PYTHIUM SCLEROTEICHUM N. SP. CAUSING MOTTLE NECROSIS OF SWEETPOTATOES[1]

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INTRODUCTION

In an extended account of mottle necrosis, a distinctive decay affecting the enlarged edible roots of the sweetpotato (*Ipomoea batatas* (L.) Lam.), Harter and Whitney (*9*)[2] in 1927 referred to one of its causal parasites as *Pythium scleroteichum* Drechs., stating in explanation that the fungus represented a new species which the present writer would subsequently describe. The promised description has been long delayed in the hope that the fungus might be induced to exhibit a zoosporangial stage in addition to the sexual stage then known, and thus make possible a statement, at least tolerably complete, with respect to the several phases of main importance in the morphology of oomycetes generally. This hope has as yet not been fulfilled, in spite of the considerable variety of substrata and cultural conditions that have been brought into play. Although past experience with some similarly refractory congeneric forms gives reason to expect that zoospore production may ultimately be induced, the degree of economic importance attaching to the parasite, and consequently the increased desirability of avoiding nomenclatorial complications in connection with it, argues against further delay in providing the nomen nudum with such a description as present knowledge permits. Fortunately the binomial in question has not been applied to any other form in the meantime; nor, on the other hand, has the fungus been described as new under some other name. Indeed, so far as can be determined, the original mycological and phytopathological literature of the intervening period contains no reference to the binomial, and makes no mention either of the fungus or of the disease caused by it such as might imply a first-hand examination. This absence of pertinent references, in spite of the unusually brisk descriptive activity of these years, provides some testimony relevant to the occurrence of *Pythium scleroteichum* in nature.

OCCURRENCE OF PYTHIUM SCLEROTEICHUM IN NATURE

Unlike most of its better known congeners, especially those commonly associated with damping-off of seedlings and rootlet decay of crop plants at later stages of development, the fungus does not appear frequently in isolation plate cultures made from miscellaneous collections of diseased or decaying vegetable materials. Although the writer has examined thousands of cultures prepared in the course of more than a decade from materials representing host plants of numerous species, *Pythium scleroteichum* has been recognized only

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[2] Reference is made by number (italic) to Literature Cited, p. 889.

among cultures obtained from sweetpotatoes, and of these more particularly among cultures derived from diseased tissues of the enlarged edible roots affected with mottle necrosis, or from rootlets directly attached to such roots. That its presence in rootlets thus attached ensues often from infection directly from the soil is suggested by positional relationships to the extensive lesions within the massive root. Probably, however, this infection does not usually occur until rather late in the growing season, as the parasite was found only rarely among the various species of *Pythium* comprising an assortment of several hundred cultures isolated from affected rootlets of young sweetpotato plants taken from the hotbed or seed bed at planting time in 1924 in connection with investigations reported by Harter (*7*).

The edible root of the sweetpotato may thus be regarded as the characteristic habitat of *Pythium scleroteichum;* yet it must be emphasized that the same habitat and pathological expression are shared certainly by the congeneric *P. ultimum* Trow, and inferentially, on the evidence of pure cultures isolated from diseased roots in the autumn of 1924, also by an unidentified species of *Phytophthora.* As might be expected, the relative proportions in which these three fungi are obtained from diseased specimens collected in the field vary in different seasons. In 1924, when mottle necrosis was evidently more widely prevalent than usual in Maryland, Delaware, and Virginia, *Pythium ultimum* predominated strongly, accounting for approximately 8 out of every 10 diseased specimens collected at the Arlington Experiment Farm, Rosslyn, Va. The remaining specimens yielded *P. scleroteichum* and the *Phytophthora* species in about equal numbers. However, in the autumn of 1928, when mottle necrosis occurred much less frequently, all of the relatively few diseased specimens found at the Arlington farm yielded cultures of *P. scleroteichum.* Similarly, a half dozen diseased specimens from Conesville, Iowa, in September 1932, and a like number from Indiana in October 1932, all yielded cultures of *P. scleroteichum* without any evidence of mixture with either of the other two fungi.

The identification of material from Iowa and Indiana extended the known distribution of *Pythium scleroteichum* into the Middle Western States and suggested that it and mottle necrosis will ultimately be discovered in other regions where sweetpotatoes are grown. A brief note entitled "New Zealand Sweet Potatoes Diseased" and published presumably by W. G. Smith (*10*) as early as 1884 supplements the historical account given by Harter and Whitney and may be pertinent in connection with the distribution of *P. scleroteichum.* It recorded the receipt in England of "tubers" of *Ipomoea chrysorhiza* Hook., which on being cut open revealed in section numerous brown places similar to those in potato tubers invaded by the late-blight fungus, *Phytophthora infestans* (Mont.) de Bary. On microscopic examination a profuse growth of mycelium together with a few oospores bearing considerable resemblance to those of the genus *Peronospora* were seen, yet the fungus was held to be *Pythium debaryanum* Hesse. As *P. ultimum* has probably more frequently than any other species been designated by Hesse's binomial, this identification would seem to be nomenclatorially intolerable, though perhaps accidental, consonance with findings concerning mottle necrosis. It is altogether unlikely, especially since species of *Pythium* as found in their natural hosts are usually in a condition quite unsatisfactory for the

recognition of many significant morphological features, that Smith's specimens could have supplied the information necessary to distinguish between *P. ultimum* and *P. scleroteichum* even if the respective specific characteristics of these two forms had then been known.

Several peculiarities, particularly the strange labyrinthine course of infection and the pronounced disintegration of host tissue and parasite alike throughout the larger lesions, except in the newly invaded marginal parts of freshly water-soaked appearance, contribute toward making mottle necrosis one of the most remarkable of diseases. As these peculiarities obtain, regardless of which of the several causal parasites happens to be involved, it may be concluded that they are due less to the specific characteristics of the fungi concerned than to the properties of the host tissues affected. The irregularity of the lesions would seem in some way to be related to the anatomical differentiation of tissues within the enlarged root, which tissues in general are, moreover, firmer and much less succulent than those of nearly any other relatively mature massive plant structure now known to be invaded by members of the genus *Pythium*. A somewhat similar though less pronounced irregularity is evident in the edible root of the radish (*Raphanus sativus* L.) affected with "black root", caused by another phycomycetous parasite, *Aphanomyces raphani* Kendrick.

As in naturally infected specimens the final conversion of invaded parts into grayish, somewhat felty, dry masses entails the disintegration of the parasite as well as of the host tissue, attempts to isolate *Pythium scleroteichum* or *P. ultimum* from the central portions of the larger morbid pockets are rarely successful. It is not surprising, therefore, that adherence to customary procedure, in failing to reveal the causal fungi, led to an earlier interpretation of mottle necrosis as a physiological trouble (*8*). Yet isolation of either of the parasites is readily accomplished by employing the technic set forth in another paper (*5*), provided care is taken to start with material excised from the newly invaded water-soaked periphery of actively developing lesions.

CULTURAL CHARACTERISTICS

In pure culture *Pythium scleroteichum* is not very striking to the naked eye, though distinguishable from some congeneric forms frequently encountered as plant parasites. On transparent media of moderate richness like maize-meal decoction agar, its mycelium is generally diffuse without marked luster and without either a pronounced radial or cumulous appearance. Aerial growth on such media is ordinarily absent, though on richer substrata and especially in tube cultures rather meager aerial development may sometimes be observed. With respect to rate of mycelial extension the fungus is to be reckoned among the many members of the genus that are intermediate between the species of unusually rapid growth like *P. butleri* Subr. and *P. ultimum* near the one extreme and the relatively slow-growing forms like *P. complens* Fischer and *P. euthyhyphon* Sideris near the other.

Nor does the vegetative thallus of *Pythium scleroteichum* present any conspicuous features under the microscope, even if the somewhat haphazard manner of branching results in a random disposition of the mycelium and makes its appearance considerably different from that, for example, of *P. debaryanum* or of *P. anandrum* Drechsl., in either of

which radially oriented, straight-forward, axial hyphae bear at fairly regular intervals freely ramifying branches of limited length and characteristically irregular course. The mycelial filaments, though in general somewhat smaller in diameter than those of the coarser members of the genus like *P. butleri*, *P. ultimum*, or *P. anandrum*, certainly do not give the impression of minuteness conveyed by such delicate congeners as *P. complens*. Appressoria are produced in relatively small numbers where the mycelium encounters the surface of a solid body like the base of a Petri dish, occurring as in most congeneric forms as swollen terminal modifications that after functional frustration may grow out into processes of connected sickle-shaped parts.

Pythium scleroteichum appears more exacting in its cultural requirements for the normal development of sexual apparatus than most of the better known congeneric forms associated with plant diseases. Familiar congeneric plant pathogenes like *P. debaryanum*, *P. ultimum*, and *P. butleri* show such satisfactory mycelial growth and form sex organs and normal oospores in such adequate numbers on maize-meal agar made by adding agar-agar to a filtered or strained decoction of maize meal that this easily prepared and readily manipulated medium has come into wide use for the cultivation of species of *Pythium*, *Phytophthora*, *Aphanomyces*, and other phycomycetous genera. On this maize-meal agar medium the fungus under consideration likewise shows good mycelial growth and gives rise to an abundance of oogonia with accompanying antheridia, but degeneration of the oogonial contents is usually so prevalent that only one normal oospore may result from several hundred female structures. If, however, the medium is modified by incorporating in it together with the decoction a considerable quantity of the finer maize-meal sediment and then adding enough hydrochloric acid to change the pH value, ordinarily about 6.0, to 5.0 or 4.5, oogonial degeneration becomes negligible and normal oospores are produced in extraordinary quantity. Similar improvement in production of normal oospores by cultivation on the more substantial maize-meal agar medium has been observed in other members of the genus, notably in the fungus described by Braun (*2*) as *P. complectens* (which, however, would appear to be the same as that on which de Bary based at least one of his figures [*1*, *pl. 5*, *fig. 4*] of his *P. vexans*), in the flax parasite identified by Buisman (*3*) as *Pythium megalacanthum* de Bary, and in the species described by the writer (*6*) as *P. polymastum*, *P. acanthicum*, *P. periplocum*, and *P. myriotylum*.

MORPHOLOGY

The use of a medium suitable for the production of normal sexual structures is especially important because the distinctiveness of *Pythium scleroteichum* attaches very largely to the morphology and relationships of oogonium, antheridium, and oospore. The antheridia vary in number from 1 to 5 and are borne on rather slender longish branches all of which usually (fig. 1, *A–C*, *E–I*; fig. 2, *A–F*, *H*, *I*), though not always (fig. 1, *D*; fig. 2, *G*) represent ramifications of a single parent filament, which may be a branch of the oogonial hypha (fig. 1, *C*, *G*; fig. 2, *F*, *I*), or have some other close connection with the oogonial hypha (fig. 2, *C*, *D*, *H*), or, again, may be merely a neighboring filament without demonstrable connection with the oogonium (fig. 1, *B*, *E*, *F*, *H*, *I*; fig. 2, *A*, *E*). In any case, when the fungus is

cultivated on agar media the hyphal elements directly bearing the male organs are wrapped intimately and extensively about the oogonium in a manner prevalent among various species of *Aphanomyces* parasitic on higher plants (*5*). Similar enwrapment of the oogonium has been noted previously (*6*) in a discussion of *P. periilum* Drechsl., which species would seem to articulate more closely than any other with the one under consideration. In *P. myriotylum* intimate application of the antheridial branches is varied with looser arrangement of these elements, and the same condition obtains, even in larger measure, in *P. graminicolum* Subr. and *P. arrhenomanes* Drechsl. Butler (*4*) found extensive enwrapment of antheridial branches about the oogonia of a fungus parasitic on tobacco in Nyasaland, provisionally designated by him as *P. aphanidermatum* (Eds.) Fitzp. Such disposition of antheridial branches, as Butler intimated, is quite foreign to *P. aphanidermatum* and to the larger *P. butleri*, so that final reference of the tobacco parasite to either of these species would seem definitely precluded. Agreement in sizes of oogonium and of oospore suggests the possibility of identity with *P. scleroteichum*, though the abundant production of zoospores provides a feature strongly at variance with the performance of the latter. In spite of some evident discrepancies, the likelihood of the Nyasaland fungus being *P. myriotylum* deserves consideration, especially as a number of cultures isolated from tobacco seedlings by S. C. J. Jochems in Sumatra and sent to the writer in 1929 were readily identified with that parasite of mostly tropical and subtropical distribution.

In emphasizing the intimate enwrapment of the oogonium by the antheridial branches as a specific characteristic of *Pythium scleroteichum*, it must be mentioned that when the fungus is cultivated under aquatic conditions, the apparatus sometimes is formed away from solid material and this enwrapment then is largely supplanted by a much looser relationship. Apparently the rigidity of a solid, or at least firm, medium in preventing the yielding of one sexual element relative to the other, is of consequence in bringing about the intimate arrangement. However, even if this arrangement were dependent entirely on the merely mechanical properties inhering in a solid substratum, it yet would constitute a feature of importance, since in nature the fungus undoubtedly develops its sex apparatus in such substratum, and even under artificial cultivation has hitherto failed to produce normal oospores in the absence of solid material.

Often the antheridial branches of *Pythium scleroteichum* show a number of sharp transverse indentations or furrows (fig. 1, *E;* fig. 2, *C, D, F, H*) the like of which has never been observed in other members of the genus. The male organ itself, just as in *Pythium periilum*, is generally upcurved from the oogonium, not only being applied distally with its somewhat flattened apex, but often, though not always, also making contact proximately by means of its narrowed basal part. As the wall of the antheridium is appreciably thinner than in some other species like *P. butleri* and *P. ultimum*, this structure, which is besides rather small in comparison with the oogonium, has a characteristically frail appearance. After becoming emptied of contents both the antheridia and the branches bearing them become exceptionally difficult to follow, so that in older cultures only the morphology of the mature oospore and of the surrounding oogonial envelop is clearly revealed.

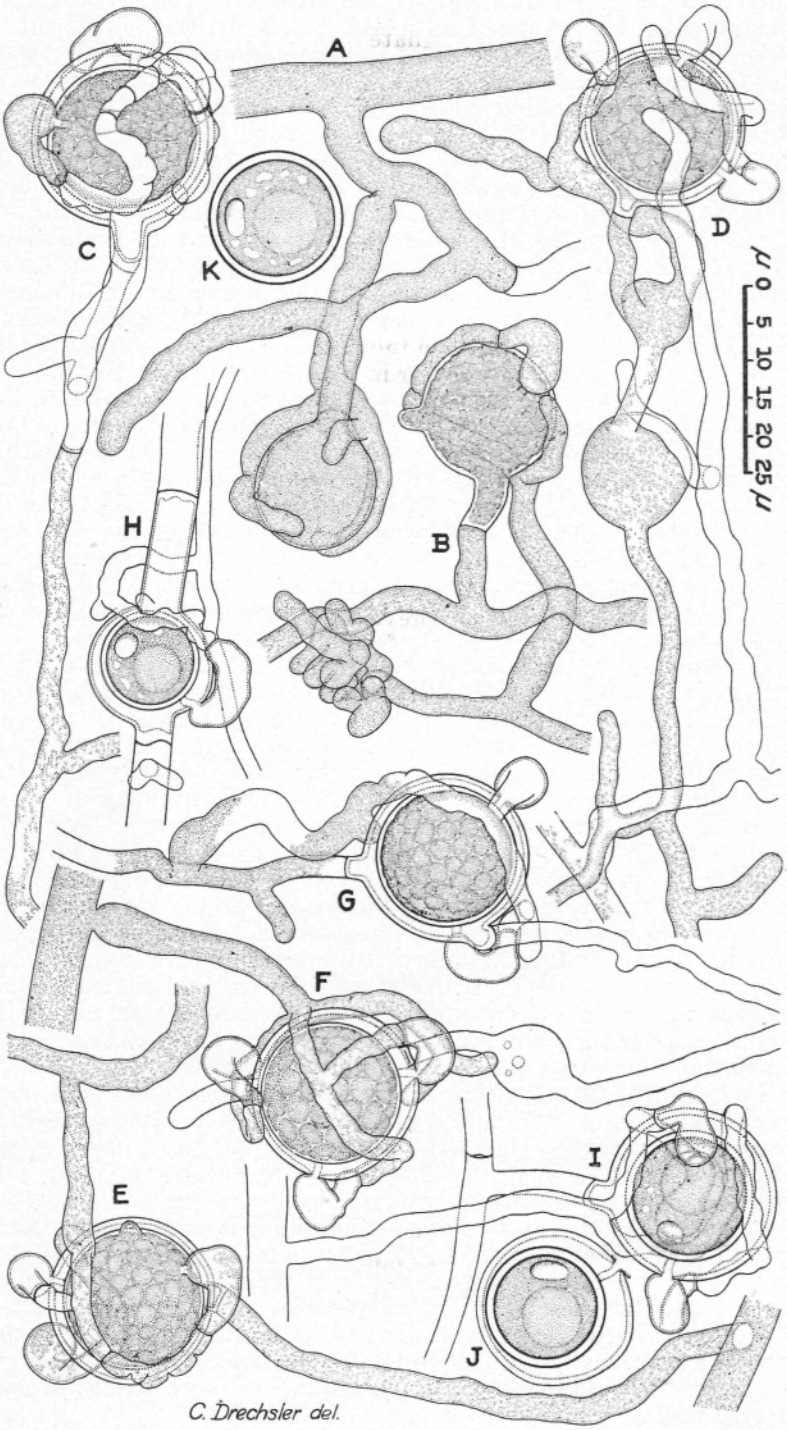

FIGURE 1.—For explanatory legend see opposite page.

With respect to size, neither the oogonium nor the oospore of *Pythium scleroteichum* is exceptional, the diameter in both cases showing a range of magnitudes shared by the corresponding dimensions in a number of better known congeners. Thus 250 oogonia chosen at random in plate cultures of acidulated agar-containing decoction and ample sediment of maize meal, 15 to 20 days after planting, yielded measurements of diameters distributed according to the nearest micron as follows: 16μ, 1; 17μ, 1; 19μ, 1; 20μ, 4; 21μ, 21; 22μ, 22; 23μ, 52; 24μ, 64; 25μ, 43; 26μ, 23; 27μ, 13; 28μ, 3; 30μ, 1; 32μ, 1. Measurements of the diameters of the 250 oospores contained within these oogonia yielded values distributed as follows: 11μ, 1; 13μ, 1; 14μ, 1; 15μ, 7; 16μ, 16; 17μ, 33; 18μ, 47; 19μ, 56; 20μ, 44; 21μ, 30; 22μ, 11; 23μ, 2; 26μ, 1. From these measurements averages of 23.8μ and 18.7μ for diameter of oogonium and of oospore, respectively, were computed, which are not far from the homologous values for *P. ultimum*, *P. paroecandrum*, and *P. aphanidermatum*.

The oospore of *Pythium scleroteichum* is somewhat unusual in being distinctly yellowish when mature, the coloration even in entirely pure culture being comparable in intensity with that usually observed in other species only in isolation plate cultures, that is, in the presence of bacteria and disintegrating host materials. Although coloration in oospores of various species of *Pythium* is often associated with relatively thick oospore walls, this association does not obtain in the fungus under consideration, as its oospore wall is rather thin in comparison with the same structure in congeneric forms (fig. 1, *J*, *K*). On the other hand, except in the areas of contact with the apices of antheridia, the oogonial wall is relatively thick and of unusual resistance to external strain (fig. 1, *J*). Because of this sturdiness the agar medium may dry up without bringing about noticeable distortion of the original spherical shape, whereas under similar conditions the oogonial envelope of *P. ultimum* usually collapses markedly, and that of *P. debaryanum* often loses nearly all semblance of its earlier appearance. As the physical character of the oogonial envelope provides a feature conspicuous even under casual microscopic inspection, it was deemed suitable for recognition in the specific term applied to the fungus.

TECHNICAL DESCRIPTION

Pythium scleroteichum, n.sp.

Intramatrical mycelium in favorable transparent medium diffuse, without lustrous or radiating or markedly cumulous appearance, capable of approximately 26 mm radial extension in 24 hours at 24° C.; consisting of hyphae mostly 2.5μ to 7μ in diameter and bearing in small or in moderate number terminal clavate

EXPLANATORY LEGEND FOR FIGURE 1

Sexual apparatus of *Pythium scleroteichum* developed in plate cultures of acidulated maize-meal suspension agar; drawn with the aid of the camera lucida at a uniform magnification. ×1,000.

A.—Early stage previous to appearance of septum delimiting oogonium and before appearance of antheridia on the clasping branches, the latter being of monoclinous origin.

B.—Somewhat later stage; oogonium and one antheridium delimited by septa; diclinous.

C.—A still later stage; one antheridium emptied and the other discharging contents into oogonium; monoclinous.

D.—A stage shortly following fertilization; one antheridium of monoclinous origin, two of diclinous origin.

E, F.—Diclinous apparatus; one of the antheridial branches in *E* showing transverse indentations.

G.—Oogonium intercalary; closely monoclinous relationship.

H, I.—Mature apparatus; diclinous.

J.—Mature oospore within oogonial wall, showing thickness of oospore wall and of oogonial membrane, as well as size and shape of reserve globule, refringent body, and fertilization tube.

K.—A mature oospore.

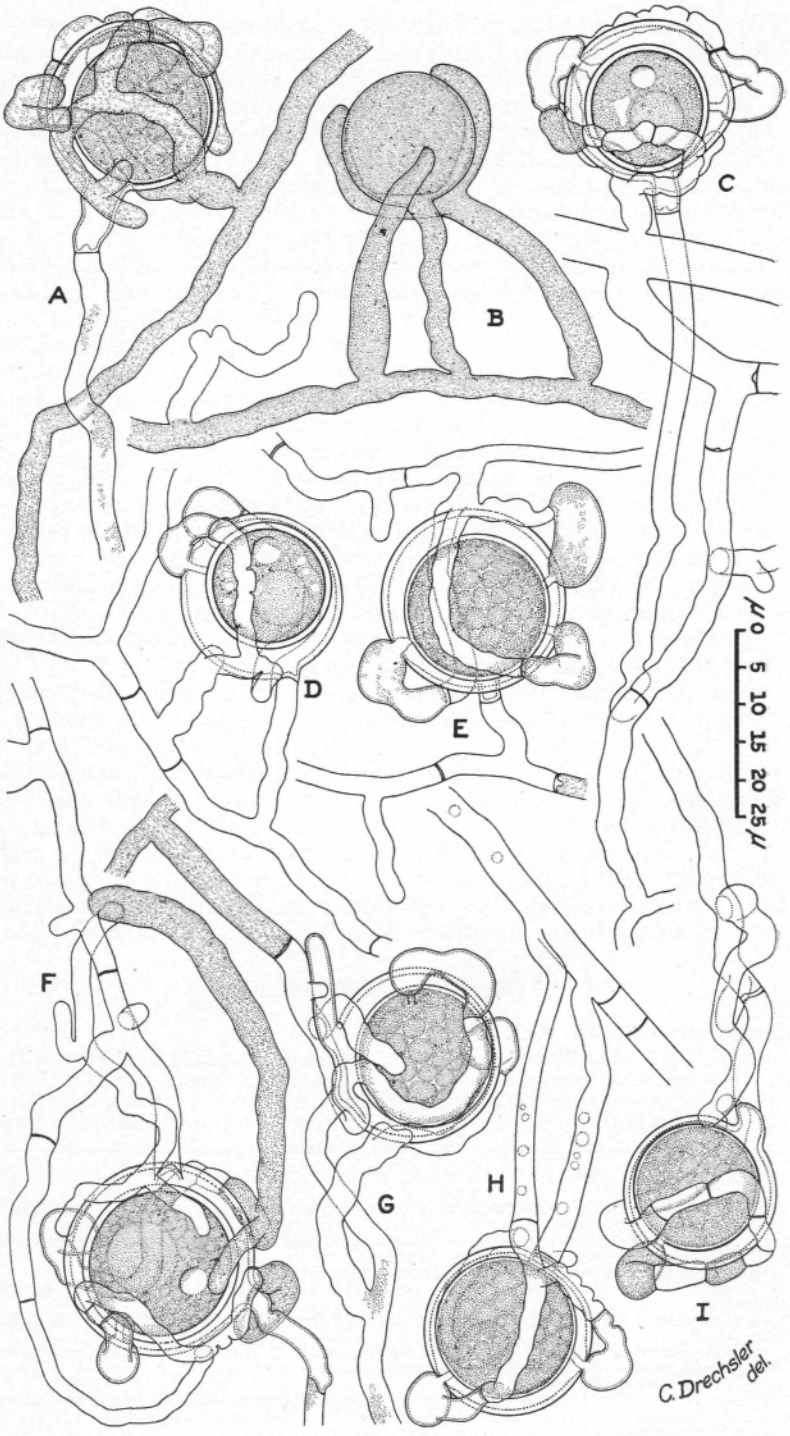

FIGURE 2.—For explanatory legend see opposite page.

appressoria mostly 5μ to 12μ in diameter. Aerial mycelium absent or present in meager quantity.

Oogonium intercalary or more frequently borne terminally on the longer hyphae or on shorter branches; provided with a smooth sturdy wall, 0.5μ to 1.2μ, mostly 0.7μ to 1.1μ in thickness, though conspicuously thinner in areas of contact with apices of antheridia; subspherical, measuring 16μ to 32μ, mostly 21μ to 27μ (average 23.8μ) in diameter, the delimiting septum or septa rather regularly inserted beyond the spherical contour so as to include a cylindrical part or parts, mostly 1μ to 10μ in length. Antheridia regularly clavate, crooknecked, measuring 4μ to 7μ in diameter in wider distal part and 9μ to 16μ in length along curved axis from apex to basal septum, the apical end bluntly rounded and making narrow contact with the oogonium or flattened and more broadly appressed, in either case giving rise to a fertilization tube approximately 1μ in diameter and 1μ to 3μ in length; varying in number usually from 1 to 5 (average 3) to an oogonium; borne usually terminally or occasionally laterally on branches which sometimes contain a number of septa and are often constricted at intervals by abrupt, relatively deep, transverse, dorsal furrows, but which in any case are frequently, together with vegetative ramifications arising from them, wrapped extensively and intimately about the oogonium; antheridial branches supplying an oogonium arising frequently from a single parent hypha, or sometimes from two parent hyphae without close mycelial connection with one another, the single parent element or one of the plural elements often constituting a ramification of the oogonial hypha, the filamentous connection between antheridium and oogonium then not usually measuring less than 75μ in length. Oospore distinctly yellowish, smooth, largely filling oogonium, measuring 11μ to 26μ, mostly 15μ to 22μ (average 18.7μ) in diameter, with a wall 0.8μ to 1.4μ, mostly 0.9μ to 1.3μ, in thickness, a reserve globule 6.5μ to 11.5μ (average about 8.6μ) in diameter, and a refringent body oblate ellipsoidal or subspherical in shape, in latter case mostly 3.5μ to 5μ in diameter.

Causes a mottled decay especially of the enlarged edible root of *Ipomoea batatas* (L.) Lam. in Delaware, Maryland, Virginia, Indiana, and Iowa.

Mycelium ramosum, hyphis 2.5–7μ crassis; oogoniis globosis terminalibus subinde intercalaribus, 16–32μ diam., membrana 0.5–1.2μ crassa; antheridiis 1–5μ, clavulatis, curvulis, 9–16μ longis, 4–7μ crassis; ramis antheridialibus longis, saepius ramosis, ex origine monoclina vel diclina, oogonium amplectentibus; oosporis sphaericis, flavidis, 11–26μ diameter, membrana 0.8–1.4μ crassa; zoosporangiis ignotis.

Hab. parasitice in radicibus Ipomoeae batatae, Delaware, Maryland, Virginia, Ohio, et Iowa.

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EXPLANATORY LEGEND FOR FIGURE 2

Sexual apparatus of *Pythium scleroteichum* developed in plate cultures of acidulated maize-meal suspension agar; drawn with the aid of the camera lucida at a uniform magnification. ×1,000.

A.—Stage during fertilization; antheridia of diclinous origin, one lateral and one terminal in position.

B.—An early stage; closely monoclinous.

C.—A mature stage, more remotely monoclinous, one antheridial branch septate, the other showing transverse indentations.

D.—A mature stage, rather closely monoclinous; one of the antheridial branches with transverse indentations.

E.—A stage during fertilization; diclinous.

F.—A mature stage, the oogonium intercalary, the three antheridia of monoclinous origin, but two of them being borne on a bifurcating branch having separate origin from the branch bearing the third.

G.—A stage showing fertilization by two antheridia, one of monoclinous, the other of diclinous origin.

H.—A stage showing three antheridia of monoclinous origin, a branch bearing one of them having transverse indentations.

I.—A stage showing fertilization by a single antheridium borne on a septate branch; monoclinous.

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