THE TEXAS ROOT ROT FUNGUS AND ITS CONIDIAL STAGE[1]

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More than twenty-five years ago Pammel ('88, '89) spent two summers in Texas investigating an important cotton disease popularly known as the "cotton blight" or "cotton dying," and as a result of his observations two reports were published upon the cotton root rot,—the latter more appropriate name being applied by him to the disease in question. He determined the causal organism to be a sterile fungus found in some abundance on every dead or dying root, and it was tentatively identified as *Ozonium auricomum* Lk. After a study of Link's type, Shear ('07) described the organism as a new species, *O. omnivorum*.

Since the work of Pammel the disease has doubtless been the object of numerous field observations, more or less extensive, but so far as is personally known to me, and so far as reports are available, the only records are those of Duggar ('09, observations made '01, '02), Shear ('07, observations beginning in '02), Shear and Miles ('07, '07[a]), Heald ('09, '11), and Heald and Wolf ('11, '12). The disease is undoubtedly one of the most destructive of the cotton fungi, and the average losses sustained in the state of Texas have been variously estimated by Orton ('06) and others to be two to three million dollars. In addition, considerable damage is sustained by such crops as alfalfa, beans, sweet potatoes, and certain orchard fruits.

It would appear that the organism is very largely confined to Texas. In that state it seems to have been commonly ob-

[1]The writer was engaged in a study of cotton diseases, especially the Texas root rot, in the Bureau of Plant Industry during the seasons of 1901–02. In the fall of 1902 the work was transferred to Dr. C. L. Shear. Now that the Missouri Botanical Garden is giving special attention to a botanical survey of a certain section of the Southwest, it has seemed appropriate to resume the studies of this fungus so wide-spread and destructive in a large part of that region.

served since 1866. Shear ('07) states that the fungus is distributed from eastern Texas to southern California, and that it has been found in southern Oklahoma and Indian Territory. The writer failed to find the organism in western Louisiana and southwestern Arkansas in 1901, but it was observed in southern Oklahoma in 1915. I am unaware of the data on which the occurrence of the fungus westward to California is reported. Nevertheless, considering the long period of time during which the *Ozonium* has been a serious disease-inducing factor in Texas, it is rather remarkable that it has not been found in Louisiana and Mississippi. In these states the cotton wilt fungus, *Fusarium vasinfectum,* is well known, but the *Ozonium* has never been reported, so far as can be learned. It is almost impossible to assume that the fungus has not been distributed to these states through the various possible commercial channels; so that one is impelled to draw the inference that the establishment of the fungus farther eastward is limited by climatic or soil factors.

It should be recalled that Pammel reported the disease common throughout all sections of Texas in which cotton was grown, with the exception of the gulf prairie region and certain alluvial soils. It seems now certain that there is no soil type in the cotton-producing section of the state which is free from the disease. Nevertheless, the percentage of loss has been invariably greater in the black prairie or black waxy soils, whether with or without outcroppings of rotten limestone. As noted later, the organism occurs on a number of native plants, both trees and herbs, but the observations thus far made give no clue as to whether or not it may be considered endemic. I have been unable to secure data on the occurrence of this fungus from Mexico southward.

From the reports of Pammel, Heald, Heald and Wolf, and from my own observations, the following host plants may be enumerated.

Trees and shrubs: *Ulmus americana, Broussonetia papyrifera, Morus alba, Ficus Carica, Acer saccharinum, Tilia americana, Fraxinus americana, Diospyros Kaki, Melia Azedarach, Pyrus communis, P. Malus, Cydonia vulgaris, Robinia Pseudo-Acacia, Prunus Persica, P. sp.* (cherry), and *Hibiscus syriacus.*

Herbaceous plants: *Beta vulgaris, Chenopodium sp., Cassia Tora, C. marilandica, Medicago sativa, Arachis hypogaea, Phaseolus vulgaris, Vigna sinensis, Linum rigidum, Croton spp., Euphorbia spp.* (three), *Sida spinosa, Hibiscus esculentus, Gossypium herbaceum, Petroselinum hortense, Ipomoea Batatas, Solanum rostratum, Ambrosia psilostachya,* and *Xanthium canadense.*

So far as I am aware, no special attempt has been made to determine all the species of wild herbaceous plants or forest trees affected. The enumeration of hosts given above makes it seem plausible, therefore, that few plants or crops may be free from the disease except the grains and other members of the grass family. At Petty, Texas, in September, 1901, the disease was found upon half a dozen species of weeds in a pasture, the sod of which could not have been disturbed for some years previous.

The chief characteristic of the disease, as far as I have observed it on herbaceous plants, is the sudden wilting and dying of the affected individuals. Occasionally a slight yellowing and unhealthy appearance is found to be due to an infection which does not encircle the main root, and less frequently to the localization of the disease in a few of the larger primary root branches. The first "dying" of cotton is associated with the beginning of blossoming, or of boll formation, commonly from June to July; but Pammel reports one case in which the disease was observed May 6. If the fungus is responsible for injuries in the early stages of growth, then either such injuries have been overlooked or have been ascribed to other causes.

In common with *Rhizoctonia Crocorum* the organism spreads radially, the rate of spread being most variable and, of course, governed by the conditions. The most rapid spread observed by the writer was in a field of irrigated alfalfa. The persistence of the larger "dead spots" season after season in much the same part of the field is accountable in large measure for the popular belief that these are "alkali" spots. The progress of the disease from one year to another is best followed by observing a perennial crop such as alfalfa, in which case new infections are usually relatively few, whereas in a field grown two years or more to cotton one notes the disappearance of some of the smaller spots of the previous year, and often the number of new infections is considerable.

If diseased cotton stalks are left standing in the field, few or no evidences of the fungus are apparent on the roots the following March. However, some of the more interested growers claim to have observed mats of the fungus turned over by the plow when bedding the land. I have been unable to obtain such material for study. As already indicated, the reappearance of the larger spots, particularly, is a strong indication of the persistence of the mycelium in the soil. This leaves out of consideration the influence of the conidial stage, discussed below, in the persistence of the organism in the same area during successive years.

On lifting wilted stalks of cotton, or stalks recently dead, it is found, from the most favorable material, that the roots are closely invested with a cinnamon-buff[1] felt of hyphae in which strands are conspicuous. The fungus may involve the smallest rootlets, and in addition, the strands of hyphae penetrate the soil and apparently extend considerable distances. The larger soil strands are somewhat darker, often cinnamon-colored. In a badly infected area the strands of hyphae may be found in any lump of soil. Pammel describes the mycelium as brown in color, and Shear as "dirty yellow, whitish when young." In the early stages of development on the host, I find the mycelium pale buff, becoming cinnamon-brown as strands are formed.

In September, 1915, the conditions were particularly favorable at Paris, Texas, for late-season infections, so that by examining the roots of many plants taken at the periphery of a diseased area, but themselves apparently healthy, comparatively early stages of infection were observed. In all cases a depression of the bark pointed out the area of penetration of the fungus on the main root. The observations also demonstrated clearly that the attack may be either what I shall designate centripetal or centrifugal. In the former case the infection converges upon the main roots from a few or many small laterals, while in the latter the main root may be com-

[1]Ridgway's 'Color standards and nomenclature' has been employed in the determination of all colors referred to in this paper.

pletely encircled before the fungus extends to the branches. If recovery of affected stalks occurs at all, it is usually by the production of very superficial laterals.

I have not made a careful study of the distribution of hyphae in the various tissues, nor of the mechanism of penetration. From the variety of plants affected it may be inferred that direct infection by the hyphae is general. The presence of lenticels on the enlarged part of the root of cotton by midsummer may possibly be related to the greater susceptibility of this plant, and may also be a factor in determining the frequency of the centrifugal type.

During the seasons of 1901–02 a careful search was made for spore stages of the *Ozonium,* and while several basidiomycetous fungi were found on old cotton stalks in areas where cotton had died from the disease, still no case was observed which, upon careful examination, proved worthy of experimental study. In the examination made of such material special care was given to the characters of the mycelium. However, while examining a semicircular area of dead cotton on the edge of a cotton field in 1902, my attention was caught by a buff-colored circular spot on the ground just outside the cotton field in an area of grass and weeds wherein several of the latter had died from the *Ozonium.* The material observed proved to be an incrustation, or light powdery layer, of spores covering about one square foot in area. One small area of a few square inches only, considerably weathered, was found between the rows of cotton. Removing the soil with some of the spore material and making an examination under the hand lens it was found that strands of the *Ozonium* pervaded the whole mass, and thus there was presented the possibility of a spore form genetically connected with the *Ozonium.* Subsequently, the spore material was studied more carefully. At that time it was clear that strands of the *Ozonium* were present under the masses of spores, but the observations afforded no evidence of the method of spore production. The masses of small spheroidal spores formed a layer sometimes 3 mm. in thickness, and while the broken bits of hyphae observed resembled those of the *Ozonium,* no light was thrown on the relation of spores to mycelium. The conditions so much resembled those under which oidial formation occurs in cultures of certain *Basidiomycetes* that I subsequently suggested the presence of such an oidial stage of this fungus (Duggar, '09). Owing to the transfer of the cotton disease work to Dr. C. L. Shear at this time the material was laid away, and not again examined until a recent reinvestigation of all material in my hands which might be considered related to *Rhizoctonia*. Then it was ascertained that the best packet of material collected in 1902 had not been studied—that from the area found between the rows of cotton. The reëxamination of this collection resulted

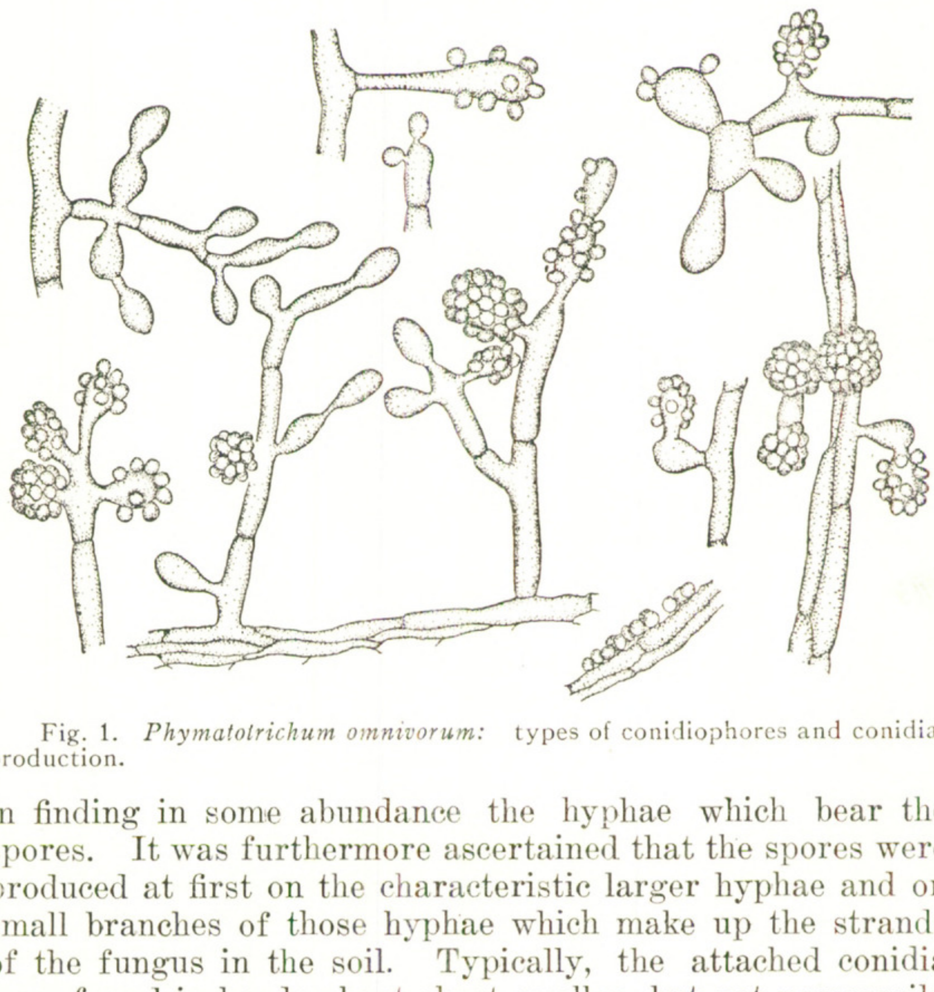

Fig. 1. *Phymatotrichum omnivorum:* types of conidiophores and conidial production.

in finding in some abundance the hyphae which bear the spores. It was furthermore ascertained that the spores were produced at first on the characteristic larger hyphae and on small branches of those hyphae which make up the strands of the fungus in the soil. Typically, the attached conidia were found in heads about short swollen, but not necessarily spherical, branches of the short-celled or strand hyphae. These branches were simple or forked, the forking being at irregular intervals, and occasionally branching was continued from a swollen cell (fig. 1). The spores adhered somewhat, but never in such masses as characterize certain species of *Sporotrichum* or *Verticillium*.

In view of the importance of this observation, and failing to secure material from correspondents, a trip to Paris, Texas, was arranged in September, 1915, with the view of securing fresh material and of making further observations on the fungus in the field. The time selected proved favorable, and an examination of the cotton fields in the vicinity of Paris revealed the *Ozonium* in unusual abundance. Nevertheless, "dead spots" in many fields were examined before the characteristic fruiting stage was found. Then it was located in some quantity in a "dead spot" of about one acre in extent, occurring in a very rich, black waxy soil in a low-lying area of the field. In this area no less than a hundred or more of these conidial circles were found. They varied in diameter from 3 to 30 cm. The majority of these were found in the furrows or "middles" between the rows of cotton, yet they also occurred on the ridge rows, and in seven cases they encircled diseased cotton stalks. In the latter cases, however, the strands penetrating the spore-bearing layer appeared to come from the soil in general rather than directly from the diseased root. In every case the typical color of the spore mass was light pinkish cinnamon, and in thin strata pinkish buff, fading somewhat on drying. At that date the circular area consisted of a more or less perfect crust of spores sometimes broken or powdery. A few of the spore areas had become overgrown with such olive-green moulds as *Cladosporium* and *Macrosporium*.

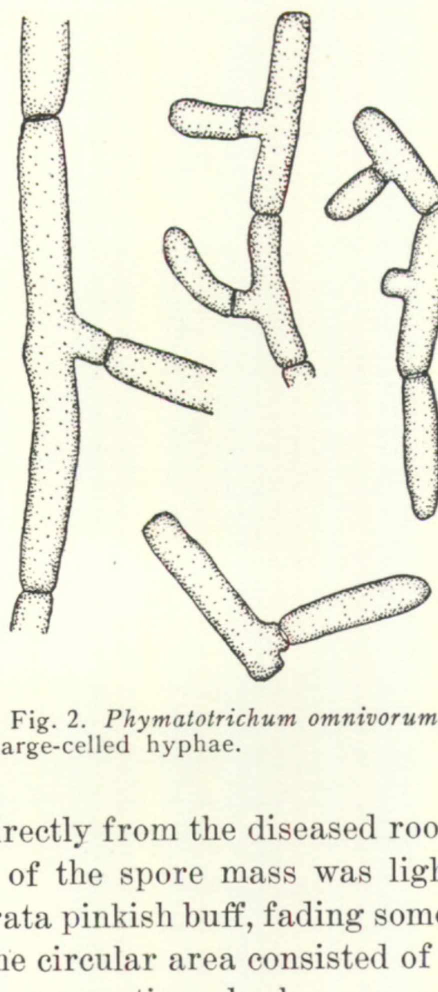

Fig. 2. *Phymatotrichum omnivorum*: large-celled hyphae.

Just below the spore crust, especially towards the center, the typical cinnamon-buff strands of the *Ozonium* occurred in abundance. A similar type of mycelium also permeated the soil to a considerable extent in the immediate vicinity and often about the periphery of the spore-bearing area.

The study of the collections made in 1915 emphasize the diversity in the form of hyphae as well as in the method of spore production. Although no circular areas were found in an early stage of development, yet some of the older ones yielded on the periphery material from which the method of spore formation could be followed. It would appear that a superficial growth of large, branched, almost hyaline hyphae is first formed (fig. 2), covering the surface with a delicate stratum. These hyphae are sometimes Rhizoctonia-like. They may also bear conidiophores at irregular intervals, the latter arising usually as short assurgent branches. These branches either produce conidia directly, or commonly after becoming variously forked (fig. 1). As further growth proceeds, however, definite strands are developed, and then swollen branches from any cell of the strand may produce spores. Later the wave of spore production appears to involve practically the whole mycelium, and the conidia are found laterally distributed in various positions on the surfaces of both the strand and simple hyphae, so that in the end there is practically nothing left but a pulverulent mass consisting of the conidia and remains of the mycelium and strands. The conidia are sessile, but occasionally cells bearing conidia exhibit a somewhat roughened surface. The true character of the fungus cannot be determined unless one is careful to secure the youngest material available, that is, from near the margin of the spore area, or otherwise a spore-forming area in an early stage of development.

The diverse characteristics of the mycelium, as found on the surface of the host and in the soil, may be briefly summarized as follows:

(1) Large-celled type. Hyphae Rhizoctonia-like, often abundant on the margins of the conidial areas, measuring frequently 20μ in diameter, with cross walls 60–120μ apart. This type should also include some of the arachnoid mycelium

on the surface of the roots, also those representing early stages of strand formation (fig. 2).

(2) Strand hyphae. In these the individuality of the hyphae is practically lost, the strands being ultimately plectenchymatic bands in which the individual cells vary considerably in diameter, the larger cells of young strands resembling somewhat the larger hyphae above mentioned. It is interesting to observe that they may serve not only to spread the fungus vegetatively, but superficial soil strands may function as a conidial stroma. They are also more or less sclerotial and are doubtless an important factor in the persistence of the fungus in the soil (fig. 3).

(3) Acicular type. The arachnoid mycelium with which the root is invested gives rise to certain fairly rigid hyphae

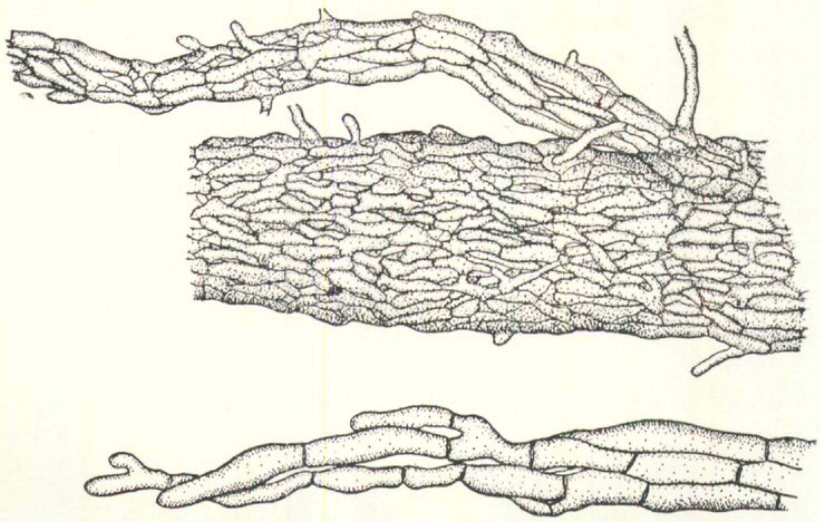

Fig. 3. *Phymatotrichum omnivorum:* mycelial strands; upper, old strand from root of cotton; lower, young strand from conidial area.

which in turn produce branches that are most frequently in pairs, that is, opposite, and at right angles. Branching is also not infrequently verticillate. In all cases such branches are characteristic in appearance, being rigid and needle-like, tapering to very fine filaments (fig. 4). This type has been found only on the roots.

It is necessary to add, however, that intermediate types between the various forms mentioned occur. In general, the mycelium is Rhizoctonia-like rather than Ozonium-like, yet no sclerotia have been found. In this connection I may add that it is proposed in a later paper to bring together certain observations which have been accumulating on Ozonium stages of *Basidiomycetes.*

Numerous germination cultures have been made with material from two weeks to three months old. While this has afforded some interesting suggestions, germination in any particular medium has been, on the whole, erratic. The data are

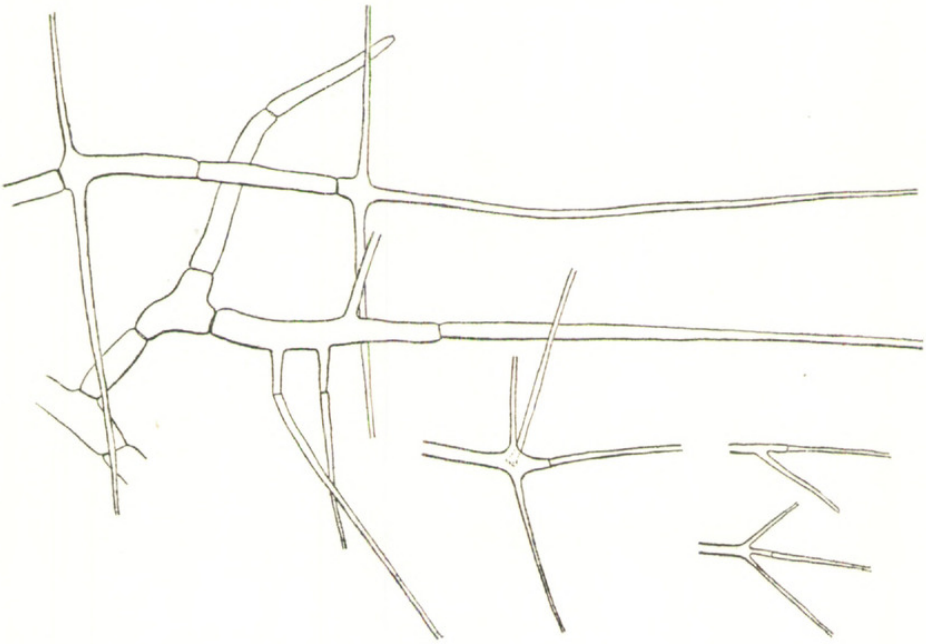

Fig. 4. *Phymatotrichum omnivorum:* acicular hyphae exhibiting characteristic modes of branching.

reserved for a later report. The cultures which have been prepared from the newly infected root, as also those from erratic spore germination, have yielded a sterile mycelium which, while in itself distinctive, resembles only in a general way the mycelium found on the roots and in the soil. The mycelium in culture is hyaline, forming on young bean stems and on various other culture media a dense, slow-growing mat, seldom rising more than 3 mm. above the substratum, and never becoming fluffy in appearance. After standing for some weeks this mycelium becomes somewhat colored, assuming a warm buff to light ochraceous buff. In culture the hyphae are likewise most diverse in diameter, varying from those 15–20μ to others extremely delicate and flexuous (fig. 5). No truly acicular branches, however, are produced under ordinary cultural conditions. With age, the mycelium somewhat collapses toward the substratum and has a greater tendency to grow along the glass tube in the form of false strands. Grown in soil, by covering a vigorous growth on bean stems with a layer of loam, hyphae similar to those just described are produced; but, in addition, there are formed here and there vesicular enlargements, and the latter are sometimes

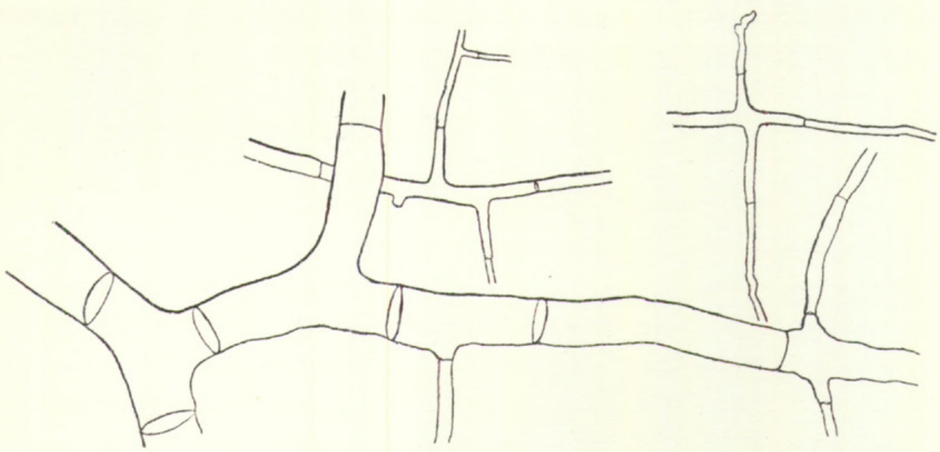

Fig. 5. *Phymatotrichum omnivorum:* hyphae grown on bean stems, from a culture 60 days old.

in clusters, the branches becoming two to three times forked. The conditions for conidial formation have not been determined.

It will be seen that the connection of the conidial stage with the *Ozonium* rests at present upon two classes of observation: (1) the presence in the conidial layer of hyphae and strands (bearing conidia) found to be identical with the characteristic mycelium on the roots of affected plants, and (2) the identity in artificial culture of the mycelium originating, on the one hand, from diseased roots, and, on the other, from the germination of the conidia. To complete the proof it would, of course, be necessary to secure positive results by inoculation with conidia, or better, positive results with a pure culture originating from conidia. Unfortunately this phase of the work has not been successfully developed. In this connection it should be said that no inoculations carried out in the greenhouse up to the present have given positive results. As a source of infection I have employed (1) diseased cotton roots fresh from the field (showing the *Ozonium* in abundance), (2) fresh conidia, and (3) cultures from diseased roots. It is apparent that the conditions for infection have not been made satisfactory. Such experiments are to be continued both in the greenhouse and in the field.

It has been found difficult to place the fungus satisfactorily in any established genus of the *Hyphomycetes*. While in the manner of conidial production it is undoubtedly related to such genera as *Phymatotrichum, Botryosporium, Rhinotrichum,* etc., it does not exhibit all the characteristics of any of these genera. Nevertheless, it has seemed best, after examining all available exsiccati material of forms which might be related, to place the fungus tentatively in the genus *Phymatotrichum,* and, if Bonorden's figure (Handb. d. allgem. Myk. *pl. 8, f. 181*) is correct, fairly close to *P. pyramidale* Bon.[1] The fungus is clearly excluded from *Botryosporium,* the conidiophores of which are erect, with conidia produced on sterigmata. Slightly emended, the genus *Phymatotrichum* would be of taxonomic convenience. In placing the Texas cotton fungus in this genus, I would not convey the impression that this fungus is considered to belong to the *Ascomycetes*. Accepting Shear's specific name, a revised description of the organism is appended.

Phymatotrichum omnivorum (Shear) Duggar, n. comb.

Hyphae diverse, forming on the host (1) a loose weft of large, branched cells, producing more rigid hyphae with acic-

[1]It should be noted that the genus *Phymatotrichum* Bonorden (Handb. d. allgem. Myk. p. 116. *pl. 8, f. 181.* 1851) was at first reduced to a section of *Botrytis* by Saccardo (Sylloge **4**:134. 1886). Later, however, he restored it to generic rank (Sylloge **16**:1033. 1902) to accommodate a species of Oudemans. Costantin (Les Mucédinées simples, pp. 44–46. *f. 12.* 1888) gives a detailed description of a fungus, which was obviously considered *Phymatotrichum pyramidale* Bon., under the name *Botryosporium pyramidale* Cost. There can be little doubt that the fungus figured by Costantin is properly placed. However, the source of Costantin's material was apparently not the original specimen of Bonorden, and since his figure differed in many respects from that of Bonorden, it is perhaps fair to question the identity of the two fungi. Lindau (Rabenhorst's Kryptogamenflora **1** (Abt. 8):117. 1904) seems to accept the views of Costantin. He also cites as exsiccati, Vestergren, Micr. rav. sel. 421.

ular branches, these last often arising at right angles and opposite, and (2) plectenchymatic strands; almost hyaline when young to cinnamon-brown in mature strands. Fertile hyphae arising irregularly from the large-celled mycelium or direct from cells of the strands, assurgent, simple or forked, with spore-bearing portion vesicular (spheroidal to ellipsoidal), often 20–28μ in length and 15–20μ in diameter. Spores finally arising also from undifferentiated hyphal and strand cells, hyaline, spheroidal to ovoidal, the spheroidal averaging 4.8–5.5μ in diameter, the ovoidal measuring $5\text{–}6 \times 6\text{–}8\mu$; extreme diameters, 3.2 and 9.8 μ. The conidial stage forms a continuous pulverulent, sometimes crust-like, area on the soil.

Hab. Hyphae on living roots of many plants and in soil, conidial stage on soil in the vicinity of diseased plants.

Specimens have been deposited in the herbaria of the Missouri Botanical Garden and the Bureau of Plant Industry, Washington, and in the collection of Professor W. G. Farlow, Cambridge.

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