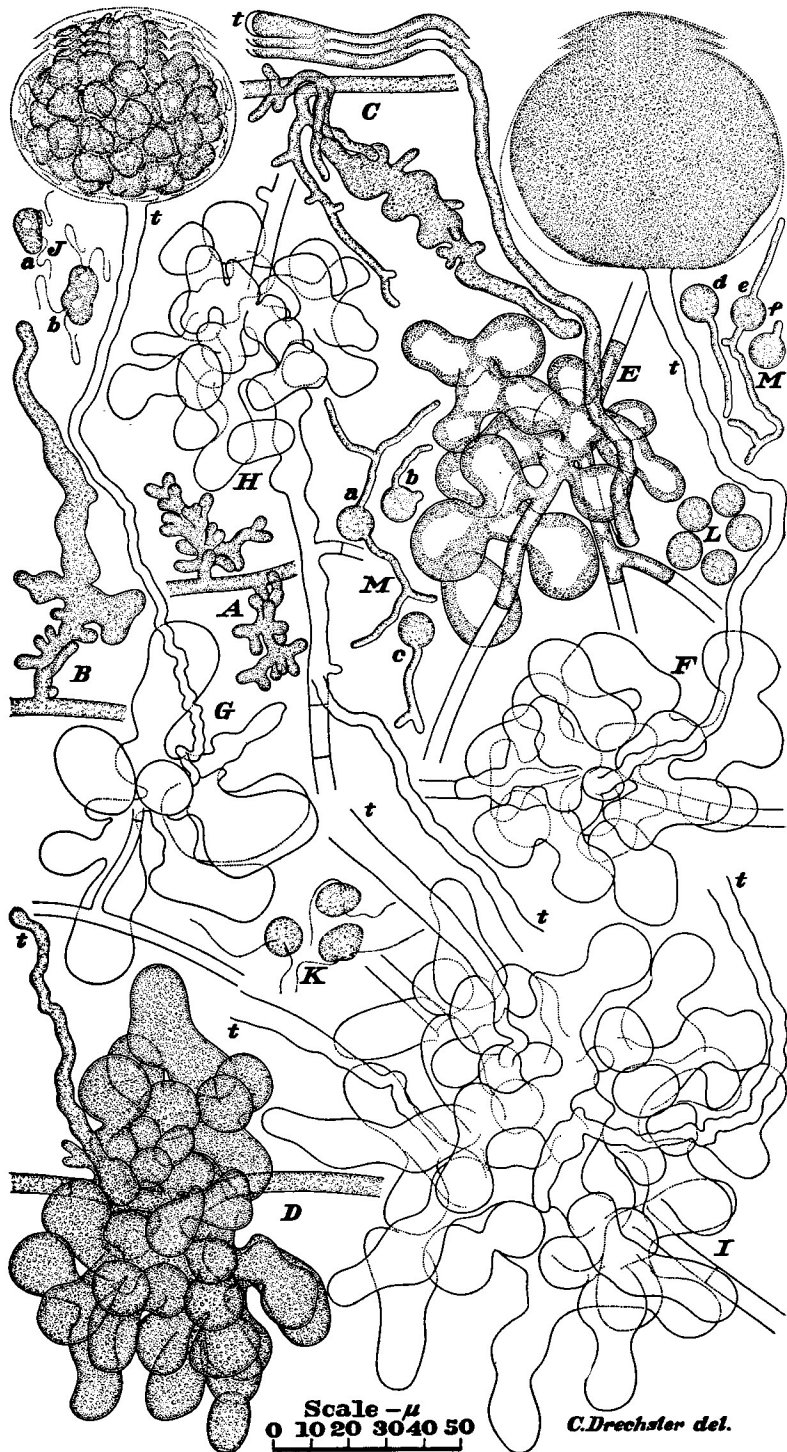


FIG. 6. Mycelium and asexual reproductive apparatus of *Pythium periplocum*; $\times 500$.

of motility the zoospores round up (Fig. 6, L) to germinate later by the production of 1 or 2 germ tubes (Fig. 6, M, a-f).

In its nutritional requirements for sexual reproduction, *Pythium periplocum* is more exacting than the more widely known damping-off parasites congeneric with it, inviting comparison rather with such refractory forms as *P. arrhenomanes* and *P. scleroteichum* Drechsl. (19). On many media of wide usefulness, as, for example, potato-dextrose agar, the mycelium may give rise abundantly to oogonia and antheridia, but the sexual organs promptly degenerate, often without producing any good oospores whatever. Normal development of oospores is better encouraged through use of maize-meal-decoction agar containing in suspension a considerable quantity of the finer maize-meal sediment. When paper-thin slices, cut with a razor from the plane surface of a culture prepared with this medium, are mounted under thin cover glasses and examined microscopically under an objective of high magnification, the rather intricate arrangement of the sexual apparatus may be made out satisfactorily.

The intricacy of the individual unit of sexual apparatus is attributable mainly to the antheridia, which, in numbers varying usually from 1 to 4, are borne on a single branch (Fig. 7, A, C, D, F, G, H, J, K) without close mycelial connection with the oogonium, or, more rarely, on 2 such branches (Fig. 7, B, E). As each antheridium consists typically of a longish structure bearing a number of ventrally protruding lobes, and, in addition, may in various ways be branched more or less, the oogonium is often surrounded by its male complement in a manner recalling certain species of the saprolegniaceous genera *Aphanomyces* and *Plectospira* (15, 16).

While in *Pythium periplocum* the female organ would seem to be formed terminally somewhat more often than in *P. acanthicum*, the difference in positional relationship cannot be considered a very pronounced one. Nor are wide departures from the morphology of the latter species evident in the spiny ornamentation of the oogonium, or in its dimensions. Measurements of the 200 oogonia selected at random, from which were derived the relevant metric data submitted in the diagnosis, showed a distribution of values for diameter expressed to the nearest micron, as follows: 15 μ , 1; 16 μ , 1; 21 μ , 5; 22 μ , 17; 23 μ , 25; 24 μ , 38; 25 μ , 50; 26 μ , 39; 27 μ , 11; 28 μ , 8; 29 μ , 2; 30 μ , 2; 32 μ , 1. Measurements of the oospores contained within these oogonia showed a distribution of values for diameter, as follows: 13 μ , 1; 14 μ , 1; 16 μ , 1; 17 μ , 2; 18 μ , 7; 19 μ , 14; 20 μ , 39; 21 μ , 48; 22 μ , 48; 23 μ , 24; 24 μ , 13; 26 μ , 1; 27 μ , 1. Comparison of the averages computed from the 2 sets of measurements with the corresponding averages computed from the homologous measurements of *P. acanthicum* indicates a somewhat greater oogonial diameter and a slightly smaller oospore diameter in the present species. Because of this dimensional proportionality the oogonial cavity here is noticeably less nearly completely filled by the oospore, which, in its fully mature state, reveals the internal organization familiar in numerous oomycetes,—a single large central reserve globule of homogeneous consistency being sur-

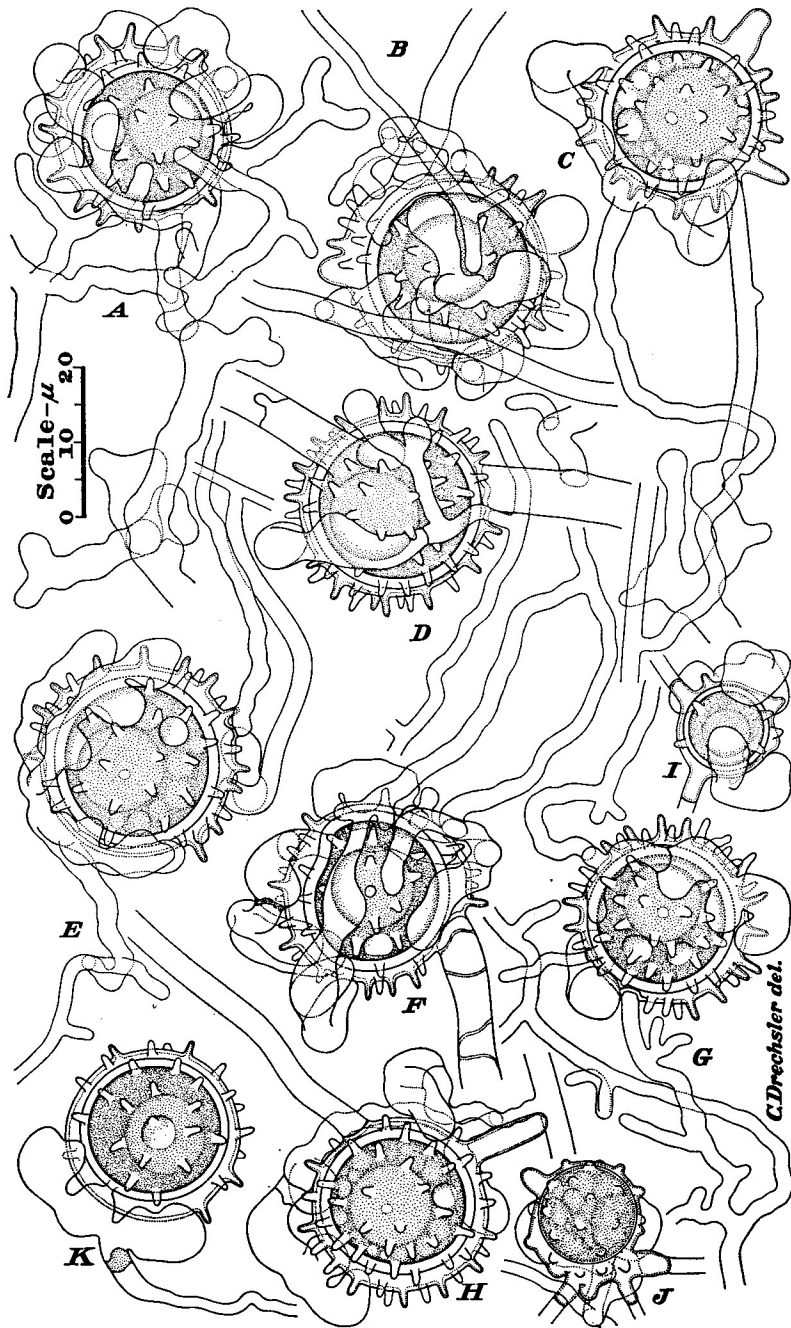


FIG. 7. A-K. Sexual apparatus of *Pythium periplocum*; $\times 1000$ throughout.

rounded by a parietal layer of granular protoplasm in which is imbedded a single subspherical or oblate ellipsoidal refringent body (Fig. 7, A-I, K).

PYTHIUM HELICOIDES

Pythium helicoides has been identified in only one lot of cultures isolated by me from watermelons affected with blossom-end rot. Of the 48 cultures in that lot, all of which were obtained from separate decaying specimens chosen at random on August 18, 1937, in an experimental field belonging to the U. S. Regional Vegetable Breeding Laboratory at Charleston, South Carolina, 7 are referable to the fungus in question. The very wet conditions that had prevailed in the field during the week before the material was collected may very probably have facilitated the unusual parasitism of the organism in a plant structure neither subterranean nor aquatic. It may be presumed that as with other fungi causing blossom-end decay the initial difficulty in gaining a foothold within the uninjured fruit provides the chief protection of the host; for, when *P. helicoides* in pure culture is introduced into watermelons by incisions through the stone-cell layer, decay manifested externally in dark-brown discoloration (Fig. 8, A, B) follows with no less certainty than after similar inoculation with *P. acanthicum*.

In pure culture on artificial media the fungus extends its mycelium nearly as rapidly as the species of *Pythium* causing damping-off. A general similarity to such forms as *P. ultimum* and *P. irregulare* is evident, too, in the macroscopic appearance of its growth on agar media; though the resemblance is often largely lost after cultures attain an age of several weeks, when the aerial mycelium of the common seed-bed parasites will ordinarily, even in the absence of contaminating bacteria, have collapsed to the surface of the substratum as a moist fibrous mat, whereas the aerial mycelium of *P. helicoides* will usually persist for years in its cottony state. This persistence of aerial mycelium, long familiar in various species of *Phytophthora*, is conspicuous also in cultures of the 3 fungi I have described (17) under the names *Pythium oedochilum*, *P. polytylum* and *P. palingenes*,—fungi that, from their morphological parallelism with *P. helicoides*, must be regarded as closely related to it.

Unlike most of the species of *Pythium* familiar to students of plant diseases, *P. helicoides* does not generally give rise either to zoosporangia or to their morphological equivalents, conidia, under conditions wholly unfavorable for immediate production of zoospores. A dry substratum usually encourages little or no development of asexual reproductive apparatus. However, when sizable tracts of well nourished vegetative mycelium in pieces of maize-meal agar or Lima-bean agar are transferred to a shallow layer of fresh water, they extend into the liquid new filaments of considerable length that often give off distally in racemose or cymoid arrangement a number of shorter branches, some of which may in turn give rise to secondary branches. On the axial filament as also on the individual ramification is borne terminally a subspherical or obovoid zoosporangium (Fig. 9, A, a-h) that, mostly from its

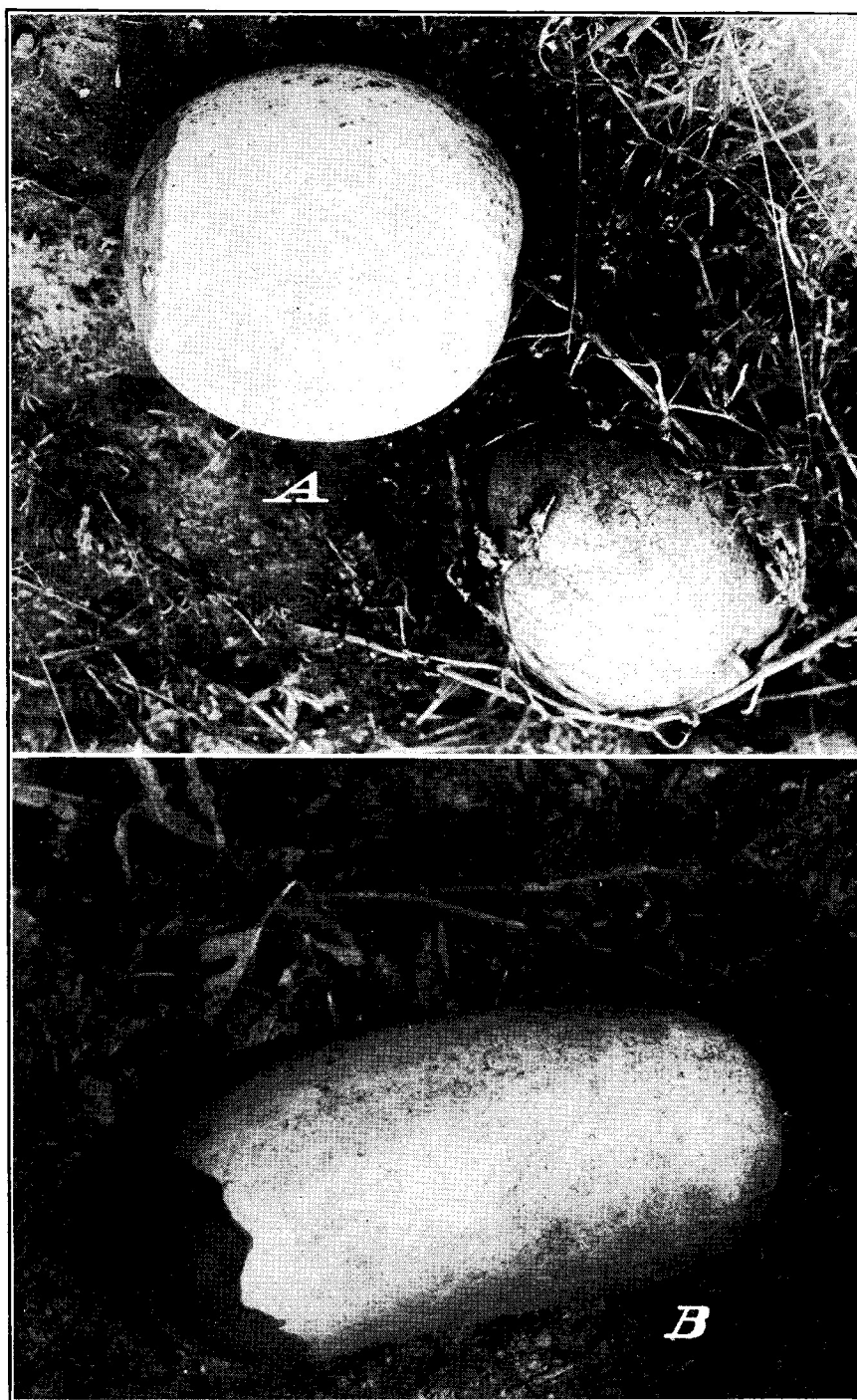


FIG. 8. Watermelon fruits 10 days after inoculation with a pure culture of *Pythium helicoides*,—the inoculation having been made in each case through an incision at the apical end of the fruit, which was kept attached to the living vine until photographed; approximately $\times \frac{1}{2}$. A. Specimen of the variety Northern Sweet. B. Specimen of the variety Burrell's Gray.

apex or from a part close thereto, thrusts out a sturdy and often rather short evacuation tube. When the undifferentiated sporangial contents are discharged through this tube they accumulate in a vesicle formed by inflation of its refractive gelatinous cap and are then fashioned into biciliate reniform zoospores after the manner typical of the genus (Fig. 9, B, L, I, M, O, Q). External conditions making for functional frustration of an evacuation tube may be reflected in somewhat unusual elongation of this structure (Fig. 9, N), or in production of one or more supernumerary tubes (Fig. 9, R, S, T, U). Following a variable period of motility the zoospores come to rest and round up (Fig. 9, X) either to produce secondary swarmspores somewhat later by repetitional development (Fig. 9, Y, a, b), or more usually to germinate vegetatively (Fig. 9, Z).

In common with many other of the aquatic or semiaquatic Pythiaceae that bear globose, ovoid or obovoid sporangia terminally on more or less submerge filaments, *Pythium helicoides* shows a proliferous tendency in its asexual reproduction (Fig. 9, A, g; H). On discharge of a sporangium the supporting hypha often gives rise to a second sporangium, which may be borne sessile within the empty envelope of the first (Fig. 9, C, J, P, V, W), or may be borne terminally on a hyphal prolongation passing lengthwise through the empty envelope and its empty evacuation tube (Fig. 9, D). By repetition of the process a third sporangium may occasionally be observed developing within the evacuated membrane of the second (Fig. 9, E). Somewhat similar multiplication comes about when from a position immediately below one sporangium, whether discharged or not, the supporting filament grows out laterally to produce another, now directly on the axial hypha, now terminally on a lateral prolongation (Fig. 9, F, G, K, L, T, U).

In all essential details of morphology and development the asexual reproductive phase of *Pythium helicoides* reveals unmistakable parallelism with the zoosporangial stage which de Bary (2) described very well in 1860, and on which, in the absence of a sexual stage, he then established his *P. proliferum*. Later, fortunately, that species was given a more precise definition in a revised characterization (3, 4) based on a fungus producing in conjunction with proliferous sporangia sex organs very closely resembling those set forth by him as pertaining to *P. debaryanum*; the usually intercalary oogonium being fertilized in most instances by 2 or 3 antheridia consisting predominantly either of adjacent portions of hypha, or of short lateral branches arising from the oogonial hypha close to the female organ. The ripe oospore of *P. proliferum* was stated by de Bary (4, p. 559) to be provided with a more prominent and more highly refractive central reserve globule than that of *P. debaryanum*. The figure (4, fig. 19) given by him of this structure shows a single central reserve globule that would seem, indeed, of relatively large size; and, what is of no less importance, depicts unmistakably a single refringent body ("heller Fleck") lying in the narrow parietal layer.

There is reason to presume that in the main the application of *Pythium proliferum* has been governed more by general agreement with the type of

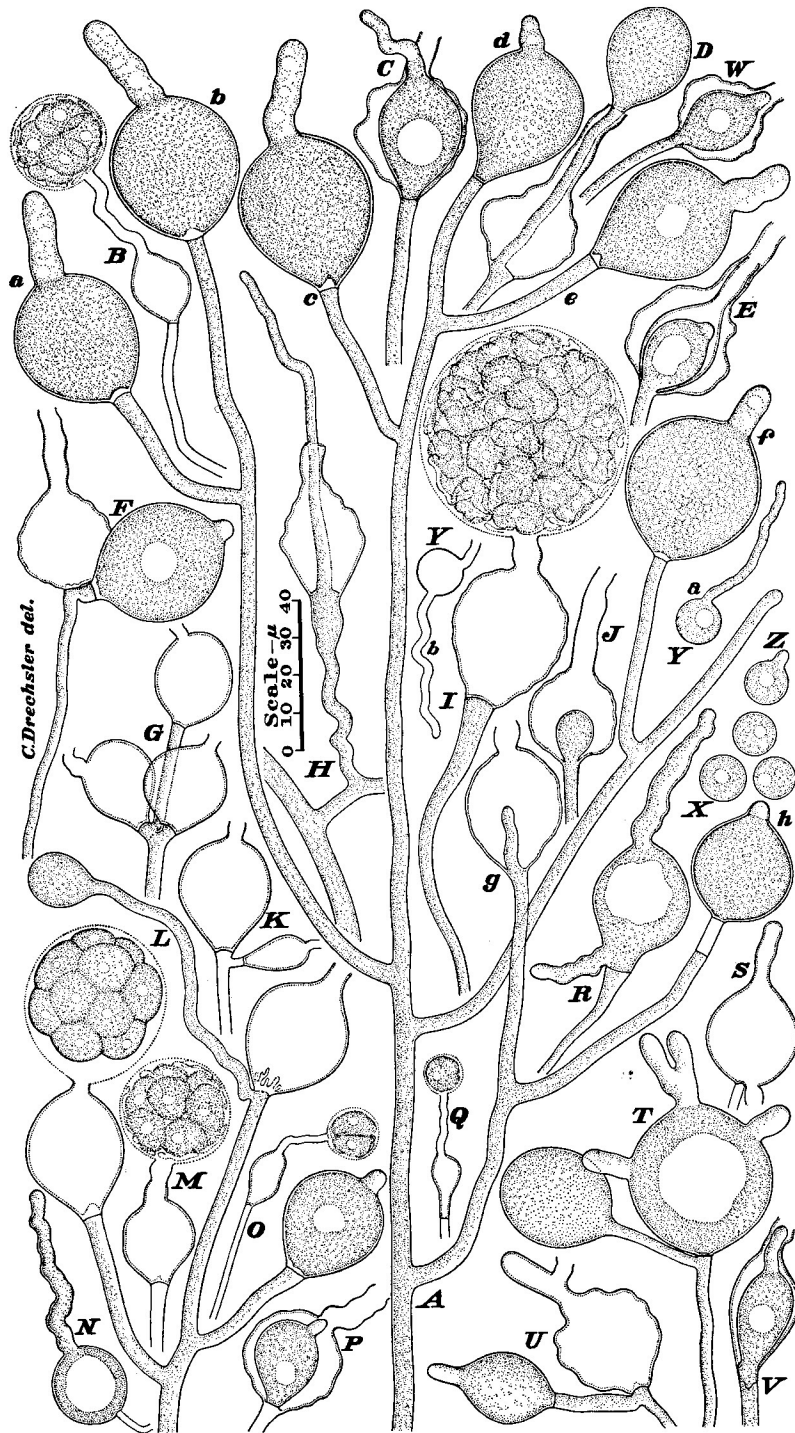


FIG. 9. Asexual reproductive apparatus of *Pythium helicoides*; $\times 500$ throughout.

asexual reproduction originally made diagnostic for the species than by conformity to the type of sexual apparatus later brought into the specific characterization. Citations of the binomial without descriptive detail in publications on the microflora of soil or of water, such as those of Harvey (21), Raper (28), Valkanov (29), and Lund (23), were more probably based on observed instances of zoosporangial proliferation than on recognition of correspondence with respect to morphology of oogonium, antheridium, and oospore. In Wardlaw's report (32) on the occurrence of the species on roots of the strawberry in Scotland, reference is made to mycelium, to sporangia, to zoospores, and to the process of proliferation, but no mention is made of oospores. Crooks (10), after discussing mycelium and proliferous sporangia in her recent account of *Pythium proliferum*, states that no sexual organs were observed.

Yet, some usage attributing to *Pythium proliferum* sexual apparatus, unquestionably of the same general type as that set forth in de Bary's final characterization of the species, is supplied in a number of descriptive publications by later writers. Ward (31), who obtained an abundance of oogonia in material he held referable to *P. proliferum*, declared their development and fertilization similar to these processes in *P. debaryanum*,—an assertion supplemented by several figures of which 2 show stages in the fertilization of terminal oogonia by antheridia borne terminally on branches (the branch in one instance arising from the oogonial hypha at some distance from the female organ), while a third shows oospores in each of which a single large reserve globule is surrounded by a parietal layer. Butler (7) found in his material of *P. proliferum* more antheridia arising from a neighboring hypha than were supplied by the oogonial filament, and described these organs as generally being short and little curved. Matthews' (24) illustrations of the species show antheridia that, in shape and mycelial relationships, as also in their frequently rather narrow apical contact with the oogonium, are reminiscent of the antheridia formed by *P. debaryanum* and *P. ultimum*; and in the oogonia fertilized by these decidedly commonplace male elements were formed equally commonplace oospores, which were stated to contain at maturity a central reserve globule surrounded by a granular layer of protoplasm having imbedded in it a small refractive body.

Conspicuous morphological departures from such very familiar type of sexual apparatus come to light in *Pythium helicoides*. The oogonium of this species, to be sure, offers little peculiarity, consisting merely of a subspherical enlargement densely filled with protoplasm and borne for the most part either laterally on an axial hypha (Fig. 10, A–D, F) or terminally on a lateral branch that frequently is very short (Fig. 10, G) but sometimes attains moderate length (Fig. 10, E). Much greater distinctiveness attaches to the elongated cylindrical antheridia that in numbers from 1 to 4 apply themselves very tightly lengthwise to the oogonium, becoming fused virtually from basal septum to rounded apex with the oogonial wall along an arc equivalent often to more than a fourth of the oogonial circumference. The frequently

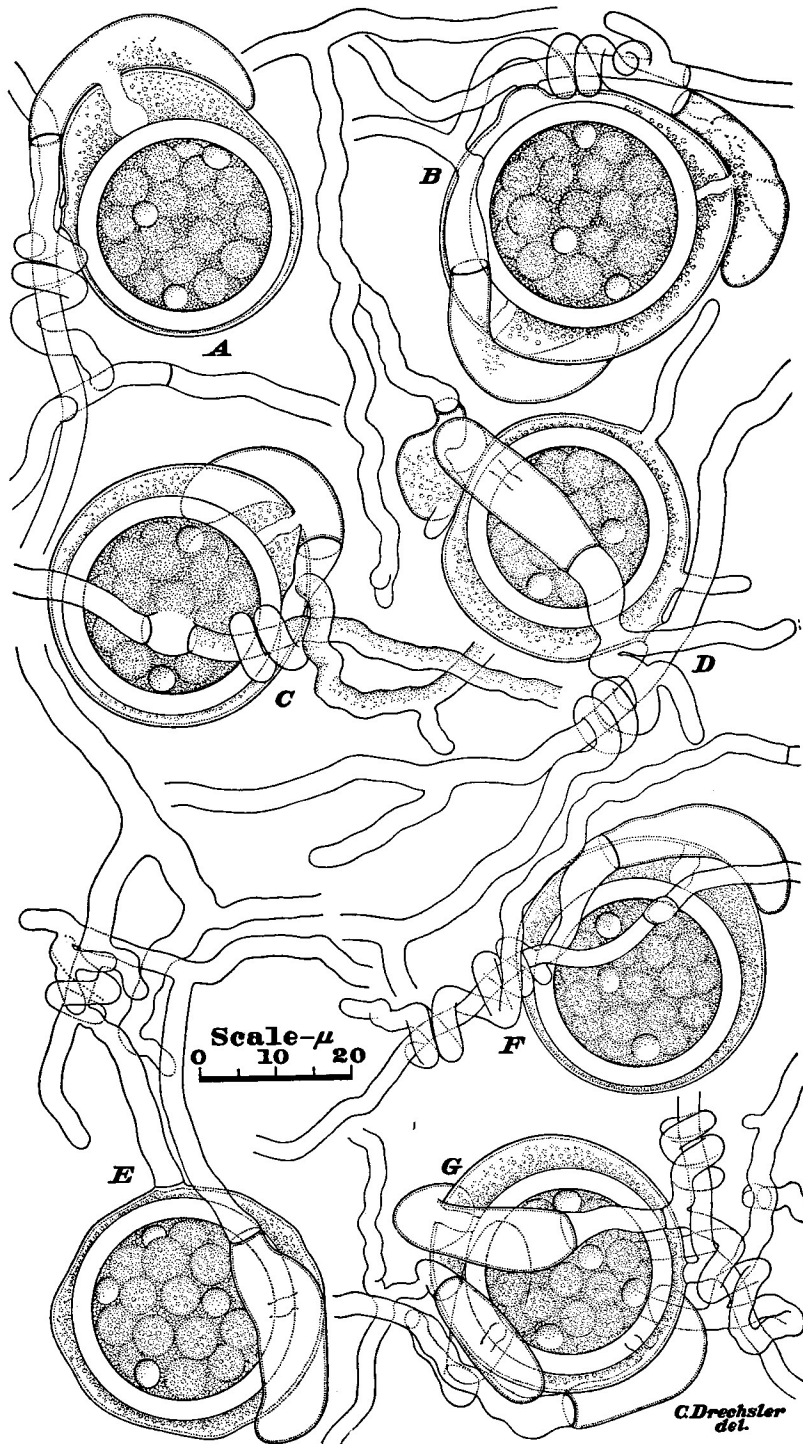


FIG. 10. A-G, Sexual apparatus of *Pythium helicoides*; $\times 1000$ throughout.

somewhat stout fertilization tube, through which the antheridial contents are transferred, arises ordinarily from a position approximately median between the base and the apex of the male organ, rather than from an apical position, as in most oomycetes. It usually attains a greater length than the homologous structures in most species of *Pythium*,—a detail of development in many instances related to a protrusion of the oogonium at the region of contact. A very characteristic relationship of the filaments supporting the sex organs is consistently present in a helicoid involvement of an oogonial hyphal element by an antheridial hyphal element. Two close turns, comparatively regular in a geometrical sense, are usually described in each spiral, though, by juxtaposition of 2 spirals, 4 turns sometimes appear as if making up a single series (Fig. 10, F, G). At least one spiral arrangement is found associated with each unit of sexual apparatus. In units where the oogonium is fertilized by plural antheridia of separate origins, only one antheridial system may participate in the involvement (Fig. 10, D, G); or, again, additional involvement may be achieved by one or another of the supernumerary male hyphae.

The mature oospore of *Pythium helicoides*, as has been pointed out earlier (17, 20), shows a very characteristic internal organization. Its content of oily reserve material, instead of being concentrated in a single large central globule, is divided into a half-dozen to a score of smaller globules distributed more or less evenly throughout the murkily granular protoplasm. Likewise, instead of a single refringent body, 2, 3, or 4 such bodies may be discerned imbedded in the granular material at some distance from one another (Fig. 10, A-G).

Sexual apparatus of the type represented in *Pythium helicoides* was set forth also in the original descriptions of the 3 closely allied species, *P. oedochilum*, *P. polytylum* and *P. palingenes*. Similar apparatus has been recognized, too, in many cultures more recently isolated from various sorts of decaying plant materials. A fungus repeatedly obtained from watersoaked portions of leaves of the white water-lilies *Nymphaea odorata* Ait. and *N. tuberosa* Paine, collected in Massachusetts, New York, and Wisconsin, shows a general parallelism with *P. helicoides* not only in its oogonia, antheridia, and oospores, but in its proliferous zoosporangia, as well; and must accordingly be considered a member of the same intimate group. Whether the fungus in question is identical with the one isolated by Dissmann (12) from leaves of *N. candida* Presl. in Central Europe and discussed by him at length under the binomial *P. proliferum*, remains problematical. The elongated clasping antheridia described and figured as pertaining to the parasite on the European water-lily, certainly show much more similarity to the male organs of *P. helicoides* and its allies than to those ascribed to *P. proliferum* by de Bary. The "sickle-shaped bodies" that Dissmann was led by resemblances in outward shape to interpret as antheridia formed independently of oogonia, appear more correctly interpretable as appressoria. The production of such bodies at the surface of hard objects, observable in cultures of numerous species of *Pythium*, and more particularly the very copious development

of homologous modifications on aerial hyphae of *P. butleri* and *P. myriotylum*, whereby unmistakably these frequently aerial parasites are enabled to force their way through unbroken epicarp of cucumber fruits, for example, seem little expressive of a sexual function.

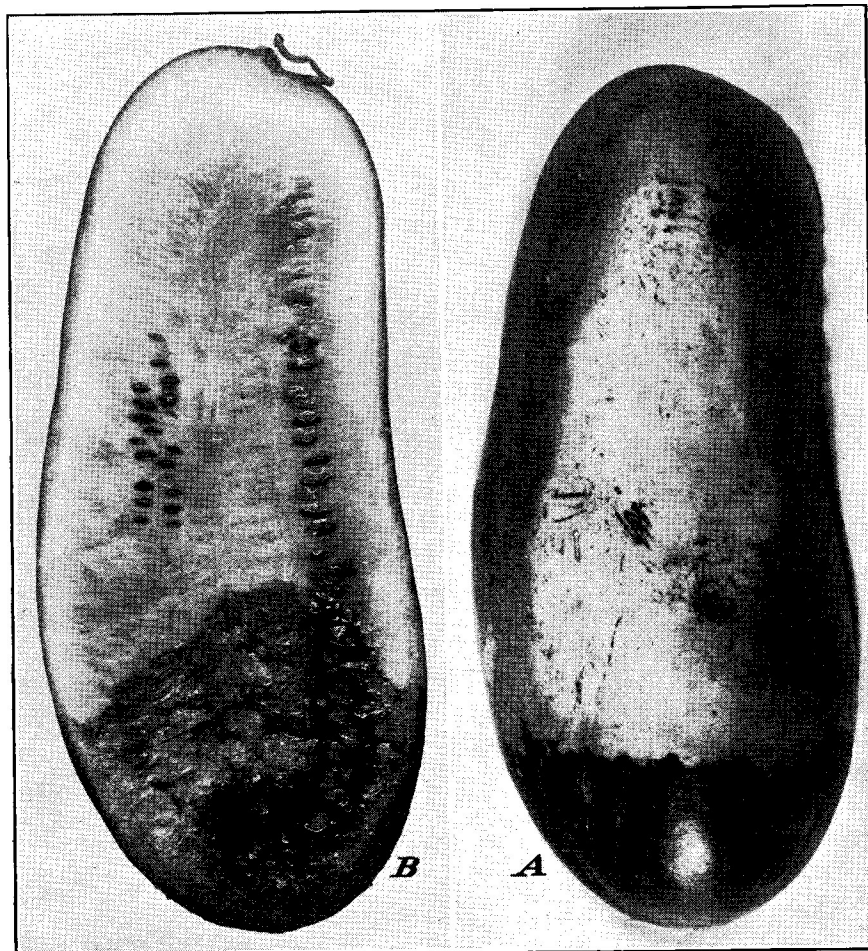


FIG. 11. Watermelon of the variety Irish Gray 8 days after inoculation with a pure culture of *Pythium anandrum*,—the inoculation having been effected by placing a bit of the culture into an incision through the stone-cell layer in the region of the flower scar, in order that the invasion might simulate the development following spontaneous infection; approximately $\times \frac{1}{3}$. A. External view. B. Longitudinal section along the axis of the fruit.

PYTHIUM ANANDRUM

A sexual stage reminiscent of *Pythium acanthicum* and *P. periplocum* is found combined with an asexual reproductive phase suggestive of *P. helicoides* in a fungus that I described (17) under the name *P. anandrum* in 1930, 6 years after isolating it from a decaying underground bud of a failing crown of rhubarb, *Rheum rhaponticum* L. As similarly affected crowns have not

since been received for examination, opportunity for determining to what extent the fungus occurs on rhubarb buds has been lacking. It has not been recognized in some thousands of cultures derived from decaying portions of numerous other flowering plants, and would seem, therefore, to represent a comparatively rare species. On artificial inoculation into watermelon fruits it causes a fairly rapid decay that, like the blossom-end rots caused by the 3 congeneric parasites already discussed, is manifested externally by dark-brown discoloration (Fig. 11, A), and internally by dilute sepia discoloration as well as by watery softening of the tissues (Fig. 11, B).

In pure culture on maize-meal agar *Pythium anandrum* reveals a handsome mycelial habit somewhat similar to that of *P. debaryanum*, *P. irregulare*, or *P. mammillatum*: the axial hyphae pursuing gracefully straightforward courses, and giving off at intervals perceptibly narrower, irregularly disposed branches that in turn bear stubby ramifications and diverticula (Fig. 12). After a few days oogonia begin to develop as globose bodies borne terminally on branches of variable lengths. At first smooth (Fig. 13, A) they soon become beautifully beset with numerous tapering spiny protuberances. Maturation brings about development of a thick-walled spherical spore showing the internal organization usual for oospores,—a single large central reserve globule being surrounded by a parietal granular layer, within which is imbedded a single subspherical refringent body (Fig. 13, B–F). Though the septum delimiting the oogonium is often convexly arched toward the spore, it is not evident that a stalk antheridium is present. Since branch antheridia are assuredly always absent, development here would seem consistently parthenogenetic. Just as in many other species of *Pythium*, occasional departures from the usual are to be seen, as, for example, production of 2 parthenospores in a biloculate oogonium (Fig. 13, G), and failure of abnormally small oogonia to put forth spiny protuberances (Fig. 13, H). Degeneration is usually not serious, even when maize-meal agar is employed that contains little maize-meal sediment in suspension; the healthy character of the development being reflected in moderate uniformity of dimensions. The measurements of 200 oogonia, selected at random, from which were derived the relevant metric data submitted in the original diagnosis, showed the following distribution of values for diameter (exclusive of protuberances) expressed to the nearest micron: 23 μ , 1; 24 μ , 5; 25 μ , 7; 26 μ , 23; 27 μ , 26; 28 μ , 47; 29 μ , 36; 30 μ , 26; 31 μ , 22; 32 μ , 6; 33 μ , 1. Measurements of the 200 oospores contained within these oogonia showed a distribution of values for diameter, as follows: 19 μ , 1; 20 μ , 1; 21 μ , 11; 22 μ , 18; 23 μ , 27; 24 μ , 47; 25 μ , 31; 26 μ , 30; 27 μ , 27; 28 μ , 7.

Pythium anandrum appears rather reluctant to reproduce asexually. Yet, now and then, following transfer of young mycelium in pieces of Lima-bean agar to a shallow layer of water, it has been observed to put forth long delicate submerged hyphae bearing solitary, terminal, prolate ellipsoidal, or somewhat ovoid sporangia, provided individually with an apical papilla of homogeneous consistency (Fig. 14, A–B, D–H). In shape, as well as in papillate condition,

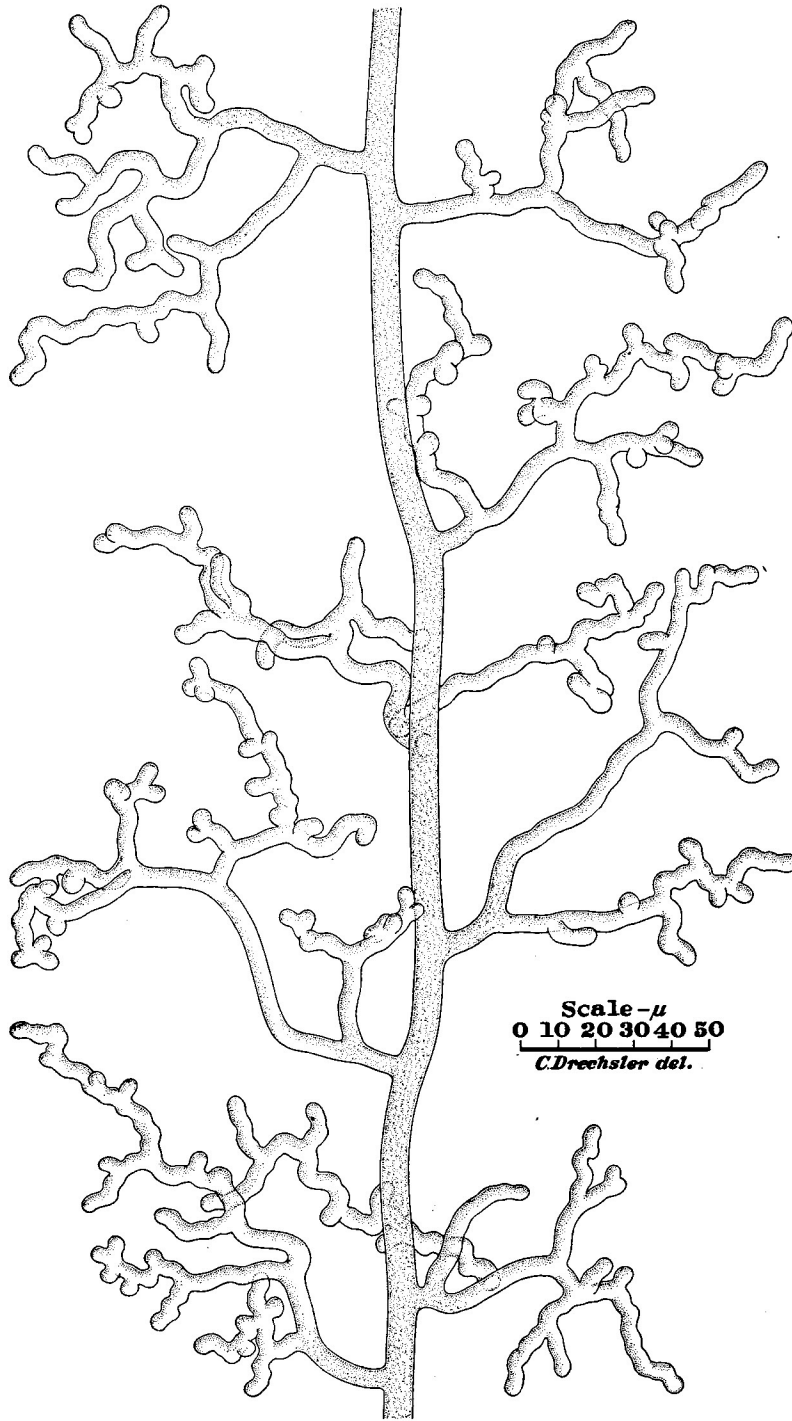


FIG. 12. A portion of submerged mycelium of *Pythium anandrum*, showing disposition of lateral branches on an axial hypha, and their manner of ramification; drawn from a maize-meal agar plate culture about 5 mm. back from the advancing margin of the growth; $\times 500$.



FIG. 13. A-H. Sexual apparatus of *Pythium anandrum*; $\times 1000$ throughout.

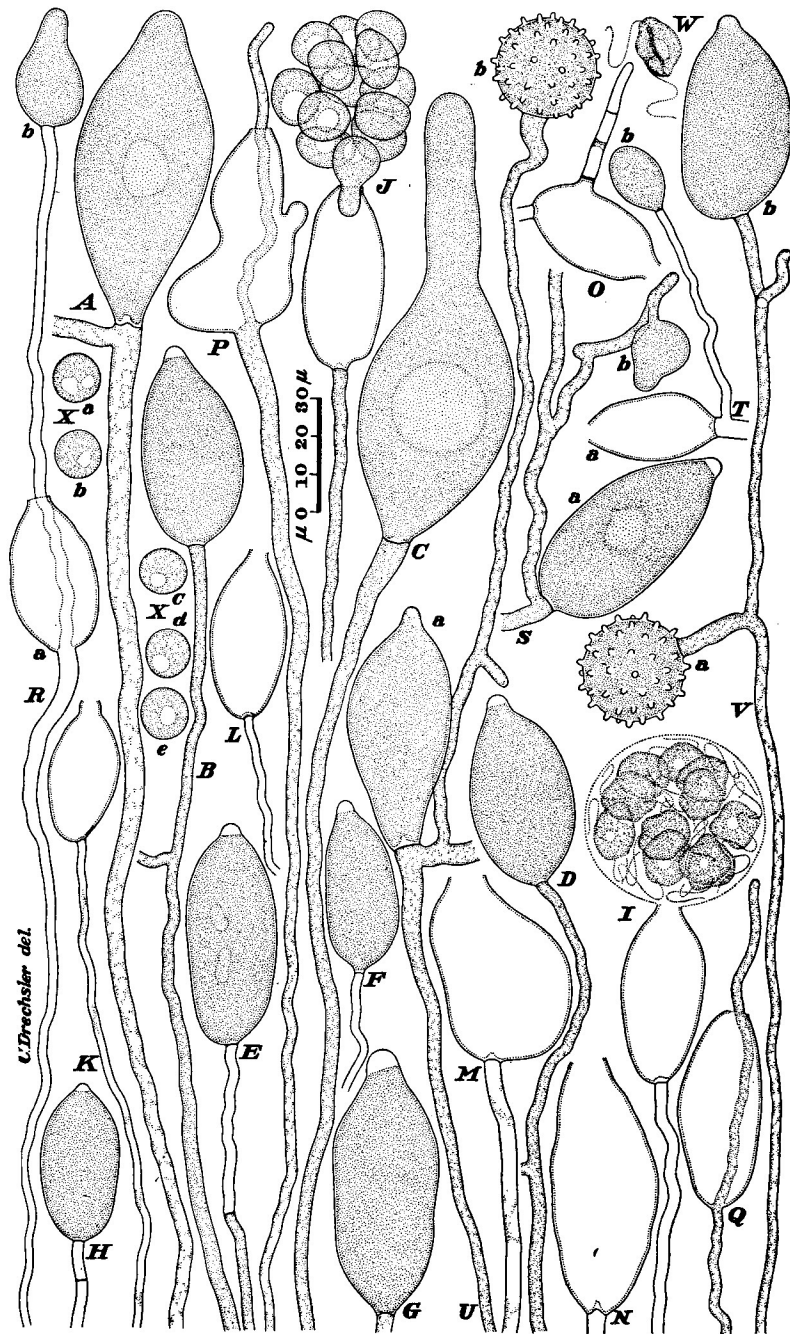


FIG. 14. Asexual reproductive apparatus of *Pythium anandrum*; $\times 500$ throughout.

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the sporangia seem comparable to those of certain species of *Phytophthora*, more particularly, perhaps, of *Phytophthora citrophthora* (Sm. & Sm.) Leonian and *P. colcasiae* Rac. Certainly, in most instances, no recognizable evacuation tube is formed preliminary to dehiscence, the sporangial contents, apparently while in a wholly undifferentiated state, being discharged directly into a sessile vesicle resulting from inflation of the papilla. Within this vesicle the relatively large biciliate zoospores are fashioned wholly after the manner usual in the genus *Pythium* (Fig. 14, I). Following rupture of the gelatinous membrane these ordinarily swim about for some time (Fig. 14, W), eventually to encyst (Fig. 14, X, a-e) in scattered positions; though, occasionally, for lack of water in sufficient quantity, they may round up in a cluster at the mouth of the sporangium (Fig. 14, J). Sometimes no further development takes place (Fig. 14, K-O), but at other times the sporangiophore resumes growth by extending itself through the empty sporangial chamber (Fig. 14, P; Q; R, a) and then bears a second sporangium farther on (Fig. 14, R, b). Often, too, a second sporangium (Fig. 14, S, b; T, b) is formed on a lateral prolongation of the sporangiophore arising from a position immediately below the basal septum delimiting the first (Fig. 14, S, a; T, a). A fertile hypha not infrequently gives rise at some little distance from the sporangium (Fig. 14, U, a; V, b) borne by it, to an oogonium (Fig. 14, U, b; V, a), which here, owing to the aquatic environment, is usually ornamented only with rather small protuberances. Because of unfavorable conditions a sporangium may suffer functional frustration, and thereupon put forth a stout process often somewhat resembling the evacuation tubes of other species (Fig. 14, C).

A combination of proliferous sporangia with spiny oogonia in one and the same fungus is recorded in de Bary's account (3, 4) of his *Pythium megalacanthum*. Since, in that account, branch antheridia from 1 to 4 in number are set forth as supplying the terminal or intercalary oogonia, sporangia are described as regularly producing evacuation tubes previous to dehiscence, and extraordinarily large zoospores are stated to round up into cysts averaging 18 to 20 μ in diameter, the species discussed is manifestly not identical with *P. anandrum*. Yet, despite the very obvious specific differences, it cannot be considered impossible that de Bary's fungus may have been a form morphologically and taxonomically less alien to *P. anandrum* than is the impressive spiny parasite from flax, *Linum usitatissimum* L., roots dealt with by Buisman (6) and by Diddens (11) under the binomial *P. megalacanthum*,—a parasite intimately related to 2 somewhat smaller species, *P. polymastum* Drechsl. and *P. mastophorum* Drechsl., neither of which has hitherto revealed any proliferous tendency in its asexual reproduction (17).

SUMMARY

Decay of watermelons caused by one or another of 9 known species of *Pythium* has been found widely distributed in the United States. In regions where the crop grows and matures under moderately dry conditions, the

losses are usually insignificant. Rather substantial losses result, however, in some parts of the Middle Atlantic States, where during wet seasons a late crop is exposed to more abundant infection. Normally, the fungi gain entrance into uninjured fruit at the flower scar, their advance through the massive berry being manifested externally either in a water-soaked appearance or in dark brown discoloration, depending in large part on the identity of the parasite concerned. Descriptive accounts of 3 species producing dark brown blossom-end rot, *P. acanthicum*, *P. periplocum* and *P. helicoides* are given herein to supplement the diagnoses previously published. Similar discussion is devoted also to *P. anandrum*, one of many species capable of causing decay when inoculated artificially into watermelons, but so far not known to occur spontaneously on fruits in the field.

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