

MORPHOLOGICAL SIMILARITY BETWEEN THE PHYTHIUM-
LIKE FUNGUS FOUND ASSOCIATED WITH DISEASED
SUGAR-CANE ROOTS IN HAWAII AND PORTO
RICO

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The first pathologist in more than two decades since 1896 to

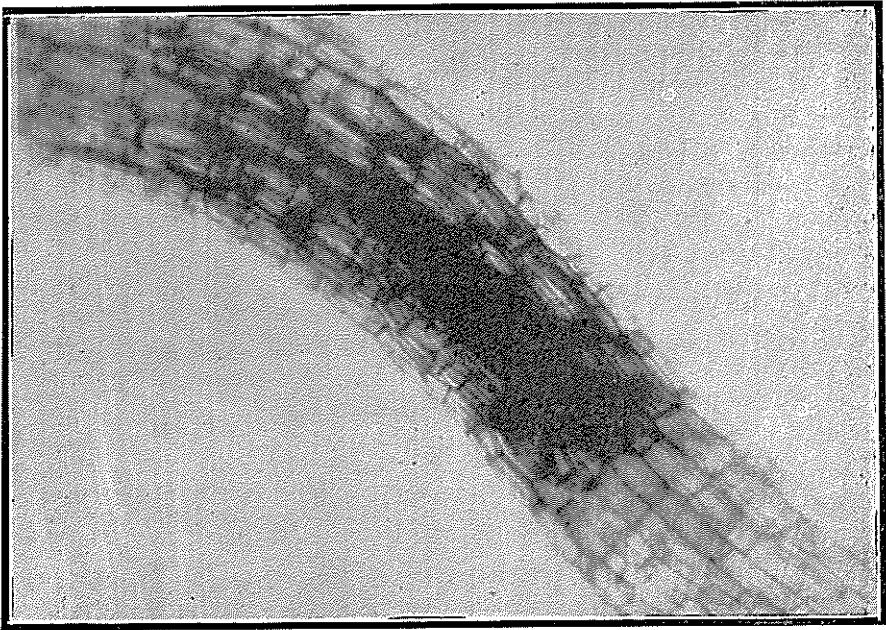


FIG. 1.—Photomicrography of secondary rootlet of sugar cane near root tip showing area of dark reddish-brown tissue found in cases of typical *Pythium* root-rot. X 165.

claim that a fungus having characters resembling the genus *Pythium* was a primary cause of that condition in sugar cane known as "root disease" was Mr. C. W. Carpenter (1) of the Hawaii Agricultural Experiment Station. In fact, the experiments he described with this fungus in 1919 leave little doubt that it is a vigorous parasite under certain conditions in Hawaii and can be made to reproduce the typical symptoms of root disease under carefully controlled experimental conditions.

Prof. Earle (3) has already pointed out that Dr. Treub in 1885 and Dr. Wakker in 1893 have both found *Phythium* associated with root disease in Java, but that the more conspicuous *Marasmius* seems to have attracted Dr. Wakker's attention as well as that of subsequent investigators and no further mention of the former fungus as being associated with a cane disease has been found in literature until that of Carpenter previously mentioned.

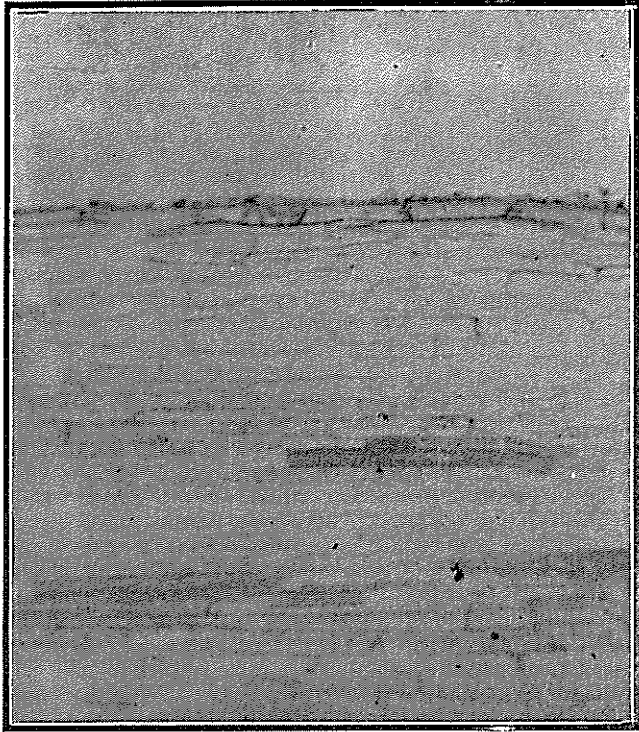


FIG. 2.—Photomicrograph of a longitudinal section through a reddish-brown diseased portion of a primary cane root affected with *Phythium* root-rot. Note the thick type of mycelium seen in one of the epidermal cells in the center of the picture. X 170.

Here in Porto Rico the first mention of *Phythium* as being isolated from roots of sugar cane suffering from root disease was recorded in 1920 by Mr. Matz (4) recently Pathologist of the Insular Experiment Station. Although Mr. Matz's experiments leave little doubt that *Phythium* is a vigorous parasite on cane roots under certain conditions and can be made to induce rot in these under experi-

mental conditions just the same as was shown by Carpenter in Hawaii, yet it has not been satisfactorily demonstrated that the species present in Porto Rico can reproduce all the true symptoms of root disease, including marked stunting of the cane such as is claimed by Carpenter for the Hawaiian fungus. So far as is known, inoculation experiments with *Pythium* sp. under Porto Rican conditions have not yet been tried with the *Lahaina* variety (probably the same as the Bourbon, Otaheite and Caña Blanca) which is known to be particularly susceptible and which was used by Carpenter in his inoculation experiments.

In the absence of definite evidence, therefore, as to whether we have here a different species of *Pythium* or a different biological strain of the same species as is found in Hawaii, it would seem desirable to present evidence relative to the morphological similarity between the two fungi as they exist naturally in cane roots and also in pure culture in the two countries. It is not the purpose of this paper to discuss the life history of the species of *Pythium* found here, since this would not clear the situation in the absence of similar studies with the Hawaiian parasite. So far no detailed description or figures of the *Pythium* found in Porto Rico have been published, and the writer believes that in presenting certain results recently obtained by a histological examination of roots of cane affected with this fungus, together with certain observations of pure cultures of the parasite, he will be throwing some light on the similarity between the organism found here and the one described from Hawaii.†

Figure 1 represents a typical secondary rootlet of cane¹ affected with *Pythium* root-rot. The dark reddish-brown area toward the central portion of the stele seems to be quite characteristic and is usually present a short distance back from the root tip, being often easily visible to the naked eye. This typical appearance is found also in tertiary roots. The exact reason why the fungus

† Since this paper was prepared, the writer has seen a copy of Mr. Carpenter's subsequent paper entitled "*Morphological Studies of the Pythium-like Fungi Associated with Root-rot in Hawaii*". Bul. Exp. Sta. Haw. Sug. Pl. Assoc. Bot. Ser. 3: 59-65. 1921, and also Dr. H. M. Fitzpatrick's paper on "*Generic Concepts in the Pythiaceae and Blastocladiaceae*". Mycologia: 15: 166-173. 1923. Not only is it abundantly clear that the Porto Rican fungus is morphologically identical with the Hawaiian type, but according to Dr. Fitzpatrick it is really a *Nematosporangium*, the thicker type of globular, mycelium-like structure found in the epidermal cells (see figs. 3 and 4 present paper) being the true sporangium or asexual stage. The latter author has suggested the name *Nematosporangium aphanidermatum* (Edson) comb. nov. for this fungus.

¹ B. 6450 variety was the one studied throughout for the purpose of writing this paper.



FIGS. 3 AND 4.—Photomicrographs of longitudinal sections of epidermal cells of a primary root of sugar cane showing typical globular and *Pythium*-like fungus present therein. X 1,650.

reacts so peculiarly with the host tissue in the region of the young root where the distinct tissues of cortex and stele are beginning to become differentiated is as yet not clearly understood. Similar reddish-brown lesions are also to be found on tender primary roots, although these are scattered irregularly and may be quite large (over a centimeter in length) owing to the gradual extension of the fungus from the original point of entrance, and perhaps also to the coalescence of individual lesions.



FIG. 5.—Photomicrograph of longitudinal section of epidermal cell of a primary root of sugar cane showing typical fine, cylindrical type of *Pythium*-like mycelium ramifying throughout the interior. X 1,650.

Figure 2 is a longitudinal section of a primary cane root showing one of the epidermal cells invaded with the thicker type of *Pythium*-like mycelium. It is interesting to note that this type of mycelium is seldom found any deeper than the first two layers of cells in the root and most commonly just in the epidermal layer as shown in figures 2, 3, and 4. Figures 3 and 4 show the typical globular and characteristically shaped mycelium of the *Pythium*-like fungus inhabiting the epidermal cells of an affected primary root. The writer

especially desires to draw attention to the great similarity between this mycelium and that figured by Carpenter in Hawaii. It is worth mentioning, moreover, that this thicker mycelium stains quite easily with safranin (1 per cent solution in 50 per cent ethyl alcohol) whereas the finer type seen ramifying in the epidermal cell shown in Figure 5 is so poorly stained with safranin that it could not be recognized except with the greatest difficulty. The iron-alum-

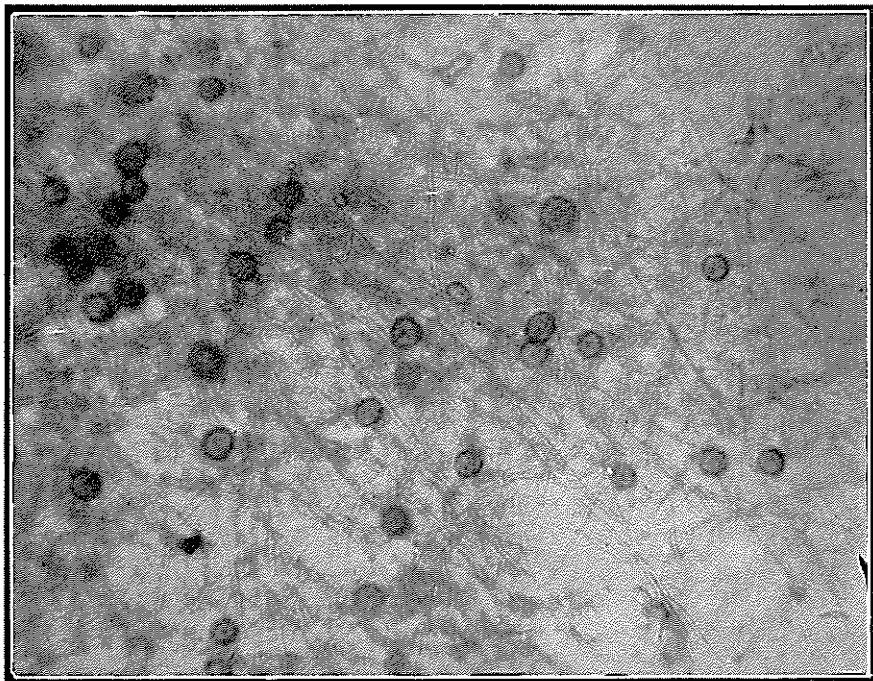


FIG. 6.—Photomicrograph of a living pure culture on corn meal of the *Pythium*-like fungus associated with cane root-rot. Note typical branching of mycelium and abundant production of oögonia. X 165.

haematoxylin cytological stain was found to be far superior for staining both types of the *Pythium*-like thallus. Carpenter also mentions and figures a similar type of fine cylindrical mycelium as shown in Figure 5 inhabiting an epidermal cell. This type of mycelium in addition to being found in epidermal cells has also been seen by the writer inhabiting root hairs, and in fact numerous stained sections show it to be present throughout the cortex and stele tissues as well as at the origin or secondary roots. As a whole this

type of thallus seems to predominate in the roots, but will easily escape recognition unless suitably stained as mentioned above.

Carpenter (1) mentions that the *Pythium*-like fungus he obtained in Hawaii produced oöspores on rice cultures, while Earle (3) in Porto Rico states that oöspores were produced abundantly in Mr. Matz's cultures. Fig 6 shows how abundantly oögonia are found in young pure culture of the *Pythium*-like fungus on corn meal isolated by the writer. Fig. 7 shows some of these oögonia more highly



FIG. 7.—Photomicrograph of living culture of the *Pythium*-like fungus on corn meal. *x*, antheridium attached to wall of oögonium; *y*, oösphere or egg cell. X 943.

magnified and in addition one of them may be seen to have a typical antheridium (X) firmly attached to its outer wall for the purpose of fertilizing the oösphere, or female egg cell (*y*) present within. Great similarity is here noted in the manner of fertilization of the oösphere as figured by Carpenter.

Fig. 8 shows the oöspores formed after fertilization. At this stage they have not yet been freed from the wall of the oögonium (*a*) and in addition to being invested with a thick inner wall (*c*), they also are surrounded by a relatively thicker layer of prei-

plasm (*b*) which is seen in the preparation to have taken the stain with difficulty. Anton De Bary (2) regards this periplasm layer in the case of the genus *Pythium* as an inconspicuous, sparingly granular mass of protoplasm surrounding the oöspore, but which cannot be seen to take part in the process of its maturation. It is possible that it may serve as a protective covering when the oöspore is liberated, or it may soon be dissolved away.

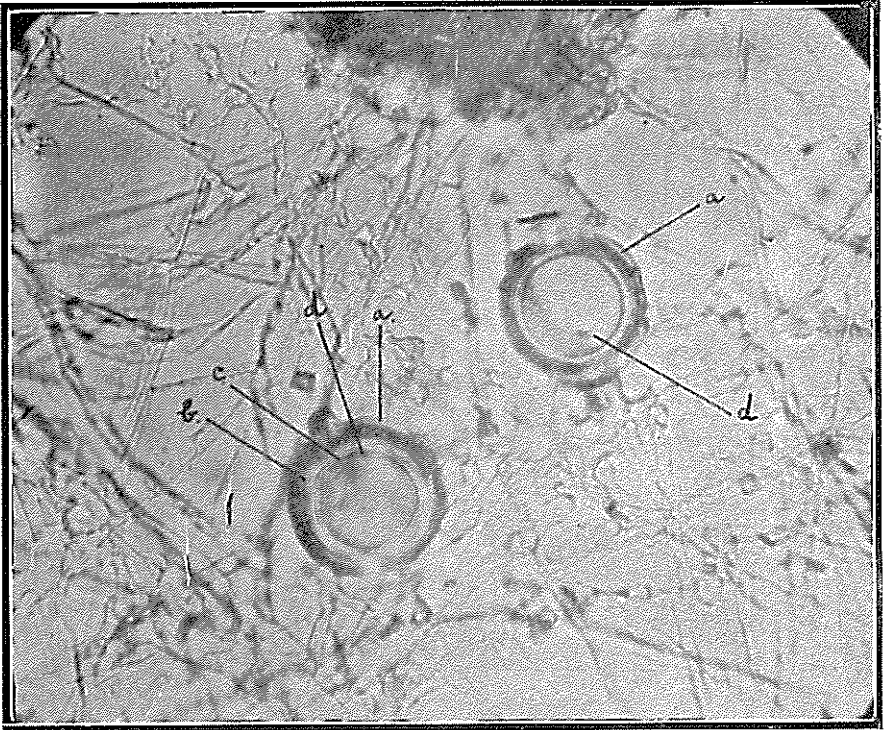


FIG. 8.—Photomicrograph of stained preparation of *Pythium*-like culture showing two mature oöspores. *a*, wall of oögonium; *d*, periplasm layer; *c*, inner wall of oöspore; *e*, probably the nucleus of the oöspore. X 1,073.

As far as comparison of the fungi from the two countries is concerned, relative to the size of mature oöpores as well as oögonia at the time of fertilization, this is hardly possible at present since the average sizes of these structures calculated from a large number have not apparently been worked out for the Hawaiian organism. Furthermore, the writer has not grown his organism on rice cultures as used by Carpenter, but on sterile corn meal as well as dextrose potato agar, so that it is feared that this difference in medium would

be a serious objection if accurate comparison in the size of these organs was undertaken. However, the similarity in size is indicated by making use of the figures of definite magnification given for the Hawaiian organism.

A typical oöspore figured by Carpenter on Plate 8, Fig. F. shows the inner granular oöspore, without the periplasm and oögonium wall, to be 15 microns in diameter. Both oöspores figured by the writer in Fig. 8 have a diameter of 12.12 microns without the periplasm and oögonium wall. Thus there is only a difference of about three microns, a variation easily possible in the same strain on different media.

SUMMARY

1. Histological investigation of roots of B. 6450 variety of cane in Porto Rico suffering from *Pithium* root-rot has demonstrated that this fungus as it exists in the tissues, bears a remarkable morphological similarity to the *Pythium*-like organism figured by Carpenter from Hawaii in the roots of the Lahaina variety.

2. Iron-alum-haematoxylin cytological stain has been found very suitable for staining both the thick and globular as well as the fine cylindrical types of *Pythium*-like thallus in cane roots. The latter type of mycelium was found to be by far the most common in the root tissues examined, the former kind being confined mainly to the outer one or two layers of cells.

3. Pure cultures of the *Pythium*-like fungus on sterile corn meal produce abundant oögonia having oöspheres which are apparently fertilized in a similar manner to the Hawaiian organism.

4. Such comparison as has been possible shows that mature oöspores of the organism from the two countries are so similar in size as to possibly come within the range of ordinary variation within the species.

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