# THE COTTONY LEAK OF CUCUMBERS CAUSED BY PYTHIUM APHANIDERMATUM

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(Contribution from Bureau of Plant Industry)

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# THE COTTONY LEAK OF CUCUMBERS CAUSED BY PYTHIUM APHANIDERMATUM[1]

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## INTRODUCTION

While several species of Pythium, notably *Pythium debaryanum* Hesse, have been found destructive to a wide variety of phanerogams in the seedling stage, and inimical to the best development of some of these hosts in later stages, the association of the genus with decay of commercial vegetable products, representing parts of plants approaching maturity, has not been frequently recorded. Perhaps the most generally known instance is represented by the "leak" of potato (*Solanum tuberosum* L.) tubers, apparently encountered by De Bary (*2*)[2] in Germany more than four decades ago, and more recently made the subject of special study in the United States by Hawkins (*8*). A soft rot of sweet pepper (*Capsicum annum* L., var. *grossum*) has been recorded by Lehman (*9*) from North Carolina as being due similarly to *Pythium debaryanum*, the decay always beginning at the blossom end, and affecting fruits not more than 6 or 8 inches from the ground. The same fungus is mentioned in the list of fungi thriving on fruit in Belgium by Él. and Ém. Marchal (*11*), who observed it on a pear (*Pyrus communis* L.) lying on damp ground.

## MATERIAL EXAMINED

This paper deals with a disease of cucumber (*Cucumis sativus* L.) fruit which the writer first observed in specimens submitted to him June 8, 1922, by the food products inspector of the Bureau of Agricultural Economics at Washington, D. C., as being representative of a type of decay found responsible for considerable damage to a carlot shipped from St. George, S. C., June 2, 1922. Each fruit was entirely encased in a luxuriant cottony mycelial weft, matted down here and there as a wet membranous layer, at first sight thus suggesting being wrapped in absorbent cotton that had become moistened in places. The tissue in the interior was found very watery and of a peculiar texture, greatly softened, and so lacking in mechanical firmness as to be divided very readily with blunt instruments. Where not occupied by secondary bacterial invaders, the juices draining copiously from the incisions were only slightly turbid. The material gave off a peculiar odor rather inadequately described by the term "marshy"—not pleasant, but having little in common with the putrid smells characteristic of the decay of vegetables due to bacteria.

Since the original discovery of the trouble no additional material has been submitted to the writer, and from inquiry it would appear that the type of deterioration in question is not frequently encountered on the Washington market, or at least not in quantity. However, early in July, 1924, J. I. Lauritzen found several carload lots in both the Pittsburgh and the Buffalo markets, of which not inconsiderable portions were affected in exactly the manner described in the preceding paragraph. Almost simultaneously G. B. Ramsey observed the same decay with its characteristic display of cottony mycelium in a carload lot of cucumbers on the Chicago market, the shipment in this instance having originated in North Carolina. It is highly probable that in the case of the cucumbers grown in the Southeastern States the destruction from this trouble will generally be found greatest in the markets of our more remote northern cities, since, other things being equal, the quantity of cucumbers affected evidently increases with the length of time the shipment is in transit.

Microscopic examination of the specimens obtained on the Washington market revealed the fresh cottony growth as a mass of mycelium composed of nonseptate hyphae. Where the weft had been matted down as a wet membranous layer closely adhering to the substratum, innumerable thou-

[1] Received for publication, Aug. 20, 1924; issued August, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 1042.

sands of oogonia with antheridia and oospores, were found in all stages of development, the entire apparatus being readily recognizable as characteristic of the genus Pythium. The softened tissue was everywhere occupied by branching mycelium, the elements of which showed little evidence of definite orientation (fig. 1). At the points where the hyphae passed through the cell walls they were constricted to approximately half their normal diameter.

Pure cultures of the fungus were readily obtained by placing pieces of diseased tissue on corn-meal agar plates, and transferring portions of

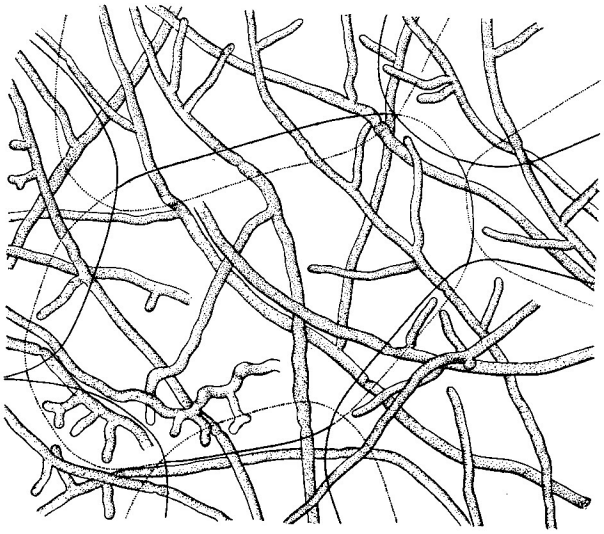


FIG. 1.—Section of cucumber affected with cottony leak, showing tissue occupied by abundance of branching hyphae, and constriction of latter where passing through host cell wall. × 250

mycelium from the margins of the resulting growth to tubes of sterile media. Through the courtesy of J. I. Lauritzen and G. B. Ramsey, transfers of the fungus isolated by them from the diseased material found in Pittsburgh and in Chicago, respectively, were also procured. In general appearance the cultures thus obtained were practically indistinguishable from cultures of the damping-off fungus, *Pythium debaryanum* Hesse. A minor but not insignificant difference could usually be made out in watching the development of the two types of parasites in parallel cultures, as under suitable conditions the cucumber fungus shows development of aerial mycelium in quantity by the end of the second day, whereas in cultures of the damping-off organism such development generally fails to ensue until the third day.

## SOME MORPHOLOGICAL FEATURES

Zoosporangia of the fungus from cucumber fruits are readily obtained by putting pieces of invaded cucumber tissue (watermelon or squash tissue occupied by the parasite serve equally well), or thin slices from the surface of Lima-bean agar cultures, into a shallow layer of sterile water, which should preferably be renewed several times to wash away soluble staling products and excessive food materials. In the course of 2 to 5 hours an abundance of new structures are proliferated from the surface and periphery of the old mycelia, consisting of stout axial elements bearing swollen digitate and short diverticulate branches, these branches frequently undergoing close successive ramification to yield somewhat involved complexes corresponding to the structure discussed by Butler (*3*) as "budlike lateral processes." At other times the branches are fewer in number and at irregular intervals in open racemose arrangement. In any case, if the entire apparatus is well developed a number of septa varying from one or several to a dozen are inserted, thus bringing about the delimitation of a variable number of units, each of which may consist, for example, of a digitate branch with its secondary lobulate branches, or of a portion of the axial element with perhaps one or more diverticulate or branching laterals. After pronounced vacuolization of the protoplasm usual

for the sporangia of Pythium, and the development of an evacuation tube from the tip of one of the digitate elements, the contents of each unit escape to form a vesicle in which the zoospores are fashioned. The latter usually vary from 30 to 40 in number, but individual vesicles developing as few as half a dozen or as many as 60 are not rare. Under favorable conditions zoospore production is extraordinarily abundant, the amount of material that can conveniently be accommodated in a 10 cm. Petri dish giving rise to numbers estimated in excess of 100,000 in the course of an hour.

The organism evidently corresponds to a fungus apparently first noted in the literature as a variety of *Pythium gracile* Schenk by Butler (*3*), who in India found it parasitic on roots and base of stem of ginger (*Zingiber officinale* Rosc.) as well as on the roots of castor bean (*Ricinus communis* L.). Later, Subramaniam (*14*) investigated what he regarded as the same form more closely and set it off as a new species, *Pythium butleri*. In the meantime it had been encountered in the United States as the cause of a disease of radishes (*Raphanus sativus* L.) and sugar beets (*Beta vulgaris* L.) by Edson (*6*), who described it as *Rheosporangium aphanidermatum*, the type of a new genus of Saprolegniaceae. The similarity and apparent identity of the American and Indian forms were pointed out by Carpenter (*4*), who found the fungus associated especially with a destructive root rot of sugar cane (*Saccharum officinarum* L.) in Hawaii. More recently Fitzpatrick (*7*) made Carpenter's inferences effective in a nomenclatorial sense by combining Edson's specific name with both generic names, Pythium and Nematosporangium, the resulting binomials being presented as alternatives, choice between which was made dependent on the advisability of retaining or abandoning Schroeter's genus Nematosporangium as distinct from Pythium.

The genus Nematosporangium as defined by Schroeter (*13, p. 104*) was intended to include the forms having sporangia represented by filaments not differing from the vegetative hyphae, as contrasted with the forms possessing subspherical sporangia, which were to be retained in the genus Pythium. The distinction thus drawn is quite similar to that made by Butler (*3*), whose subgenera Aphragmium and Sphaerosporangium correspond closely to the genera recognized in the "Pflanzenfamilien." Recognition of the subspherical sporangium as a common characteristic of one group of forms is supported by excellent morphological evidence. However, the general view that the sporangia of the remaining forms consist of a simple or branching filament, analogous, for example, to the sporangium of Aphanomyces among the Saprolegniaceae, would appear to be in need of drastic revision. The sporangia characteristic of the parasite causing the cucumber decay discussed in this paper are represented, as has been pointed out, by units resulting from the septation of conspicuously swollen elements, corresponding to the structures which Ward (*15*) first figured and described in his account of a fungus he designated as *Pythium gracile* De Bary, and which later Butler discovered in all the members of the subgenus Aphragmium examined by him. Neither of these authors appears to have observed the participation of these structures in the formation of zoospores, Ward supposing them to serve as reservoirs of protoplasm for mycelial growth or the development of oogonia, while Butler assigned to them a probable capacity for surviving unfavorable conditions. In their studies of what presumably were forms identical with the one attacking cucumbers, Edson, Subramaniam, and Carpenter illustrated and discussed the same type of structures as "presporangia," "buds," and "sporangia," respectively, although perhaps without observing them in their most luxuriant development. It may be mentioned that even more distinctive development of this lobulate type of sporangium is exemplified in one of the two species with spiny oogonia found parasitic on watermelon fruits, the larger examples here being represented by a mulberrylike aggregation consisting frequently of more than a score of subglobose communicating elements, from which the contents are delivered through an evacuation tube into a vesicle giving rise to more than a hundred zoospores. The other spiny form (*5*) associated with decay of watermelons exhibits sporangia that may be regarded as a modification of the subspherical type, consisting generally of a subspherical part together with an adjacent part of one or both hyphal elements between which it is intercalated, the evacuation tube arising from the venterlike part, or from the filamentous part, or very frequently from near the juncture of the two. Because Schroeter's disposition makes no provision either for this transitional type of sporangium or for the distinctive lobulate type, his scheme to be usable

would seem to require modification either by appropriately amending the two genera recognized by him or including one or more additional genera. For the present, therefore, it seems best to retain the genus Pythium in its more inclusive sense, as employed in the writings of DeBary and Butler.

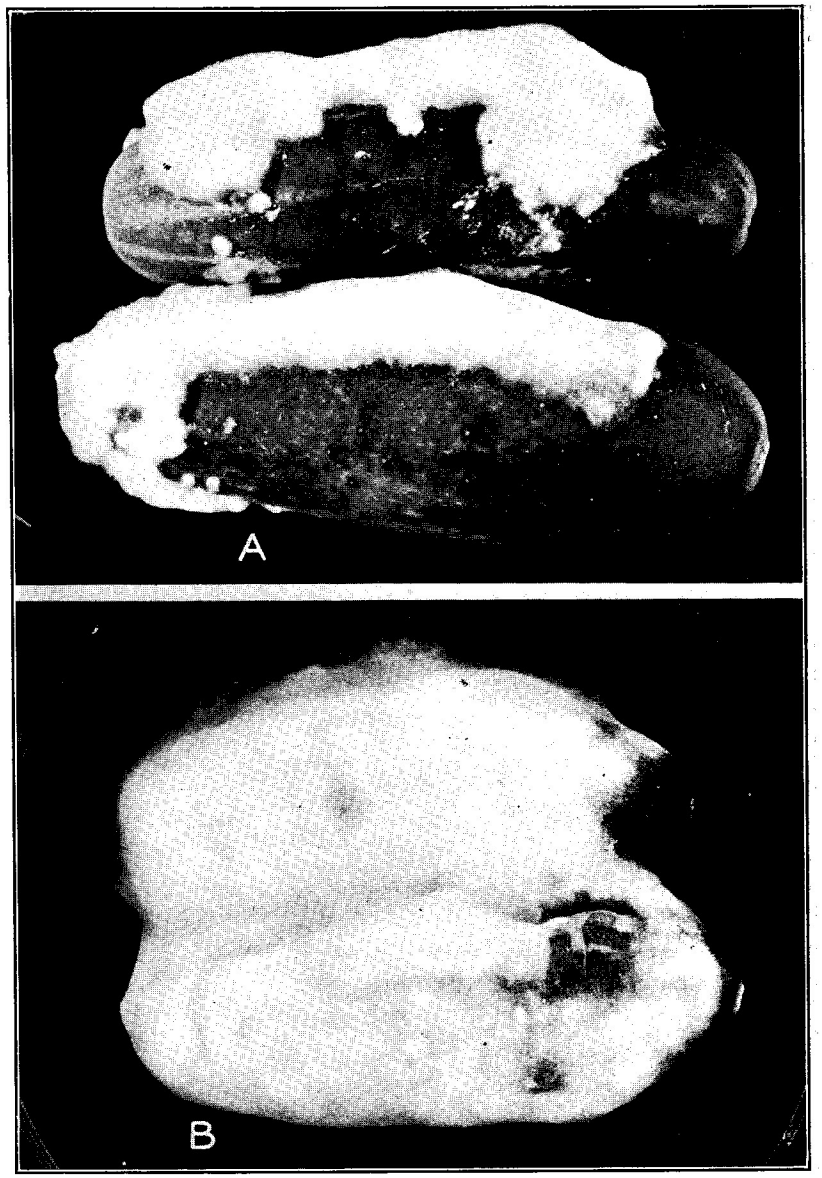
## PATHOGENICITY

The pathogenicity of the three strains isolated from material obtained on the markets of Washington, Pittsburgh, and Chicago was repeatedly demonstrated by inoculation into healthy cucumber fruits. Pieces of mycelium from pure cultures were inserted into aseptic incisions, which then were sealed with sterile vaseline, and the cucumbers placed in glass chambers without additional water. Softening, involving the tissues usually for a radius of several centimeters, was manifest within 24 hours; in 48 hours the larger part of the cucumber was involved and aerial mycelium was present in quantity near the point of inoculation, while farther away it appeared in numerous small patches and minute white flecks that marked individual spots where the vigorous crowded hyphae had burst through the confining epidermis (pl. 1, A). At the end of the third day the whole fruit was frequently entirely clothed in cottony mycelium (pl. 1, B). That we are not dealing here with a specialized parasite became evident when altogether similar results were obtained by the use of strains morphologically identical with those derived from the cucumber but isolated from other sources: (1) From dead female nematodes, *Heterodera radicicola* (Greef.) Müller, in material supplied by N. A. Cobb and G. Steiner, where the occurrence of the fungus as a saprophyte or a possible parasite invading moribund specimens could not be clearly determined; (2) from pea (*Pisum sativum* L.) roots exhibiting symptoms of root-rot; and (3) from watermelon fruits affected with the buff blossom-end rot.

The cucumber parasite was tried out on a number of other economic cucurbitaceous fruits. As might be expected, watermelons were found highly susceptible to attack, the resulting decay being entirely similar to the buff blossom-end rot familiar to the writer as a field trouble apparently widely distributed in the Middle Atlantic States, and for the most part due to the identical fungus. Pattypan, vegetable marrow, and summer crookneck squashes, all representing varieties of *Cucurbita pepo* L., are as promptly attacked and destroyed as the cucumber and with the same luxuriant development of extramatrical mycelium (pl. 2, A, B). Experiments with muskmelons (*Cucumis melo* L.) have not been quite as satisfactory, owing to the difficulty of avoiding bacterial contamination, especially in riper specimens. In general, it appears that in the green condition in which this fruit is frequently found on the market, the muskmelon does not provide a substratum very suitable for the fungus, but that as maturity is approached the soft edible pulp is more readily invaded. It is possible that the fungus participates in the destruction of rejected muskmelons left in the field; a considerable portion of the abandoned muskmelons in some Delaware fields visited by the writer in 1922 exhibited, as the initial stage in decomposition, a very watery condition of the interior, associated with the peculiar marshy odor fairly presumptive of the presence of some species of Pythium.

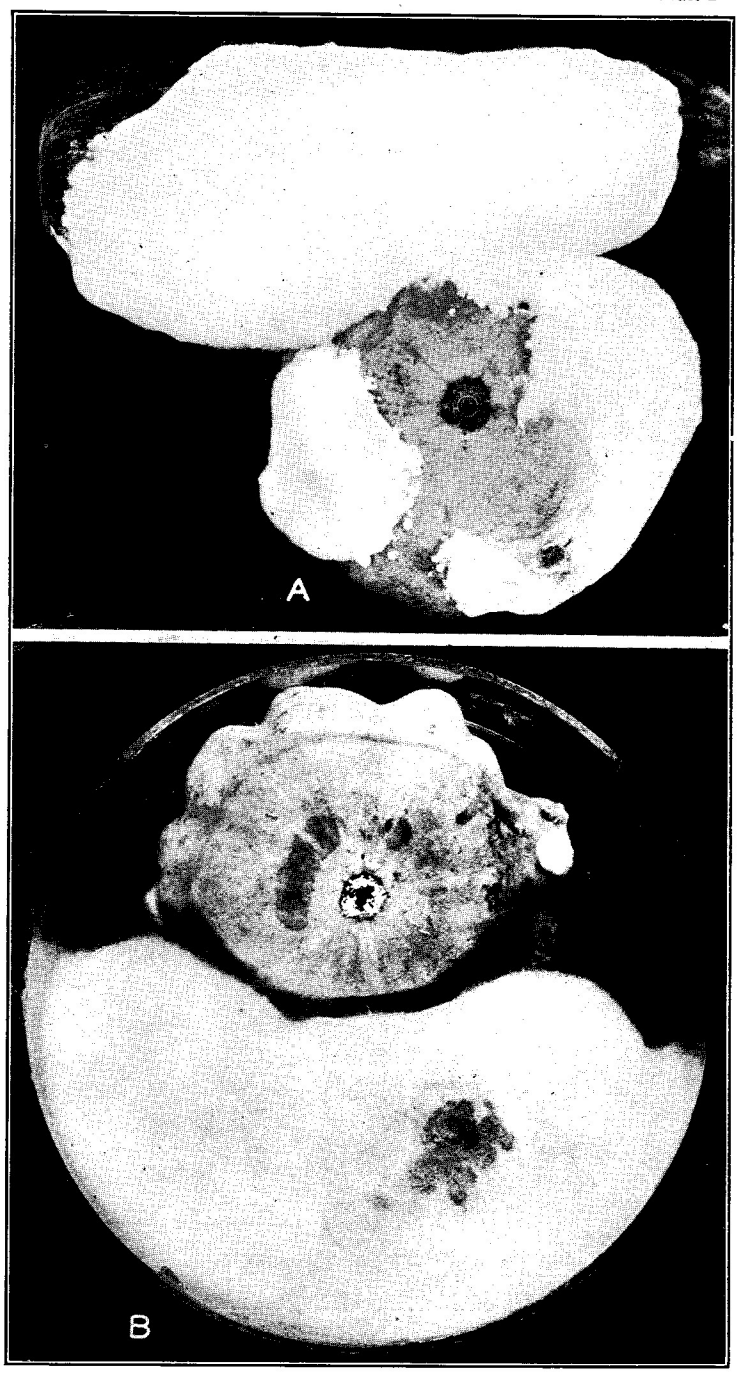
When inoculated under the rind of honeydew melons and cassaba melons (*Cucumis melo* L.), the fungus is able to establish itself, but subsequent development is markedly slow, sometimes being scarcely one-tenth as rapid as in cucumbers. The mycelium found in the tissues is of a compact, densely branching type, similar to that obtained on artificial media excessively rich in food materials, indicating that the juices of these fruits are too concentrated to permit of normal growth. Several inoculations into the flesh of Hubbard squash (*Cucurbita maxima* Duchesne) failed to result even in incipient infections, although the possibility that this vegetable is amenable to attack under other and more favorable conditions is not be to excluded.

While decay of cucumbers in transit has hitherto been found associated with only one species of Pythium, this is not because cucumbers are resistant to congeneric forms. In the course of routine procedure for obtaining the production of zoospores, for which purpose the tissue of cucurbitaceous fruits is not without merit, the writer has inoculated cucumbers with scores of strains having smooth oogonia, subspherical sporangia (or conidia), and fluffy aerial mycelium, belonging evidently to a number of related species—in short, with strains of the type traditionally and no doubt often correctly designated in papers on plant diseases as *Pythium debaryanum* Hesse. These strains have been isolated, for example, from the




A.—Two cucumbers 45 hours after inoculation with pure culture of strain of *Pythium aphanidermatum* isolated from diseased cucumber collected from carload lot at Washington, D. C., June, 1922. × ½

B.—Same two cucumbers as in A, but 24 hours later. × ⅔




A.—Vegetable marrow squash and pattypan squash 60 hours after inoculation at three points with pure culture of Washington strain of *Pythium aphanidermatum* isolated from diseased cucumber. × ½

B.—Three pattypan squashes 72 hours after inoculation with strain of *P. aphanidermatum* isolated from diseased cucumbers at Chicago, 1924. The profuse cottony mycelium in the lower part of figure has completely invested the two younger and more tender specimens. × ½

stems of cucumber plants affected with the trouble described by Atkinson (*1*) as canker; from diseased roots of herbaceous hosts, including garden peas, sweet peas (*Lathyrus odoratus* L.), rhubarb (*Rheum rhaponticum* L.), sweet potatoes (*Ipomoea batatas* Poir), cress (*Lepidium sativum* L.), and spinach (*Spinacea oleracea* L.); from roots of diseased seedlings of woody plants like *Pinus ponderosa* Dougl., *P. banksiana* Lamb., *P. sylvestris* L., *P. aristata* Engelm., and *Picea sitchensis* Trautv.; and from rose, pear, and geranium cuttings which had become diseased after being put in propagation beds. With relatively infrequent exceptions, the inoculated cucumber was attacked and the tissue invaded in much the same way as when *Pythium aphanidermatum* was employed, the rate of destruction for some forms being about equally rapid, while in the case of others advance was slower. All of the strains of Pythium isolated from separate lots of "leaky" potatoes and made available to the writer through the courtesy of G. K. K. Link have proved uniformly destructive, as have also about a dozen similar strains of the *P. debaryanum* type isolated from watermelons affected with a decay not readily distinguishable from the buff blossom end-rot due to *P. aphanidermatum*.

Although the several species of Pythium with subspherical sporangia (or conidia) and smooth oogonia show certain minor differences, in that some bring about greater softness in affected tissues, or a more watery condition than others, their effect in the interior of cucumber fruit is markedly similar to that produced by the parasite isolated from naturally infected material. The much more profuse development of aerial mycelium nevertheless provides a conspicuous characteristic by which attack by *Pythium aphanidermatum* can be distinguished from attack by the congeneric species that have been tried out. This feature appears sufficiently distinctive to merit attention in considering a common name for the disease under consideration. The term "cottony leak," descriptive of the most obvious symptoms of the malady, is proposed in this connection.

In its copious extramatrical development, moreover, is apparently to be found the characteristic to which *Pythium aphanidermatum* owes much of its destructiveness to cucumbers when packed as in commercial containers. The aerial mycelium of an individual fruit bearing an original infection grows out between adjacent fruits, partially or completely investing them. Laboratory experiments leave no room for doubt that such investment results in the infection of cucumbers, immediately if the epidermis is wounded, but without any considerable delay even if the epidermis is, as far as can be ascertained, altogether free of wounds. With the infection communicated from one fruit to another, each infected specimen gives rise in the course of 5 to 10 days to a "nest" of decaying cucumbers, including perhaps from a dozen to a score of individuals. Other species of Pythium with relatively feeble extramatrical development under conditions of only moderate humidity, such as generally prevail in produce cars, fail of passage from fruit to fruit, at least within reasonable periods of time. It is thus possible that if losses due to such species occur, the restriction of infection to single individuals might have occasioned their being overlooked by inspectors and others concerned in the examination and handling of food products.

In addition to cucumbers, the patty-pan squash, the summer crookneck squash, as well as the more delicate-skinned specimens of vegetable-marrow squash, have proved subject to infection by contact or investment with extramatrical mycelium of *Pythium aphanidermatum*. In cucurbitaceous fruits having a rind of indurated tissue like the watermelon, the cassaba, the honeydew melon, and the muskmelon, attempts at inoculation by means of surface contact have not given positive results.

It has been mentioned that some of the forms usually assigned to *Pythium debaryanum*, comprising, however, a relatively small minority, have failed to attack cucumbers when inoculated into incisions. A species not yet identified, provided with lobulate sporangia and hence closely related to but not identical with *P. aphanidermatum*, which was isolated from diseased corn roots, has shown no evidence of pathogenicity on cucumber fruit. The same statement holds true also of the two species with spiny oogonia (Artotrogus) responsible for the widespread chocolate blossom-end decay of watermelons, strains of these forms isolated from fruit thus affected as well as from pear cuttings, sweet-potato rootlets, and pea rootlets proving equally ineffective in producing decay of cucumbers. A third spiny species, in which the considerably larger oogonia are regularly borne on lateral branches, the swollen, somewhat contorted distal portion of which apparently serves as an intercalary antheridium, isolated only once from moribund rhubarb buds, similarly proved innocu-

ous to cucumbers. After securing negative results with three spiny forms, the writer was interested to discover that a fourth form derived from pea roots affected with the root-rot due to *Aphanomyces euteiches* Dr., and apparently different from the other three, attacked cucumbers with moderate vigor, the tissues becoming soft and watery.

In considering means of controlling losses from cottony leak, it is unfortunate that no information is available concerning the incidence of original infections. Knowledge as to whether such infections take place in the field or subsequent to picking would appear to be of primary importance. As the progress of the parasite at lower temperatures is relatively slow, and extramatrical development is reduced to small proportions in the absence of water of condensation and high humidity, attention to proper ventilation combined where practicable with refrigeration might be expected to check the spread of the infection to stock in good condition at the time it was packed.

The attention of students of plant diseases is directed to the very evident partiality of species of Pythium for the fruit of many Cucurbitaceae. Losses in the field due to their parasitism is undoubtedly more considerable than the paucity of references in the literature might lead one to suppose. Parisi's record (*12*) of the occurrence of *P. debaryanum* on the fruit of chayote (*Sechium edule* Sw.) in the botanical garden at Naples in December, 1920, is pertinent in this connection. Not less interesting is the very recent report by McRae (*10*) of the association of strains of Pythium with the decay of *Luffa acutangula* Roxb., *L. aegyptiaca* Mill., *Trichosanthes anguina* L., and *Lagenaria vulgaris* Ser. in India, where these members of the Cucurbitaceae are grown as vegetables.

## SUMMARY

Cucumbers grown in the Southeastern States have, on arrival at the northern markets, shown occasional losses due to a disease for which the term "cottony leak" is proposed. It is caused by a species of Pythium identified as *Pythium aphanidermatum* (Eds.) Fitz., the infection being communicated from diseased fruits to adjacent healthy ones by copious production of extramatrical mycelium.

The fungus is strongly parasitic on watermelons, on which host it is responsible for one of the blossom-end rots widely prevalent in the Middle Atlantic States. On inoculation it is rapidly destructive to patty-pan, vegetable-marrow, and summer crookneck squashes.

The sorting out of cucumbers harboring the fungus and the lowering of humidity and temperature by adequate ventilation, combined possibly with refrigeration, are indicated as means for controlling the trouble.

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