[Reprinted from PHYTOPATHOLOGY, July, 1936, Vol. XXVI, No. 7, pp. 676–684.]

PYTHIUM GRAMINICOLUM AND P. ARRHENOMANES

CHARLES DRECHSLER

(Accepted for publication August 7, 1935)

In a recent paper Carpenter (3), after a consideration of literature on species of Pythium responsible for root injury, more particularly to graminaceous hosts, set forth the conclusion that the parasite associated in Hawaii with growth failure of sugar cane (*Saccharum officinarum* L.)—a parasite he had first referred to as *P. butleri* Subr., and later as *P. aphanidermatum* (Eds.) Fitz.—was identical with both *P. graminicolum* Subr. and *P. arrhenomanes* Drechsl. Subramaniam's statement (12) that the fungus isolated in India from diseased roots of wheat (*Triticum aestivum* L.) agreed with the description and drawings of the Pythium destructive to sugar cane in Hawaii (2), together with Matthews' corroborative citation of the "*P. aphanidermatum* of Carpenter" as a synonym of *P. graminicolum* (9), was held to establish identity with the one species; while the inclusion recently by Rands and Dopp (11), of an actual culture isolated in Hawaii from diseased sugar-cane roots among the strains of *P. arrhenomanes* studied by them, was interpreted quite justifiably as establishing identity with the other. Not illogically, this dual identification was held to imply identity of the two species themselves; wherefore, it presumably remained only to determine which of the two had been described the earlier to decide upon the name by which the fungus might be correctly designated. As priority appeared to favor Subramaniam's description over my own, *P. graminicolum* was adopted as the valid binomial; *P. arrhenomanes,* at the same time, being reduced to synonymy.

If the dispositions thus made were to be found in satisfactory agreement with the facts of morphology, the resulting simplification in taxonomy would be more welcome even than the abasement of a species name somewhat indecent in its spiritual connotation. It can not be doubted that the identification of the Hawaiian fungus used by Rands and Dopp as *Pythium arrhenomanes* was altogether correct. The detailed study by these authors, of numerous strains of the species both in comparison with each other and with many strains referable to a dozen other species of Pythium, could hardly have failed to supply more than adequate information and experience. On the other hand, Subramaniam's judgment as to the scope of his species relative to the fungus set forth as a parasite on sugar cane in Hawaii, was apparently based solely on information conveyed in Carpenter's early account. With respect to what would seem to constitute the most decisive morphological feature concerned here, the origin and mycelial connections of the antheridia, this account unfortunately is somewhat less clear than

might be desired. In the illustrations accompanying it, the parts probably meant to represent antheridia show no indication of being set off by basal septa; and many of the elements thus meagerly differentiated are shown juxtaposed to the oogonium in a manner leaving doubt as to whether structural continuity with, or disappearance beneath, the female organ was intended to be depicted. A degree of ambiguity is thereby introduced that would seem to make reference of the description to any particular one of several likely species about equally uncertain. Comparison of Carpenter's figures with those illustrating the original account of *P. graminicolum* reveals so little correspondence in origin of antheridial branches that Subramaniam's ready acceptance of specific identity appears somewhat surprising. Nor is a closer agreement evident when comparison with regard to antheridial connections is extended to Matthews' figures of presumably the same fungus.

Since the culture on which the description of *Pythium graminicolum* was based has long been lost, it it fortunate that the description in itself leaves little uncertainty as to the proper application of the species. The close mycelial connection between oogonium and antheridia, evident in Subramaniam's figure, is paralleled in many cultures that R. D. Rands, beginning in 1927, isolated from diseased roots of sugar cane, together with numerous strains of various other forms, among which, as was intimated in my earlier account (4), *P. arrhenomanes* was abundantly represented. These cultures were, from the beginning, recognized as belonging to a species so distinct from *P. arrhenomanes* that confusion between the two was not expected. The species in question had become known to me some years earlier through a single culture received from B. A. Bourne, who had isolated it in Puerto Rico from a diseased root of sugar cane in connection with investigations reported by him (1) in 1924.

The relationship of parts in the sexual apparatus to the Puerto Rican fungus is illustrated in figure 1. This figure, it may be explained, was drawn in 1925; so that its preparation was influenced neither by Subramaniam's paper, which appeared 3 years later; nor by Matthews' book, issued 6 years later; nor, least of all, by considerations growing out of the issue raised in Carpenter's recent contribution. A parallelism in antheridial connections, with the sexual apparatus of *Pythium graminicolum* as figured by both Subramaniam and Matthews, is, I believe, so readily apparent as to require no further exposition. In appropriately irrigated material the fungus gives rise to lobulate zoosporangia and zoospores likewise corresponding well to the homologous structures of the wheat parasite. It was therefore assigned, together with the morphologically similar cultures obtained from sugar-cane roots in the southern United States, to *P. graminicolum* as soon as the description of that species made its appearance.

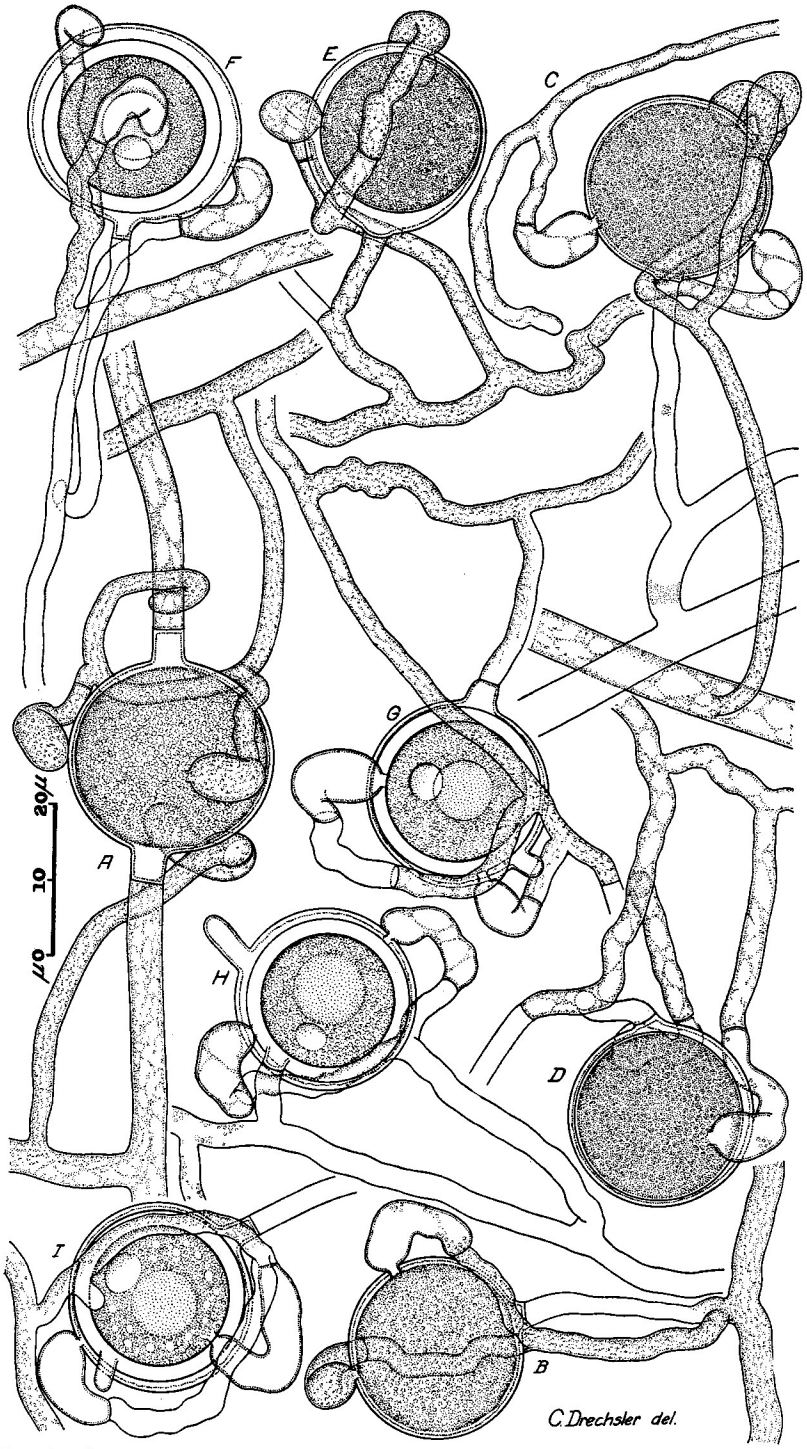

FIG. 1. Sexual apparatus of a Puerto Rican strain of *Pythium graminicolum* developed in a maize-meal agar plate culture, and drawn with the aid of the camera lucida. × 1000.

FIG. 2. Sexual apparatus of the type culture of *Pythium arrhenomanes* on maize-meal agar; drawn with the aid of the camera lucida. × 1000.

A conspicuously different relationship of parts is evident in figure 2, representing sexual apparatus of the type culture of *Pythium arrhenomanes* isolated from diseased roots of maize (*Zea mays* L.) by Helen Johann and employed in investigations reported by Johann, Holbert, and Dickson (7, 8). The mycelial connection between oogonium and antheridium here is much more distant than in *P. graminicolum;* usually, indeed, being too distant to be traced in the tangle of rather irregularly disposed filaments that make up the somewhat characteristic growth in agar plate cultures. In especially favorable material the continuity of hyphal elements between the male and the female organs can occasionally be seen, and, as in homothallic forms generally, such continuity is necessarily always present when a single mycelium occupies a tract of substratum. Rands and Dopp have well pointed out that the type culture produces antheridia more abundantly than most strains of the species. Yet the similarity in origin and arrangement of the male organs is recognizable without difficulty in strains producing these structures in more moderate quantity; as, for example, in the strain represented in figure 3, which was isolated from diseased maize rootlets submitted by W. D. Valleau as being typical of rootrot described from Kentucky by Valleau, Karraker, and Johnson (13). Figures 2 and 3 were drawn in 1927, and may, therefore, also be assumed to have been prepared in the absence of any possible prejudice bearing on the taxonomic issue under discussion.

Associated with the differences in arrangement of sexual apparatus are other differences, which, if less easily expressed in words or in drawings, are often experienced vividly enough when *Pythium graminicolum* and *P. arrhenomanes* are grown side by side on artificial substrata. On agar media containing maize-meal decoction with some of the finer maize-meal sediment, *P. graminicolum* develops a mycelium that in due course usually gives rise to oogonia and antheridia. For the most part each oogonium develops a normal oospore, which at maturity consists of a fairly thick wall, a parietal layer of granular protoplasm, a subspherical or ellipsoidal refringent body imbedded in the parietal lawer, and a central vacuole-like reserve globule (Fig. 1, F–I). The oogonial wall, the membrane of each antheridium, and the wall of each supporting hyphal element are so substantial that, after maturity, they, for the most part, long retain their respective original shapes without much alteration from collapse, and long remain very clearly visible to microscopic inspection. Asexual reproduction likewise is easily induced. When pieces of an agar plate culture with vigorous vegetative mycelium are appropriately irrigated, lobulate sporangial complexes are developed that freely give rise to zoospores. Although some strains are more refractory than others, the species, on the whole, readily reveals both of its reproductive stages, which, because of the continuing visibility of the sexual apparatus, are easy to study and to delineate.

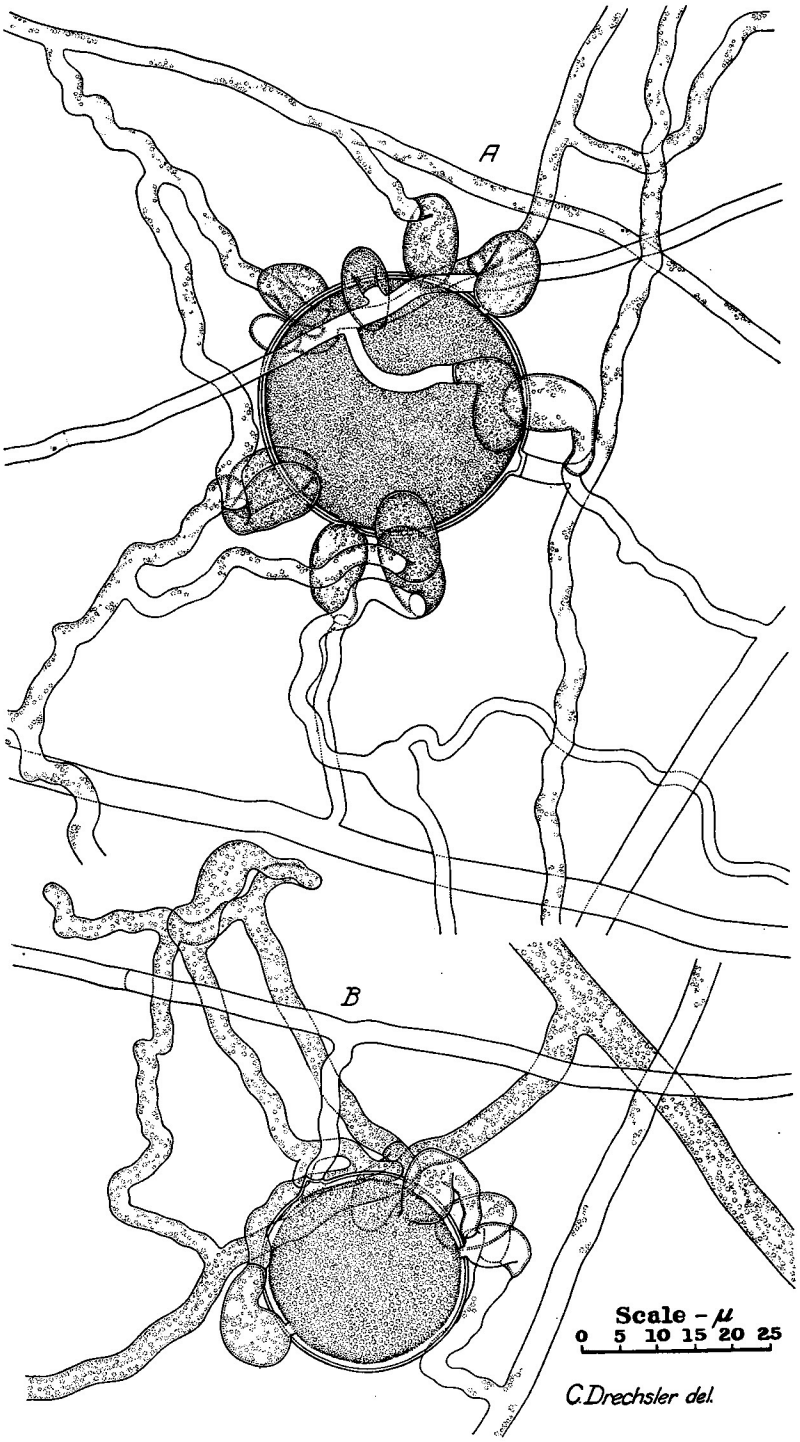

FIG. 3. Sexual apparatus of a Kentucky strain of *Pythium arrhenomanes* developed in a maize-meal agar plate culture, and drawn with the aid of the camera lucida. ×1000.

Pythium arrhenomanes, on the other hand, offers many exasperating difficulties. On ordinary maize-meal agar vegetative growth is profuse, and with many strains sex organs as well as massive lobulate sporangial complexes are soon produced in quantity. But the oogonia, together with the antheridia supplying them, almost without exception undergo degeneration, their contents either breaking down or migrating into hyphal outgrowths. Through the use of special media, like those devised by Johann (6) and by Rands and Dopp (10), the production of sexual apparatus can be stimulated, and its degeneration reduced; some strains, as, for example, the one represented in the type culture, then yielding oospores of normal structure in more than half of the numerous oogonia. Yet, even after normal development has been obtained, the frequently haphazard and rather intricate disposition of the rangy antheridial filaments is not always readily made out. Moreover, after fertilization has been completed, the antheridia and the branches supporting them become increasingly difficult to see; with the result that on maturity of the oospore they have become for the most part nearly indiscernible. The hypha bearing the oogonium similarly becomes less clearly visible, and even the oogonial wall will often lose much of its optical distinctness.

Though massive lobulate complexes are often formed abundantly when *Pythium arrhenomanes* is cultivated on maize-meal agar, these structures, on being irrigated in a manner successful for zoospore production in many congeneric forms, exhaust themselves in a promiscuous proliferation of aerial or submerged filamentous outgrowths, without giving any sign of normal sporangial development. Zoospore formation, it is true, has been observed a few times on irrigating pieces of Lima bean decoction agar occupied by vigorously growing mycelium; the output in all such instances being, however, very meager in comparison with that ensuing when freshly invaded maize roots are placed in water. The strong tendency toward degeneration of reproductive bodies, noticeable on most artificial media in common use, combined with the intricacy of the sexual apparatus and the early evanescence especially of the male parts, has undoubtedly been responsible in no small degree for the unimpressive descriptive treatment that long permitted the species to remain in obscurity.

A closer approach to the morphology and cultural traits of *Pythium arrhenomanes* than is seen in *P. graminicolum,* or, for that matter, in any other form known to me, is evident in a species I have described elsewhere (5) as *P. myriotylum.* The extraordinary haustorial development associated with the frequently aerial parasitism characteristic of this species, and the less pronounced inflation of its sporangial complexes, which, after developing on artificial media, rather readily give rise to zoospores, should suffice, nevertheless, to distinguish it from *P. arrhenomanes. P. myriotylum* has

not hitherto been recognized in any collections of ultures derived from diseased roots of graminaceous plants; and in a soil at all compact, would seem incapable of operating at a sufficient depth to attack plant parts far under ground. Its remarkable parallelism in parasitic habit and host range to *P. butleri* may be expected to make for its orientation with reference to that species of much different morphology, rather than for orientation with reference to the fundamentally more similar and apparently more closely related graminicolous form.

SUMMARY

Though *Pythium graminicolum* and *P. arrhenomanes* are both parasitic on sugar cane, being associated abundantly with root rots of this host in the southern United States, they represent separate species rather than a single species, as has recently been averred. A close mycelial connection between oogonium and antheridium, very frequent in *P. graminicolum,* is rare in *P. arrhenomanes;* and in parallel cultures the sturdy, more substantial membranous parts of the sexual apparatus of the former species remain clearly discernible long after the evanescent antheridial envelopes and supporting branches of the latter have become nearly or wholly invisible.

BUREAU OF PLANT INDUSTRY,
U. S. HORTICULTURAL FIELD STATION,
BELTSVILLE, MARYLAND.

LITERATURE CITED

1. BOURNE, B. A. Morphological similarity between the *Pythium*-like fungus found associated with diseased sugar-cane roots in Hawaii and Porto Rico. Jour. Dept. Agr. Porto Rico 8, no. 2: 61–70. 1924.
2. CARPENTER, C. W. Morphological studies of the *Pythium*-like fungi associated with root rot in Hawaii. Hawaii. Sugar Planters' Assoc. Expt. Sta. Bull. Bot. Ser. 3: 59–65. 1921.
3. ————. Predisposing factors in *Pythium* root rot, VII. Hawaii. Planters' Rec. 38: 279–338. 1934.
4. DRECHSLER, C. *Pythium arrhenomanes* n. sp., a parasite causing maize root rot. Phytopath. 18: 873–875. 1928.
5. ————. Some new species of *Pythium.* Jour. Wash. Acad. Sci. 20: 398–418. 1930.
6. JOHANN, H. Grated carrot agar favorable for studies of *Pythium.* Phytopath. 18: 710. 1928.
7. ————, J. R. HOLBERT and J. G. DICKSON. A *Pythium* seedling blight and root rot of dent corn (Abstract). Phytopath. 16: 85. 1926.
8. ————, ———— and ————. A *Pythium* seedling blight and root rot of dent corn. Jour. Agr. Research [U. S.] 37: 443–464. 1928.
9. MATTHEWS, V. D. Studies on the genus *Pythium.* 136 pp. University of North Carolina Press, Chapel Hill. 1931.
10. RANDS, R. D., and E. DOPP. Humus extract agar favorable for oospore production in *Pythium.* Phytopath. 23: 745. 1933.

11. RANDS, R. D., and E. DOPP. Variability in *Pythium arrhenomanes* in relation to root rot of sugarcane and corn. Jour. Agr. Research [U. S.] **49**: 189–221. 1934.
12. SUBRAMANIAM, L. S. Root rot and sclerotial diseases of wheat. Agr. Research Inst. Pusa Bull. 177. 1928.
13. VALLEAU, W. D., P. E. KARRAKER, and E. M. JOHNSON. Corn root rot—a soil-borne disease. Jour. Agr. Research [U. S.] **33**: 453–476. 1926.