SOME FUSARIA FOUND ON THE COWPEA, WATERMELON AND CARNATION

by

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SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF ARTS

in the

GRADUATE SCHOOL
of the

UNIVERSITY OF MISSOURI

1916

Approved May 15, 1916
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5/18/16 C.B. Lutchison

The economic importance of the Fusarium problem affords a sufficient reason for the study of this genus of fungi. Various wilts, rots and blights are caused by parasitic and saprophytic Fusaria. It is true that wilts may be caused by organisms other than Fusarium but the wilt diseases of the south are primarily a Fusarium problem.

Throughout the cotton growing states, but more especially along the coast from North Carolina to Louisiana, the wilts of cotton, watermelon, and cowpeas are destructive. In severe cases whole fields may produce no crop. In moderate infections scattered plants may die, the others showing more or less reduction in vegetative growth and fruiting. The failure of a cotton crop means the failure of the staple crop of the south. The importance of the cowpea crop is indicated when the statement is made that cowpeas are rapidly becoming the "clover" of the cotton growing states. They are exceedingly valuable as a means of increasing the nitrogen content of the soil, improving its texture and at the same time providing a profitable forage crop.[10]

Though Fusaria flourish under high temperatures, these diseases are not confined to the southern states. Cabbage wilt or "yellows" in both Ohio and Wisconsin

has threatened the destruction of the cabbage growing industry. Potato wilt and rot have, in recent years, been very destructive in Ohio, Colorado and Oregon.[12] In 1909, at the Ohio Agricultural Experiment Station, at Wooster Ohio, as a result of Fusarium disease, the yield of potatoes was reduced from 180 bushels per acre, the preceeding four-year average, to 69 bushels. In that year, in Ohio alone, 151,611 acres were planted to potatoes.[9] If the further fact be taken into consideration that most of the tubers produced by diseased plants, before the time of digging have been penetrated by the fungus, which under the proper storage conditions of moisture and temperature continues its growth, resulting in the rotting of the tuber, it is easily seen that the total loss from this disease may be great. In the same state the losses from Fusarium wilt of cabbage range from two per cent in lightly infected new fields to ninety-five per cent in older and badly infected fields.[8]

The wilt diseases are due to soil organisms, so persevering that if resistant varieties of the crops are not planted, an extensive rotation is the only means of curing the "sick" soil. In no case is a rotation covering less than five years recommended and in

the case of cabbage yellows eight years has not been sufficient to clear the soil of the organism. Well drained sandy soil appears to be a predisposing factor to the disease in the case of cotton, cowpeas and watermelon. That such is the case with potato wilt is by no means proved, for in Oregon and Colorado the disease has been found extensively on the heavier soils. Moisture aids in the distribution of the fungus but drouth increases the aggressive nature of the disease.

So far as known the fusarial wilts of different crops are caused by separate and distinct though closely related species or varieties of the genus Fusarium. The form producing the disease on the cowpea does not cause either cotton or watermelon wilt. Entrance is obtained through the root system. Wounds apparently are not necessary, the parasite having the ability to gain admission to the host by dissolving the cell walls with some enzyme. The growth of the fungus mycelium within the vascular bundles of the host finally cuts off the water supply causing yellowing and dropping or wilting of the leaves and eventually the death of the plant. The yellowing or browning of the xylem in advance of the mycelium gives to the roots and stems a characteristic appearance. Verticillium albo-atrum, another wilt producing fungus, also darkens the vascular bundles of the plants it attacks. The color however is more nearly black than in the case of Fusarium.

The presence of cowpea wilt (Fusarium tracheiphilum) ordinarily is not evident before the plants are six weeks old. The field assumes a mottled appearance, the weakest plants yellowing and dropping their leaves first. The term wilt as applied to the cowpea disease is somewhat misleading. The plants do not wilt. The leaves turn yellow and drop from the stem leaving the bare stalk standing upright. The name wilt has been retained in this instance because the organism causing the disease is so closely related and similar to that causing wilt of cotton and watermelon in which the reduced water supply causes the leaves to wilt before death.

"Yellows" describes the effect of the wilt fungus (Fusarium conglutinans) on the leaves of the cabbage plant. The growth of the plant is stunted and the leaves drop from the stem at the least touch. A keen eye can often detect the disease even in the seed bed. Healthy plants, after transplanting, may be attacked at all stages of growth. The outer leaves yellow and drop first, the others follow in order. This shedding of the leaves is characteristic of the Fusarium wilt of cabbage.

Potato wilt (Fusarium oxysporum) gives no evidence of its presence until the plants are about a foot high, when there is a cessation of growth. The destruction of a portion of the root system tends to retard the development of the top. The restricting of the water supply yellows the leaves and produces a wilting or rolling of the leaves some-

times mistaken for leaf roll, (Blattrollkrankheit) The leaf roll of wilt differs from true leaf roll in that the leaves lack turdidity and die within a few days.[12] Plants that have died from this disease are easily pulled up, the diseased roots not affording a firm anchorage in the soil. Infection of the tubers takes place while they are still attached to the parent plant. Stem end browning is a visible indication of the presence of the organism within the vascular tissue of the tuber. Wollenweber[18] has been of the opinion that the tubers afford simply a place for the wintering of the wilt fungus, that F. oxysporum does not cause tuber rot as well as wilt, but Manns[9], and more recently Carpenter[3], have produced typical cases of rot by inoculation with the wilt organism. The type of rot produced depends upon environ mental conditions. With a high temperature and a maximum of moisture a wet rot results quickly, i.e. in two or three weeks. The lowering of the temperature and decreasing the moisture gives a slow dry rot. Bad smelling rots are not produced by Fusaria. The vascular parasite F. oxysporum is not the only species producing potato tuber rot. The saprophytic forms associated with these troubles are numerous. The storage of potatoes at a temperature of less than 50° F prevents, to a large extent, the action of the rot producing Fusaria.

Fusarium disease of cotton (Fusarium vasinfectum) is perhaps the most destructive malady affecting cotton. Atkinson[2]

in first describing it called it "Frenching". The leaves yellow and finally wilt and die in the heavy soils of the south. In sandy soils wilting of the leaves is more pronounced, the yellowing less so. The stalks do not fall and usually, throughout the stems, there is the characteristic discoloration of the vascular bundles. The darkening of the xylem is always present in the root tissues. Though the wilting and death of the plants may appear to be sudden they are the result of a period of growth of the fungus within the host, during which time the vessels were filled with hyphae.

Watermelon wilt (Fusarium niveum) was described by E. F. Smith[15] in 1894 as an exceedingly destructive disease in Georgia and South Carolina. Entrance is obtained through the root system, as is the case with all the wilts, and within four weeks, when the soil is badly infected, the disease becomes manifest. The vines wilt suddenly and die within a few days. Though the fungus mycelium may extend for a yard or more into the stem, in the case of large vines, it does not reach the melons to infect the seed. The discoloration of the xylem is not as pronounced as in the other wilts.

Tomato wilt, caused by a Fusarium (Fusarium lycopersici) is one of the worst tomato diseases of the south. In Louisiana in 1913 it was considered the most destructive of the ten prevalent tomato diseases.[4] The disease makes its appearance about the time of flowering. The symptoms usually

accompanying wilt are present, yellowing and wilting of the leaves and the discoloration of the vascular bundles in the roots, stems and even the petioles of the leaves. The fruit, if formed, is small and lacks the normal flavor. Practically all the popular commercial varieties are quite susceptible to the disease.

Stem rot of carnations is another disease due to a species of Fusarium. The method of entrance has not been definitely determined but the organism is probably a wound parasite. In the first stages of the disease the roots appear healthy, the disease spreading from the stem, which becomes softened near the surface of the ground, into both roots and leaves, rotting the roots and yellowing, wilting and drying up the leaves. The death of the plant, though caused by the invasion of the fungus, is not due to a clogging of the xylem as in the wilts mentioned above. The disease may attack the carnation at any stage of its growth, high temperature and excessive moisture being particularly favorable to the development of the disease. While the disease is widly distributed it need not become serious if proper cultural methods are employed.

A consideration of Fusaria involves also a mention of the Imperfect Fungi, the class to which they belong. The Imperfect Fungi are a heterogenous collection of forms, organisms whose complete life cycle is not known and, consequently, with their relationship to the natural genera undetermined.

Further investigation will probably link up a considerable number with the Ascomycetes. Imperfect Fungi produce conidia in abundance but no perithecial stage has been connected with them. Within the Imperfect Fungi several families are recognized. The Hypomycetes bear their conidia on exposed conidiophores which may be single, branched or united into coremium-like structures. The genus Fusarium has its place within this family.

In his "Fusaria of Potatoes,"[14] Sherbakoff enumerates the following characters as being of generic value:

"1. Color of conidia and mycelium, never of a plain gray or black but mostly of various brilliant hues.

2. Conidia dorsiventral, attenuate, pedicellate, not appendiculate, smooth, normally not constricted at the septa, distinctly three, or more, septate, acrogenous, not in chains.

3. Conidiophores with single to irregularly whorled branches, never truly dichotomous, nor of a strictly penicillate or verticillate type. The conidiophores, typically give rise to macroscopically observable dense tufts of conidiophores, covered more or less deeply with a somewhat slimy mass of spores. Such fruiting bodies, tuberculate in form, (sporodochia), may be with or without a plectenchymic flat or wart-like base, without any differentiated enclosure.

4. Chlamydospores, (endogenous double walled bodies), terminal and intercalary or only intercalary, or none, and produced by both mycelium and by spores.

5. Mycelium composed of hyphae which are distinctly but never closely septate and irregularly, never dichotomously branched, secondary branches usually thinner than the primary ones; protoplasmic content of the mycelium for the most part plainly present and distinctly vacuolate. The rate of growth in artificial media, when compared with the Fusarium like organisms studied, is comparatively high".

The following terms are used in the discussion of Fusaria:

The "stroma" is the bed at the base of the sporiferous stages and is always present. It may be reduced to scattered hyphae, within or without the host, or may form an aerial or immersed mycelial layer, the consistancy of which is either loose or plectenchymatic. The basal part of the stroma sometimes develops coremia.

A "sclerotium" is a ball like plectenchymic mass, either sterile or serving as the base of a sporodochium or peritheceum. It functions as a resting stage.

"Pionnotes" is the term applied to an excessive conidial production where there is a minimum of aerial mycelium. (Plate XI). The spores when young form a slimy layer on the surface of the host which becomes more or less powdery

with age.

When the slimy layer of spores is to some extent marked by aerial hyphae it becomes a pseudopionnotes.

Sporodochia differ from pionnotes in their rounded wart-like form. The numerous spores are borne on elaborately branched conidiophores, (Plate), or on more simple ones crowded together. Occasionally several mycelial threads running more or less parallel become united into an upright columnar structure, coremium-like, bearing conidia along the side.

Microconidia are non- one- or two-septate spores, normally present in some sections, absent in others.

Macroconidia, smooth, sickle-shaped, three or more septate spores are notmal for all sections of the genus except the section Dimerum.(Sherbakoff)

Morphological as well as pathological characteristics are made the basis upon which Wollenweber divides the genus into sections. The section Elegans, comprises the vascular parasites. Elegans, Martiella, Discolor, Roseum, Gibbosum and Ventricosum contain more or less uniformly parasitic species causing rots. Scabs and blights are produced by the section Discolor.

SECTION ELEGANS----Wollenweber.

Fusaria with microconidia: Macroconidia hypically septate with bottle shaped apex and pedicellate base. Chlamydospores terminal and intercalary and in conidia. Color of conidia usually a pinkish buff. Substratum in agars rich in dextrose of various vinaceous hues:

on rice from pink to red, vinaceous and purple.

SECTION MARTIELLA-Wollenweber.

Microconidia typically present on the aerial mycelium macroconidia chiefly three septate, of nearly even diameter, the apex not sharply pointed and the base often apedicellate. Terminal and intercalary chlamydospores present. Conidia, in masses, drab to greenish blue; substratum on neutral or slightly acid dextrose agar, drab, vinaceous red or purple, or blue but never carmine red.

SECTION GIBBOSUM---Wollenweber

Microconidia rare. Macroconidia mostly five septate with hyperbolic to elliptic dorsal curve, broad in the middle with a long whip like apex. The pedicellate base shows a prominent heel. Intercalary chlamydospores always present, singly, in chains and clusters. Mycelium from hyaline to brown; conidia in mass from pale buff to cinnamon and sepia.

SECTION ROSEUM--Wollenweber

Conidia typically five septate, slender, gradually attenuate at both ends. True chlamydospores always absent. On dextrose agars from honey yellow to Eugenia red and sometimes nearly hyaline.

SECTION DISCOLOR--Wollenweber

Microconidia typically absent. Five septate macroconidia dominant, distinctly pedicellato and with an abruptly attenuate apex. Chlamydospores few, intercalary only. Substratum on dextrose agar typically carmine red,

occasionally yellowish, but never gray, green or blue.

SECTION VENTRICOSUM--Wollenweber

Conidia with slight dorsiventrality, more or less wedge shaped, apedicellate. Three septate type dominant not produced in sporodochia. Chlamydospores always present but variable.

SECTION EUPIONNOTES--Wollenweber

The only section that possesses a true pionnotal fruiting form. Conidia typically three-septate, apedicellate slightly broader towards the apex. Terminal and intercalary chlamydospores present.

SECTION SPOROTRICHIELLA--Wollenweber

In this section o-septate, pyre form to globular microconidia are dominant. Macroconidia are always present, at least in young cultures.

In addition to the foregoing sections established by Wollenweber Sherbokoff has proposed three more.[14]

SECTION DIMERUM--Sherbakoff

Conidia dorsiventral, one septate; chlamydospores may be present.

SECTION ARTHROSPORIELLA--Sherbakoff

Microconidia short and broad, spindle shaped, o-3 septate; sporodochial conidia mostly five septate of Roseum type: pionnotal macroconidia 5-9 septate from slightly curved to straight: chlamydospores absent: aerial mycelium from white to buff, pink and red: substratum from clay to red.

SECTION FERRUGINOSUM--Sherbakoff

Conidia intermediate between sections. Roseum and Gibbosum 3-7 septate: only intercalary chlamydospores typically present. Color varies from white to red.

The variability of Fusaria is so great that pure cultures are necessary to an adequate study of the members of the genus. They respond with various modifications to environmental conditions. Spores, macroconidia, produced under excessive moisture conditions either lack septa, or, the cells becoming turgid make the septa appear like indentations in the wall. On the other hand under excessively dry conditions the cells may collapse, leaving the septa projecting like rings.(Page). The normal, most constant form, with the greatest vitality, is midway between these two forms. Light, temperature, moisture and nutrition are all factors influencing very materially the growth and appearance of the fungus. Unless the influence of these factors is known, identification of the various forms is difficult. Diffuse light, a temperature between 25 and 30 C, stems of various woody and herbaceous plants such as raspberry cane, sweet clover, potato, lupine, etc., for the culture media, and a humidity of from 50 to 75 % have been found to give the most nearly normal growth. High temperatures increase growth and fruiting but tend to disturb the ratio between few and many septate conidia by the production of an unusual number with few septa. Direct sunlight gives on an average smaller and less regularly septate spores than when the

culture is grown in diffuse light. For the color effects produced by Fusaria potato tuber plugs are more satisfactory than stems. Sterilized rice gives even more brilliant and characteristic colors, especially in the case of the section Elegans. Age also may modify the color.(Plate III) The origin of the colony is another factor to be considered. Transfers of mycelium give an excessive vegetative growth.(Plate IV) Colonies arising from macroconidia tend to produce that form of spore early. Only when the environmental conditions are known can forms be compared and described with certainty.

As was stated before, Fusaria are imperfect fungi, effuse in conidial production but without a known perfect stage. Ascomycetes, however, having a perithecial stage, may produce fusiform conidia in such abundance that at certain stages of growth it is difficult, if not impossible, to distinguish the two forms by their conidia alone. Conidia of Giberella are indistinguishable from Fusarium culmorum of the section Discolor, and, depending on that character, a seperation is doubtful. Another difference between the two fungi aside from the production of blue or violet perithecia, which may be prevented, is the absence of chlamydospores. The Giberella fungus seems to have lost the power of producing them, depending on the plectenchymata to carry it through adverse conditions. Hypomyces produces conidia very similiar to members of the section Martiella, but also develops red perithecia con-

taining one septate ascospores, and chlamydospores. In a few cases, such as Hypomyces, occur the exceptions to the general rule that the presence of chlamydospores indicates the absence of perithecia. Other perfect forms resembling Fusaria in their conidial stages are Nectria whose perithecia, neither blue nor violet, contain two celled ascospores and Calonectria whose strongly colored perithecia are not blue and whose ascospores are one-three septate. To the genus Ramularia Wollenweber[19] has transferred those forms with cylindrical conidia, rounded at both ends, and possessing chlamydospores but no perithecia.

Neocosmospora rarely produces the sickle shaped septate conidia so typical of the genus Fusarium. The conidia are unicellular and ellipsoidol. The perithecia are red, as in Nectria, containing many asci, each with eight globose or elliptical ascospores. In 1899 E. F. Smith[14] described Neocosmospora as having micro- and macro-conidia and perithecia. The three fruiting stages were considered forms of the parasite which caused the wilt of cotton, watermelon and cowpeas. More recently, (1908), Higgins[6] has cast doubt on the genetic connection of the perithecial stage with the vascular parasite. Experimenting with all stages of the fungus he found that from the ascospores perithecia were always produced, never macro-conidia and chlamydospores; the mycelial growth was slow, scanty and never pure white; no bright colors were pro-

duced in the substratum, nor was there any growth below the surface of the gelatine. On the other hand, in cultures from the parasite found within the xylem, perithecia were never present; macroconidia and chlamydospores were always found in cultures sufficiently mature; the growth of the aerial mycelium was rapid and in most cases it was white; bright colors were produced on starchy media and a mycelial growth was produced below the surface of the gelatine. Though the ascigerous fungus, when grown in pure culture, produces conidia very similar to the microconidia of the internal fungus, the fact that perithecia of many other forms are found on the same host stems, that the perithecial form has not been connected with Fusarium wilts reported from Europe, and, that, so far, it has been impossible to change one form into another, leads to the conclusion that Neocosmospora vasenfectum is not the perithecial stage of the Fusarium that causes cowpea wilt.

The problem of the control of Fusarium diseases presents two phases, (1) The keeping of land hitherto uninfected free from such organisms and (2) the growth of a crop on infected land. The latter may be accomplished either by a system of crop rotations or by planting disease resistant varieties.

The methods of distribution of these diseases are various. Drainage water may carry the spores from higher to lower lands. Farm implements and the feet of animals may transfer infected soil from one field to another. Manures, into which diseased material has been thrown, are a source of danger. Seed grown in diseased fields may become contaminated in its preparation for the market. Recently Fulton and Winston[5], in attempting to explain the sudden and extensive appearance of watermelon wilt in districts where it had never appeared a source where wilt had prevailed the previous year. Examination and germination of the seed proved Fusarium niveum present. Seeds treated for one-half hour with formaldehyde (1 part to 200 parts of water) produced healthy seedlings, proving the advisability of such disinfection in case the seed comes from a questionable source. The better practice is, of course, to use only seed that is above suspicion. The location of seed beds upon an area on which that particular crop has recently been grown is another dangerous procedure, aiding in the spread of disease.

Crop rotations are recommended as a means of combating these diseases. In severe cases rotations of from five to ten years duration will more or less effectually clear the soil of the troublesome organism. Usually the more widly different the crops grown the shorter need be the period of rotation.

The growth of resistant varieties of crops is the best means of controlling these diseases. The difficulty has been to get varieties which are resistant and at the same time possess all the desirable qualities of the most popular commercial varieties, a problem upon which the Unites States Department of Agriculture has been working for some years. The Iron Cowpea, completely wilt resistant in the south, is a half bushy plant, maturing in ninety to one hundred days and retaining its leaves late. Its chief weakness is its moderate seed yield. Brabham, a cross between Iron and the popular variety Whippoorwill, is wilt resistant but requires a poor soil for seed production. On rich soil its vegetative growth is excessive.

The North Carolina Station Report of 1913-14 announces the production of a watermelon highly wilt resistant. It is excellent for local marketing and further efforts are being made to obtain a rind sufficiently tough for shipping. At that time the seeds had not been placed on the market.

The Louisiana Experiment Station (1913)[4] has developed a wilt resistant strain of tomatoes from a wilt resistant plant of the Acme variety. Fruiting is about two weeks later than the Acme which is a rather serious fault in a variety to be used for the early market.

So far no varieties of potatoes resistant to Fusarium have been developed. Crop rotations and the selection of tubers which show no evidence of the browning of the vascular bundles and which have been grown in fields free from wilt must be depended upon as yet to control the disease.[12]

Wilt resistant cotton is being grown in the south and recently decided progress has been made in Wisconsin toward the development of resistant cabbages.[7] In the best commercial varieties, tested on soil, twenty-seven per cent headed, while from the best selected seed ninety-three per cent headed.

The stem rot of carnations may be controlled by proper cultural methods, using cuttings from healthy plants, fresh or disinfected soil in the greenhouse, avoiding extreme changes in environmental conditions and excessive heat and moisture.[17]

LABORATORY STUDIES

The Fusaria studied in the laboratory were isolated from cowpea, watermelon and carnation plants. The cowpea and watermelon plants were sent from diseased

fields near Morley, Missouri and the carnation from a commercial greenhouse in Kansas City.

The media used were prepared as follows:

Potato dextrose agar. This was made by cooking 200 grams of sliced potatoes in 1000 cc. of distilled water for twenty-five minutes in an autoclave at 5-7 pounds pressure. The liquid was filtered through cotton, made up to 1000cc. by the addition of distilled water, twenty grams of agar added and the mixture returned to the autoclave for twenty minutes. Twenty grams of dextrose were then added, the whole again filtered through cotton, tubed and sterilized for twenty minutes at 8-10 pounds pressure.

Oat meal agar was prepared by soaking 100 grams of oat meal in 1000 cc. of distilled water over night, decanting the liquid and restoring it to its original volume. Twenty grams of agar were added and the mixture heated in the autoclave for thirty minutes at from 8-10 pounds pressure, tubed and sterilized.

Rice was placed in test tubes to the depth of about one inch, distilled water added until the column of water stood twice as high as the rice, and the contents of the tubes sterilized for thirty minutes at 110 pounds pressure.

Tapioca, to the depth of ¾ of an inch, was put into test tubes and distilled water added. The tubes were

allowed to stand for an hour. At the end of that time enough water was added to cover the swelled grains for ½ an inch and the tubes sterilized for thirty minutes at eight pounds pressure.

Rye bread, cut into suitable sized pieces, without crust, and with sufficient distilled water to keep it moist, was sterilized for thirty minutes at 8-10 pounds pressure.

The natural media used were lupine stems and potato tuber plugs, made by cutting cylindrical pieces from potatoes and dividing each into two parts diagonally. Sufficient water to make the media moist was added and the whole sterilized for thirty minutes at ten pounds pressure.

A few cultures were made by transferring the spores found on the surface of the melon root and stem directly to agar. All other cultures were obtained by planting bits of diseased tissue in agar in Petri dishes.

The stem, or root, was prepared by first washing in Mercuric chloride solution (one per cent) and afterward in distilled water; it was then broken and with a flamed scalpel a small portion of tissue removed and placed on the agar in a Petri dish. The agar was acidified with two drops of twenty-five per cent lactic acid to prevent the growth of bacteria. Usually within four days a growth of mycelium was visible around the particles. As soon as spore formation was copious, poured plates were made. If these were mixed cultures plates

were again poured and from these the germinating spores were transferred to the different culture media.

Potato dextrose agar proved much more satisfactory as a culture medium than the oat meal agar. The latter supported a very slow, scanty, almost colorless mycelial growth with a subnormal spore production. Rice was the best medium for brilliant color effects. With all the forms isolated from cowpeas it gave various pinks reds and purples with a good growth of mycelium and spores. The forms isolated from the carnation and watermelon were vinaceous, yellow and brown.

Tapioca as a culture medium gave a slow and scanty mycelial growth. The color effects were not as brilliant as those on rice. Fusaria from the cowpeas tinted the medium from indigo and purple at the top to various pink and salmon shades farther down the tube, while those from the carnation showed only an olive buff line on the surface of the medium.

The bread gave a good growth of mycelium, not as strongly colored as on rice, except perhaps in strain A. from carnation where orange and brown predominated.

The lupine stems were small, drying out quickly, and on that account not as satisfactory as were the potato tuber plugs. They showed no striking color effects. The spore production was normal.

Potato tuber plugs were suitable both for mycelium and spore production and also gave more or less strik-

ing colors, a carmine plectenchymata in the case of one carnation Fusarium, and with another form, blue sclerotia. The pionnotal fruiting was more evident on potato than on agar or rice.

No alkaline or acid series of the different media was used. The addition of small quantities of lactic acid to the agar of the dilution plates, intensified the purples or lilacs produced by the forms from the cowpea. It also had a tendency to decrease the aerial mycelium of the colony.

Excessive moisture increased the granular appearance of the conidia, the septation in some cases being invisible.

The cultures were all kept at laboratory temperature which was fairly uniform except for two or three weeks during exceptionally severe winter weather. At this time growth was quite slow, but the cultures were not permanently affected. They were grown in diffuse light, never receiving the direct sunlight.

The strains isolated from cowpeas have all been listed under the letter B, those from carnations under A and those from watermelon under W. The strains from each host were quite closely related, those from the cowpea having the Elegans shape and coloring while the forms from the watermelon with two exceptions, (Wc and Wl) showed the typical conidial form as well as coloring of the Section Martiella. Of the three strains from the carnation two, Ac and An, possess conidia with the long slender apex typical of the Section Gibbosum. Neocosmospora vasinfecta was also isolated from the cowpea roots.

DESCRIPTIONS OF STRAINS ISOLATED

Strain Ba

In plate cultures the aerial mycelium is dense and white, the substratum cream with lily green sclerotia in old agar cultures a blue green plectenchymata often is present: on rice, the color is various shades of red and purple (PlateI). Terminal and intercalary, non- or one-septate chlamydospores and microconidia, but rarely three septate conidia, are present.

Measurements of conidia from cultures:

On potato dextrose agar, 9 days old:

0-septate conidia 6-12 x 2-3u

1-septate conidia 14-16 x 2.5-3u

2-septate conidia (few) 18-20 x 3u

On potato tuber plug, 17 days old:

0-septate conidia 4.5-10 x 2-4u

1-septate conidia 8-17 x 3-4u

On occasional 2-septate conidium present.

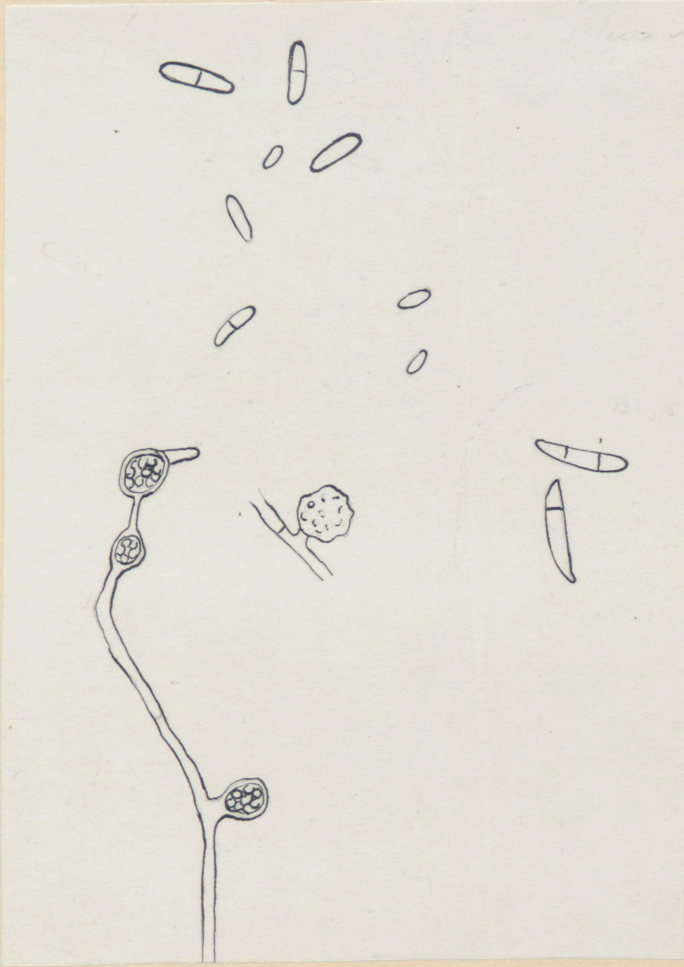
On potato dextrose agar, 76 days old:

Microconidia but no 3-septate conidia present.

On potato tuber plug, 102 days old:

Microconidia present, numerous terminal and intercalary chlamydospores. 5-7.5u in diameter.

Fig. 1, Page 25.



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Strain Bx

Aerial mycelium dense white on plate culture of potato dextrose agar. Stroma in center of colony light vinaceous purple; on rice, congo pink; on potato, white to chamois; chlamydospores present, no pionnotes, sporodochia or sclerotia.

Culture on potato dextrose agar, 5 days old.

Micro and macroconidia in about equal numbers.

Chlamydospores in conidia, none in mycelium.

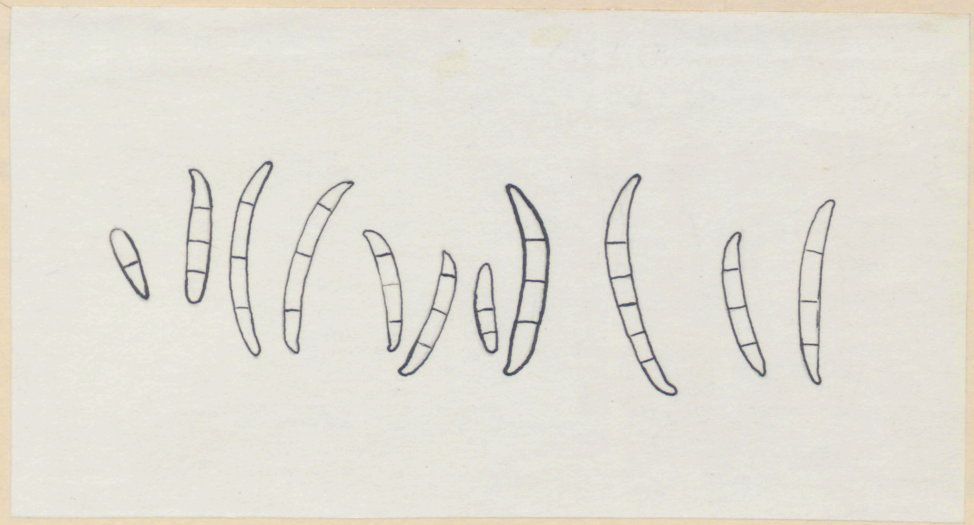
On potato tuber plug, 28 days old.

3-septate conidia 20-36u x 2.5-4u

Chlamydospores 6-8u in diameter, numerous, terminal and intercalary.

Fig. 1 Page 27. Conidia taken from an 8 day old culture on potato dextrose agar.

Fig. 2 Page 27. Conidia from a 29 day old culture on agar.



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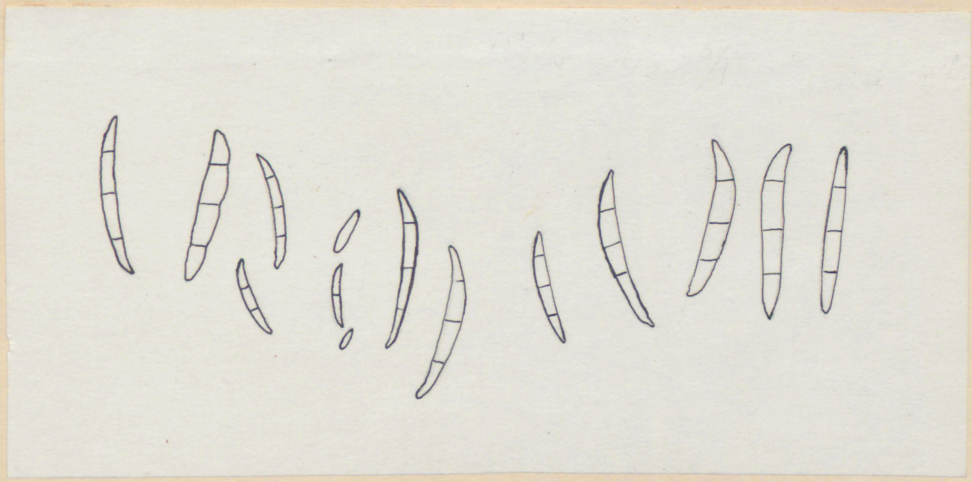


2

Strain Bs

In plate cultures there is a zone of aerial hyphae at margin of colony, the center having only submerged hyphae. Aerial mycelium white to deep cream, substratum cream; on rice and bread pale pink (Plate II); colorless sclerotia present; chlamydospores terminal and intercalary.

Potato tuber plug 8-day old culture:

0-septate conidia 4.5-14u x 2.5-4u

1-septate conidia 9-18u x 2.7-4u

3-septate conidia(rare)21u x 4u

In a 20 day old culture less than 50% of the conidia were 3 septate; 25u in length. Colorless to buff sclerotia in abundance. Chlamydospores, single, 7.5-11u in diameter. Potato dextrose agar 109 days old culture microconidia present, no three septate conidia.

Strain Bz

On potato dextrose agar plate culture aerial mycelium not well developed, white; submerged mycelium vinaceous to purple; on rice pink (Plate IV); microconidia and macroconidia present; terminal and intercalary chlamydospores present; no sclerotia.

Measurements of conidia:

On potato tuber plug, 10-day old culture;

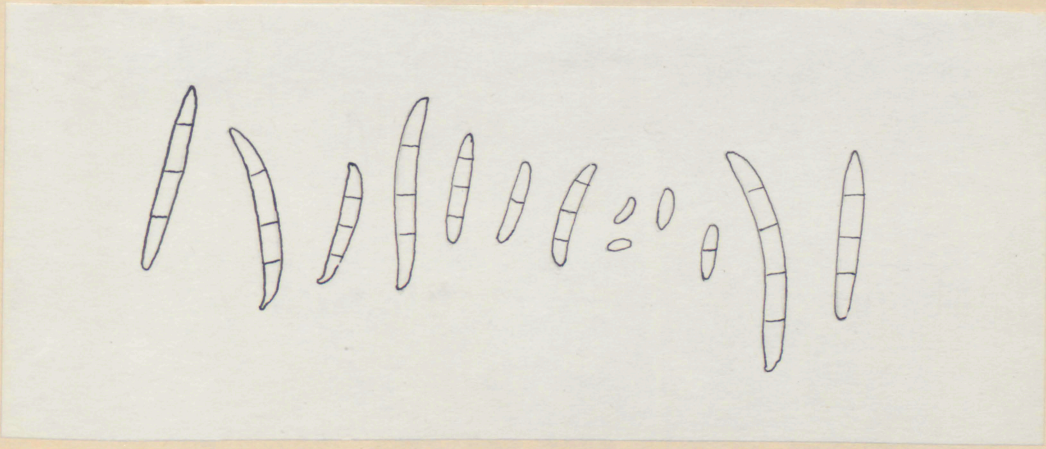
0-septate conidia 5-12u x 2-3u

1-septate conidia 10-20u x 25-3u

3-septate conidia 24-42u x 3-4u

Chylamydospores 6-8u in diameter.

Fig. 1(Page 30). Conidia taken from an 11-day old culture on potato tuber plug.



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Strain Bc

Micro-conidia and 3-6 septate macro-conidia present. On potato dextrose agar the colony has little aerial mycelium except in the center if one or more distinct purple zones appear in the submerged mycelium. Various color effects are produced on different media.(Plates III and IV). Terminal and intercalary chlamydospores are present.

The measurements of conidia are as follows:

On potato tuber plug 7-day old culture:

0-1 septate conidia dominant

0-septate conidia 4-14u long

1-septate conidia 10-20u long

3-septate conidia 25u x 3-3.6u

On potato dextrose agar 12-day old culture:

0-septate conidia 5-16u x 2.5-3.6

1-septate conidia 15u x 3-3.6u

3-septate conidia 23 x 3.6

On potato tuber plugs 12 days old

Psendo uionnotal conidia

3-septate conidia 28-40u x 3.5-5u

4-septate conidia 38u x 5u

5-septate conidia 50u x 5.2u Few

(Continued on following page).

On potato tuber plugs, 20-day old culture:

Pseudopionnotes:

3-septate conidia 18-38u x 35-45

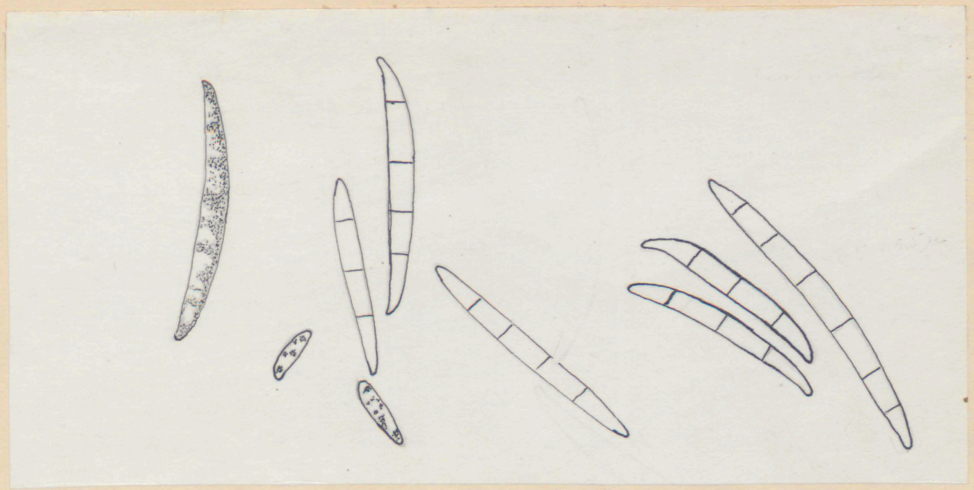
4-septate conidia 32-42u x 3.5-4.5

5-septate conidia 36-54u x 4-5u

Fig. 1; conidia from an 8-day old culture on potato dextrose agar.

Fig. 2; Conidia from a 26-day old culture on potato tuber plug.

Fig. 3; Conidia from a 47-day old culture on potato tuber plug.



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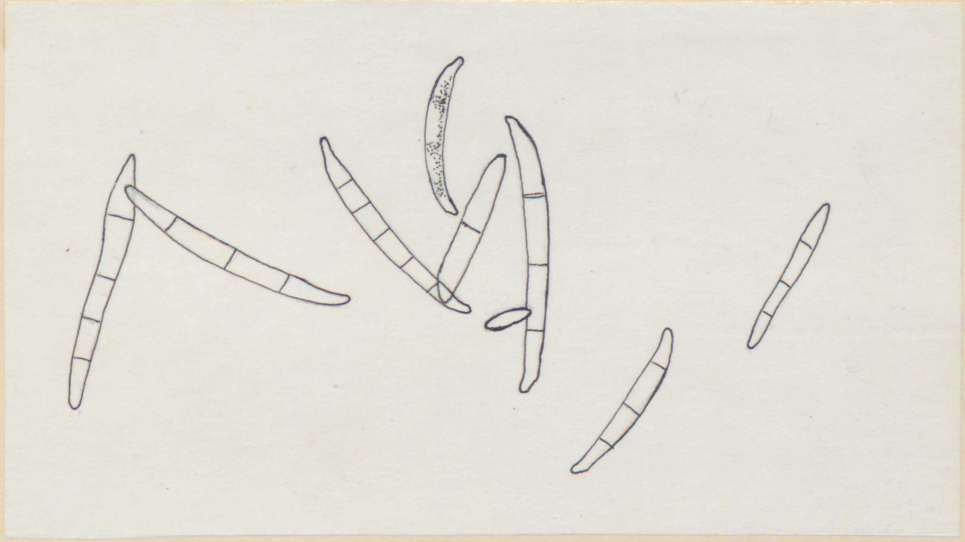

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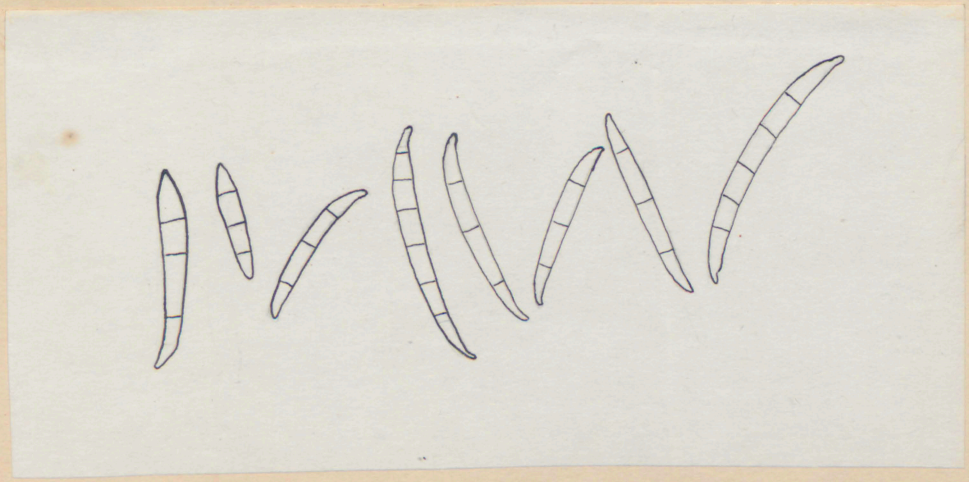


3

B

Microconidia and 3 septate macroconidia present. Scanty aerial mycelium, white, on potato dextrose agar, on potato tuber plugs the aerial mycelium is well developed white to cream; on rice congopink to vinaceous (Plate IV.); chlamydospores, terminal and intercalary, not in long chains or clusters; no sclerotia.

The measurements of the conidia are as follows:

On potato tuber plug, 15-day old culture:

0-septate conidia 5-10u in length

1-septate conidia 12u in length

2-septate conidia 18-20u in length (very few)

3-septate conidia 23-32 x 3-4u

On potato tuber plug,-26-day old culture:

Microconidia dominant.

0-septate conidia 5-18ux 2.5-3.5u

1-septate conidia 12-18ux 3u

3-septate conidia 23-34ux 3-4u

Chlamydospores 8-12u in diameter.

(Continued on following page).

On potato tuber plug, 61 day old culture:

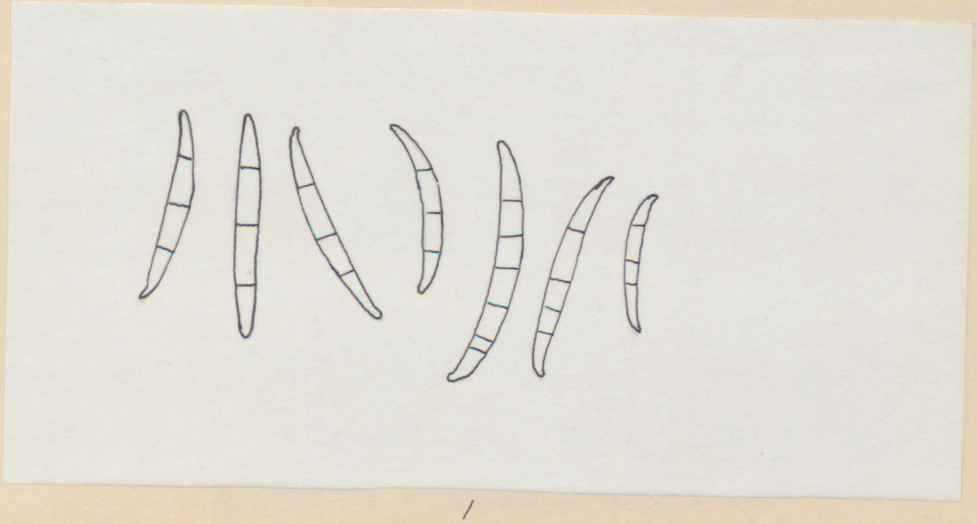
0 septate conidia 4-8 x 3-35u

1 septate conidia 10-15 x 3-4u

3 septate conidia 25-40 x 3.7-4.2u

Fig. 1; conidia from a 7 day old culture on potato dextros agar.

Fig, 2; conidia from a 26 day old culture on potato tuber plug.



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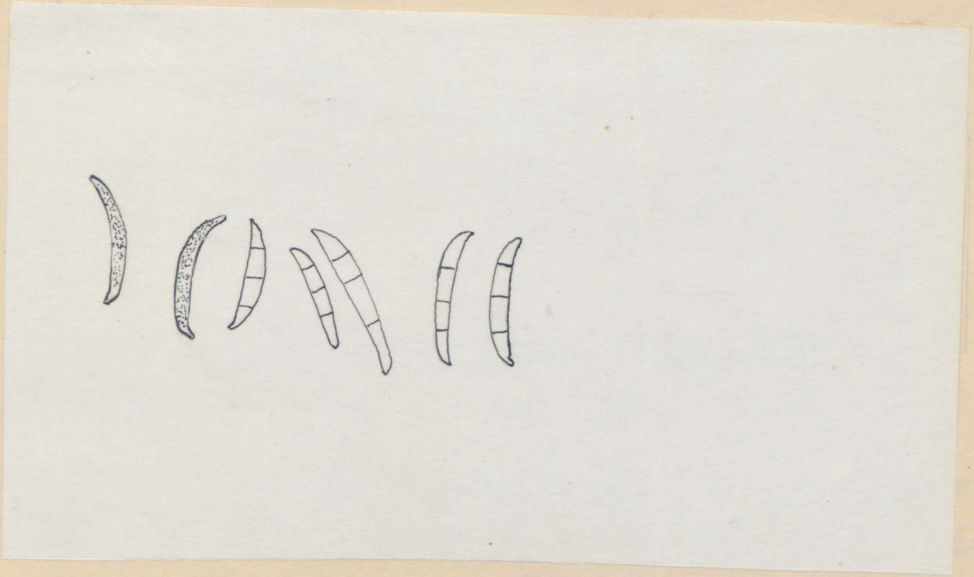


2

Forms isolated from carnation

STRAIN A

On potato dextrose agar the mycelium is well developed, flesh to salmon; on potato tubers, bread, tapioca, and rice distinctive colors are developed. (Plates IV and V)

Measurements:

Potato tuber plug, 19 days old culture:
3 septate conidia 18-28u x 4-5u
An occasional 4 and 5 septate conidium

Potato dextrose agar, 26 days old:
3 septate conidia 20-38u x 4-47u
5 septate conidia 36-40u x 4-5

Lupine stem, 6 days old culture:
3 septate conidia 20-36 x 3.5-4u

Potato dextrose agar, 119 days old culture:
3-5 septate conidia still adhering in clusters to the mycelium.

This strain does not agree exactly with any of those described by Mr. Wright in his Stem Rot Disease of Carnations. The plate cultures always developed a

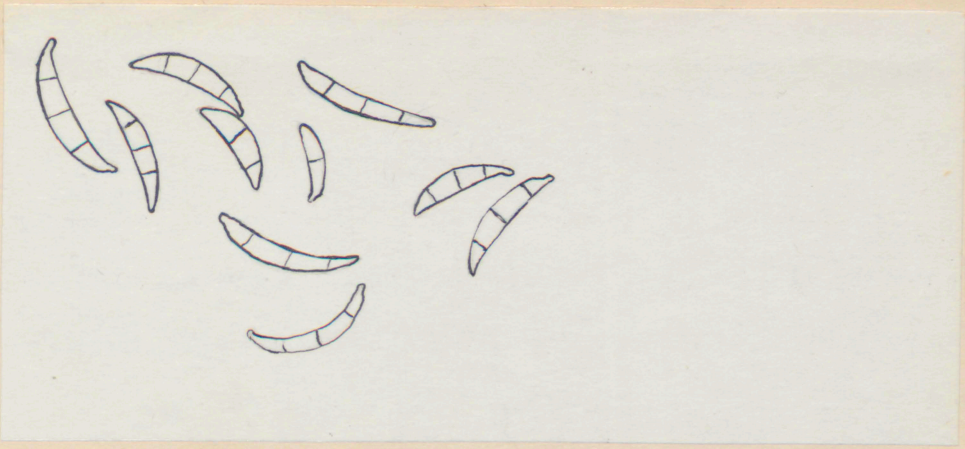
salmon color, never a wine red or yellow. They did, however, develop the yellow and pink in young cultures on rice in about equal intensity.

Fig. 1. Conidia taken from an 18-day old culture on potato tuber plug.

Fig. 2. Conidia taken from a 63-day old culture on lupine stem.

Fig. 3. Cluster of conidia still adhering to the aerial hypha, which produced them, in culture on agar 107 days old.

Fig 4. Conidiophores from 9 day old culture on potato tuber plug.



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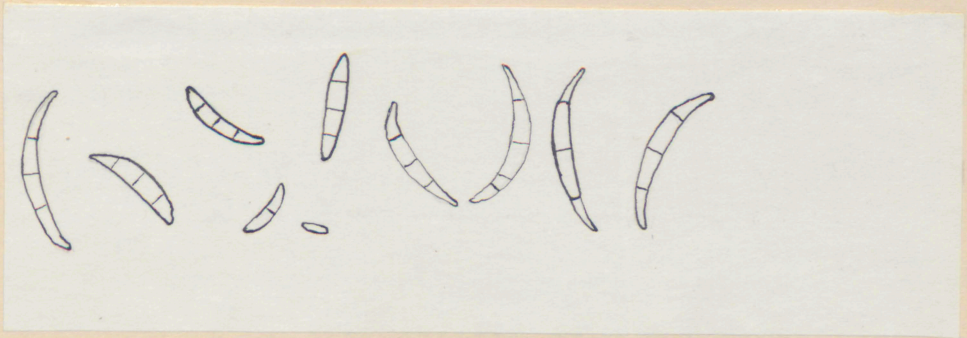

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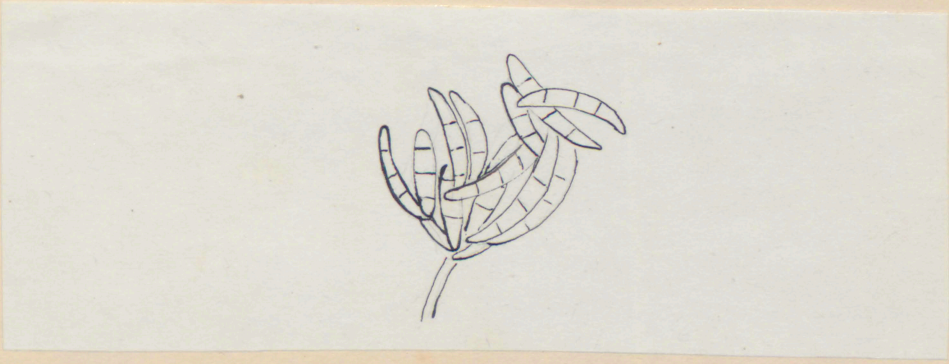

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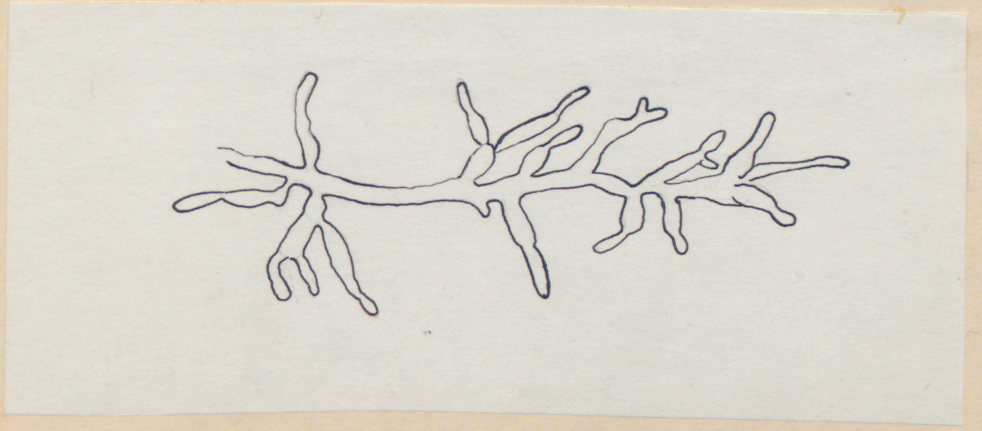

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Strain Ac

On agar aerial mycelium very slight or wanting; mycelium and spores from flesh when young, to buckthorn brown; pseudapionnotes; on potato tuber plug a scanty aerial mycelium from hyaline to drab; chlamydospores intercalary in mycelium and in conidia, 5 septate conidia dominant.

Measurements from cultures

On potato dextrose agar, 7 day old culture:

5-septate conidia 32-57u x 3.5-5

On potato tuber plugs, 15 days old culture:

3-septate conidia(few) 25-38 x 3.5-4u

4-septate conidia 28-40 x 3.5-4u

5-septate conidia 36-43 x 3.5-5u

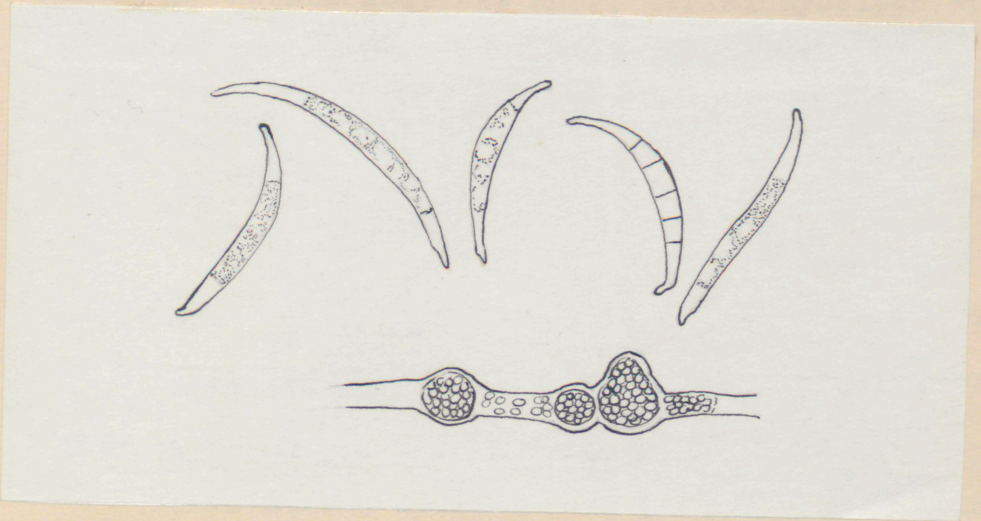
Chlamydospores single and in chains

9-12u in diameter.

The 5 septate conidia are shorter than given by Sherbokoff or Appel and Wollenweber for Fusarium falcatum, otherwise the two forms are quite similar.

Fig. 1, page . Conidia and chlamydospores taken from a 5 day old culture on potato dextrose agar.

Fig. 2, conidia and chlamydospores taken from a 16 day old culture on potato tuber plug.



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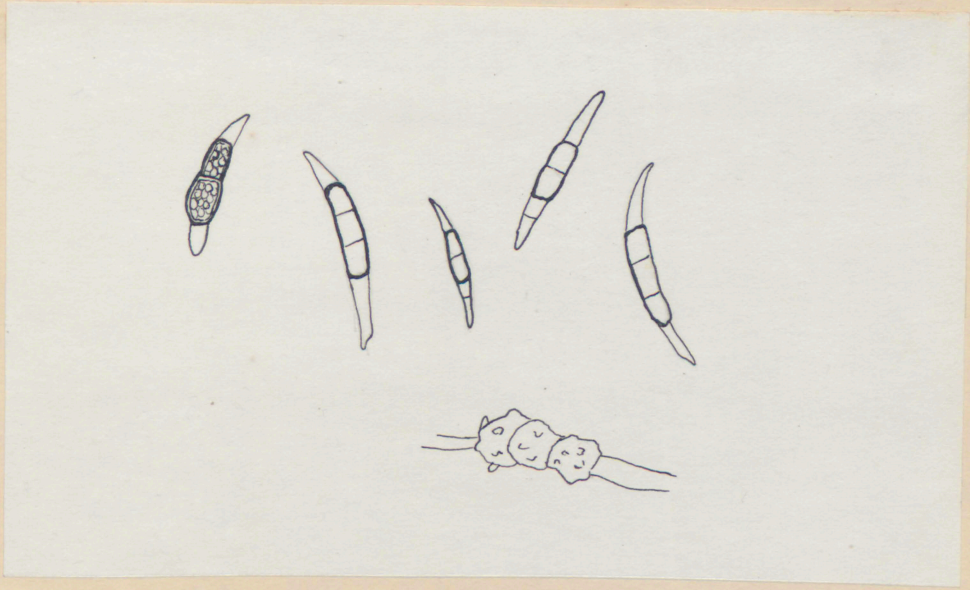


2

Strain An

On potato dextrose agar, aerial mycelium well developed, from hyaline, when young, to honey yellow, olive buff and buckthorn brown; on rice, salmon to various shades of yellow and brown (Plate IV, Fig. 6) No microconidia are present; 5 septate conidia not in pionnotes of the Gibbosum type dominant. The production of conidia ceases early; chlamydospore production is excessive.

This strain resembles very closely Fusarium falcatum Op. and Wr. var fuscum Sherb.

Measurements of conidia from cultures on potato tuber plug, 14 days old:

3-septate conidia 18 x 3.6-4u

4-septate conidia 25 x 3.7-4u

5-septate conidia 26-42 x 3.6-4u

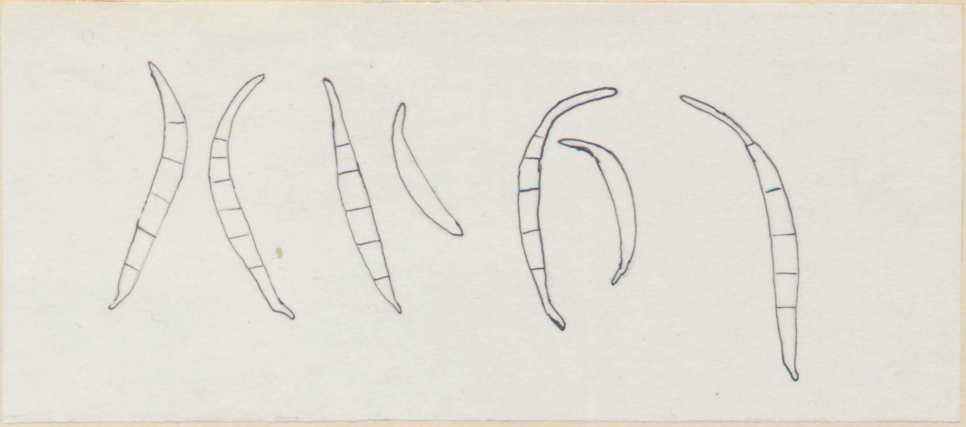
On rice, culture 6 days old:

5-septate conidia 30-59 x 4-5u

Fig. 1, page . Conidia from a 6 day old culture on rice.

Fig. 2, Conidia taken from a 7 day old culture on potato dextrose agar.

Fig. 3, Chlamydospores taken from a culture on potato dextrose agar 65 days old.



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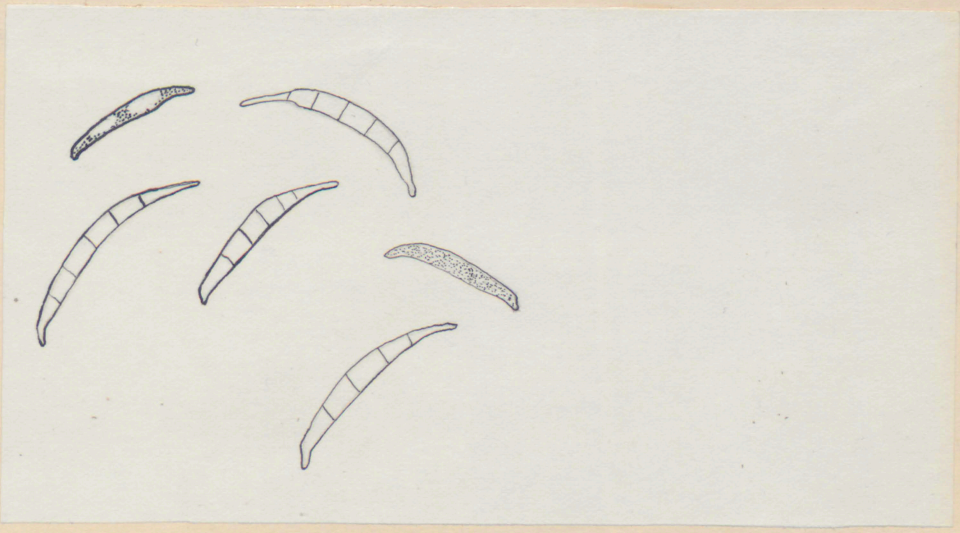

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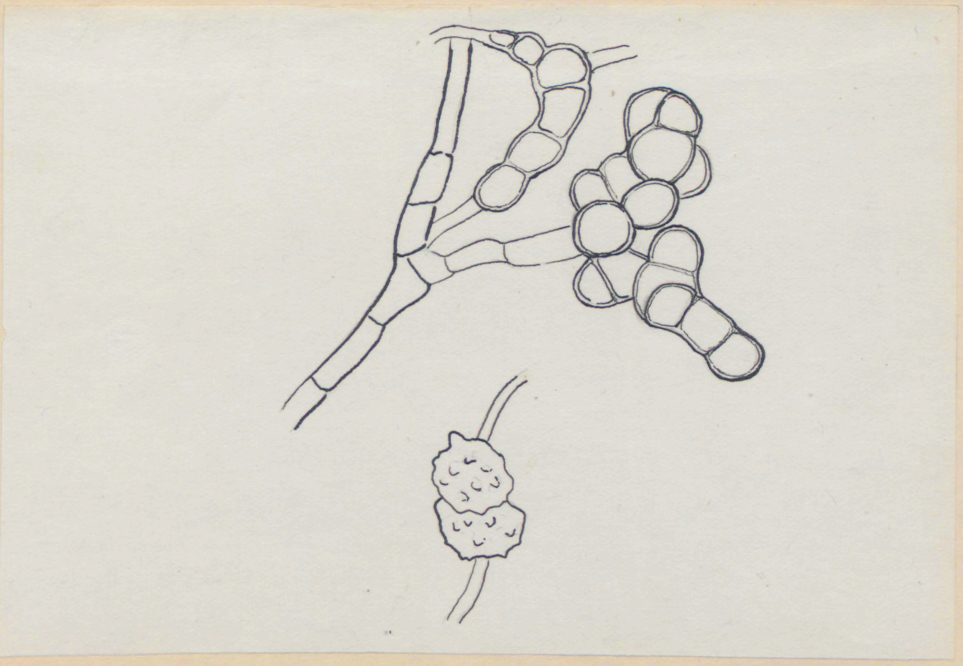


3

Strains Isolated From Watermelon

Strain W

On potato dextrose agar the aerial mycelium is low and scanty, white to a grayish cream; substratum pale olive buff to olive buff becoming light porcelain green with age. On potato tuber various brown colors develop; numerous microconidia are present; 3 septate spores are dominant; 4 and 5 septate spores few.

Measurements on different media:

On potato dextrose agar, culture 5 days old:

0-septate conidia 7-12 x 3.7-4.2u

1-septate conidia 18-25 x 4-4.5u

3-septate conidia 32-40 x 4.2-5.5u

On potato dextrose agar, culture 5 days old:

3-septate conidia 25-40 x 4.5-6u

On potato tuber plug, 10 day old culture:

3-septate conidia 28-40u x 4-6u

Chlamydospores terminal and intercalary.

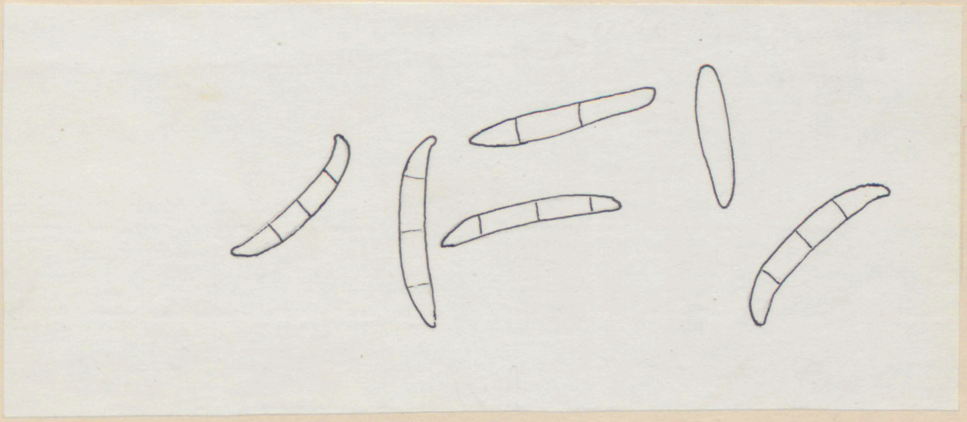
This strain resembles <u>Fusarium striatum</u> sherbokoff in habit of growth and color effect on agars rich

in glucose. The conidia are slightly thicker than in *Fusaria striatum*.

Fig. 1, page . Conidia taken from a 5 day old culture on potato dextrose agar.

Fig. 2, Conidia taken from a potato tuber culture 14 days old.

1



2

Strain Wk

The aerial mycelium in cultures on potato dextrose agar is from hyaline to a pale olive buff, low and rather scanty; the conidia are borne in tall coremium-like sporodochia; the substratum is cream color. On rice the color ranges from pale brownish vinaceous to dark vinaceous brown and liver brown(Plate VI).

Measurements:

Conidia taken from a 34 day old culture on rice:

Microconidia present

3 septate conidia 26-42ux 3.5-4.2u

From a 45 day old culture on potato tuber plugs:

3 septate conidia 25-36ux 3.5-5u

4 septate conidia 43ux 5u

The three septate conidia are doninant; chlamydospores 6-8u in diameter.

Fig. I, page 48. Conidia taken from a 34 day old culture on rice.

Fig II. Conidia taken from a 45 day old culture on potato tuber plugs.

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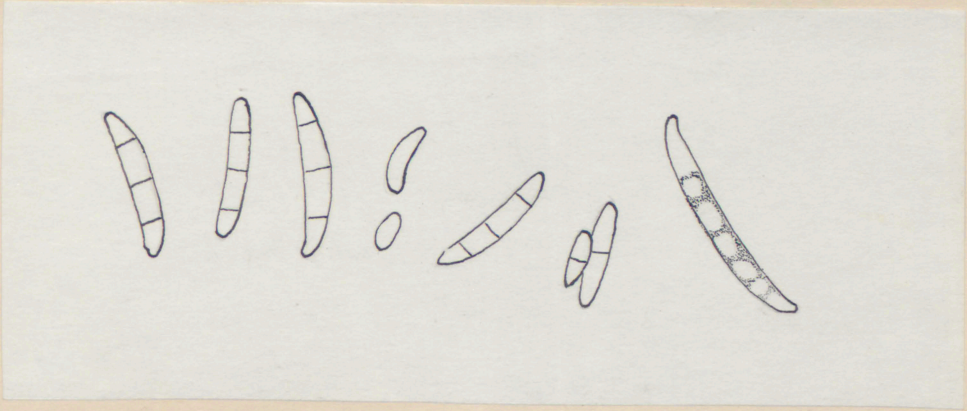

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Strain Wf

This strain differs little from Wk and except for the more fragile white aerial mycelium on potato dextrose agar; substratum cream; on potato tuber plugs yellow to brown, (Plate VI). Chlamydospores terminal and intercalary; Micro conidia in abundance on aerial hyphae.

Measurements of conidia from culture:

On potato tuber plugs 7 days old:

0 septate conidia 7-15 x 3-4u

1 septate conidia 14-25 x 4-5.5

2 septate conidia 20-28 x 4-5.5

3 septate conidia 28-36 x 5-5.5

Strain Wj

In cultures on potato dextrose agar, the substratum has a vinaceous buff color; the aerial mycelium, which is scant is a cream to pale vinaceous fawn color, differs little from Wk except in color on agar; on potato tuber plugs the aerial mycelium is dense, white to chamois; the substratum colorless to honey yellow with occasional blue-green pseudoparenchymic masses. The conidia are quite granular. Chlamydospores, terminal and intercalary, are present.

Measurements:

Potato dextrose agar, 5 days old culture:

3 septate conidia 30-40 x 4-5.5u

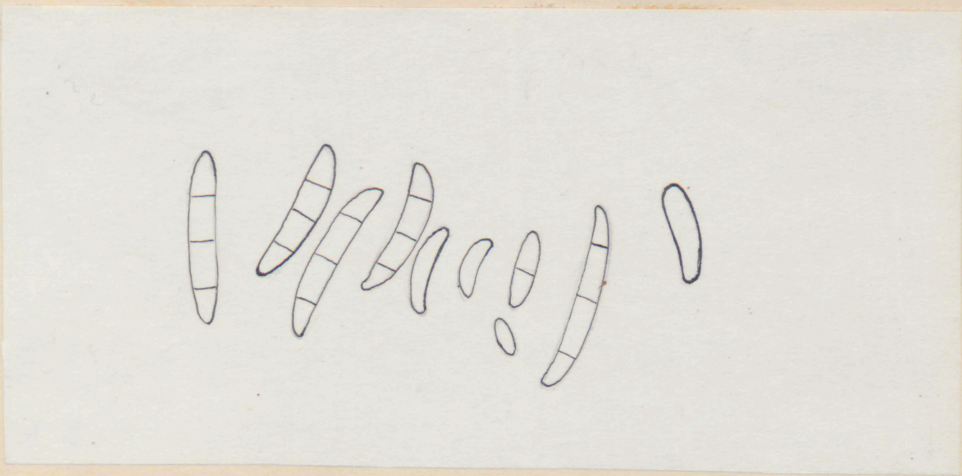
Potato tuber plug, 39 days old culture:

3 septate conidia 28-36 x 4-5.5u

Microconidia are present; 3 septate conidia are dominant.

Fig. I, page 51. Conidia from a 39 days old culture on potato tuber plug.

Fig. II, Conidiaphores from a 5 days old culture on potato dextrose agar.



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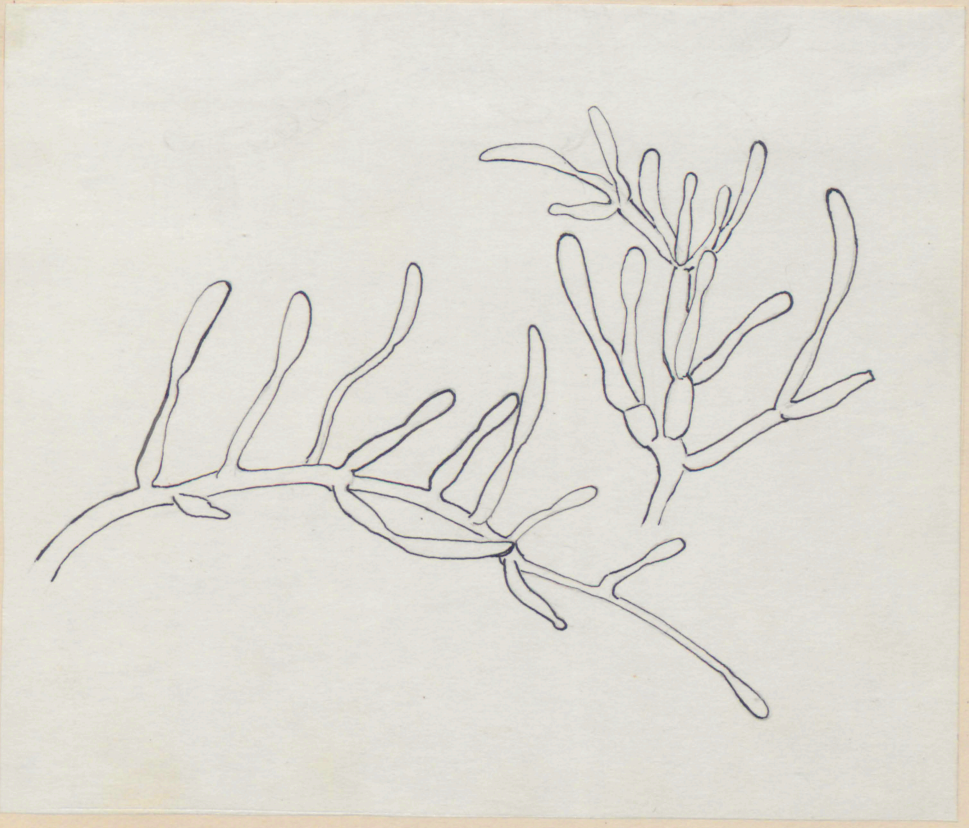

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Strain Wc

On potato dextrose agar mycelium a deep cream; on potato tuber aerial mycelium white, short and poorly developed; spores in an a continuous slimy layer, wood brown in color; on rice honey yellow to clay colors; no macroconidia, no chlamydospores present.

Measurements:

On potato dextrose agar 3 days old culture:

0 septate conidia 5-9u x 3-3.6

On potato tuber plug, 43 days old culture:

0-1 septate conidia 6-12u x 2-3.2

Aside from the solor of the spore mass, wood brown instead of salmon colored, this strain agrees with the description given by Sherbakoff for Fusarium affine.

Strain W1

This strain differs from all others isolated from watermelon in its white growth on rice; on potato tuber plugs the mycelium is short and white. Chlamydospores are formed; also small blue-green plectenchymic masses.

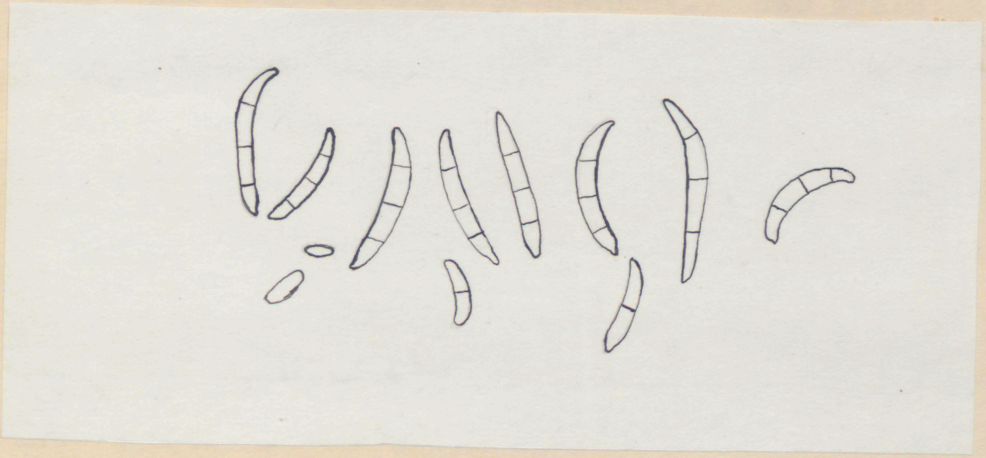
Measurements of conidia from an 11 day old culture on potato tuber plug:

0-septate conidia 5-12ux 2-3u

1-septate conidia 8-25ux 2-3u

3-septate conidia 21-36ux 3.6-4u

Fig. I, page 54. Conidia taken from an 11 day old culture on potato tuber plug.



1

Greenhouse Experiments

Seeds of Whippoorwill and New Era cowpeas, popular commercial varieties, were planted in the greenhouse, in very sandy soil, the middle of February. After the seedlings were above ground, March 3, the soil was inoculated with the different strains of Fusaria isolated in the laboratory from the roots of cowpeas. Two weeks later, other seedlings were inoculated with the same forms but by means of wounds in the hypocotyl.

March and April are not particularly favorable months for cowpea culture so a very vigorous growth of the cowpeas was not expected. None of the inoculated plants have died (May 10) but on the whole they are not as large and vigorous as the controls. The leaves of some of the plants are quite yellow and fall easily.

Two of the sickliest looking plants were removed to the laboratory and plantings of their root tissue made in agar. In both cases Fusarium cultures were obtained resembling in appearance the one used in inoculation. They have not been running long enough in the laboratory to warrant any statement as to the identity of the two forms.

The writer wishes to acknowledge her indebtedness to Doctor George M. Reed under whom this work was done and to express her appreciation of his interest and many helpful suggestions.

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Plate I

Strains Isolated From Cowpeas.

1. Ba, on rice, 23 days old culture.
2. Ba, on rice, 33 days old culture.
3. Ba, on rice, 48 days old culture.
4. Ba, on potato tuber plug, 28 days old culture.
5. Ba, on rye bread, 21 days old culture.
6. *Neocosmospora vasinfecta* (Smith) on potato tuber plug, 19 days old culture.

PLATE I

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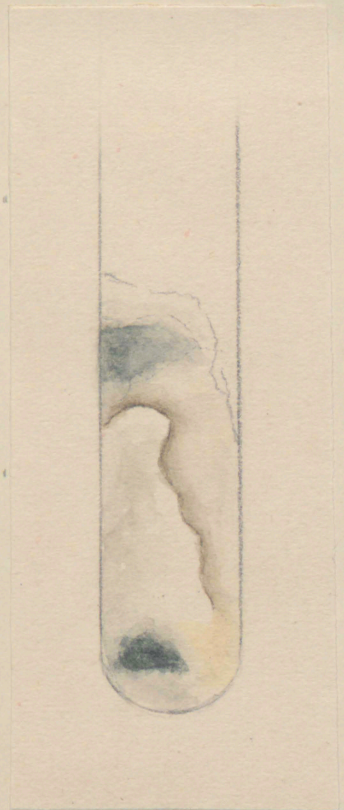

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Plate II

Strains Isolated From Cowpeas

1. Bx, on rice, 13 days old culture.
2. Bs, on rice, 23 days old culture.
3. Bf, on rice, 17 days old culture.
4. Bf, on rice, 7 days old culture.
5. Bs, on rye bread, 22 days old culture.
6. Bs, on tapioca, 27 days old culture.

PLATE II

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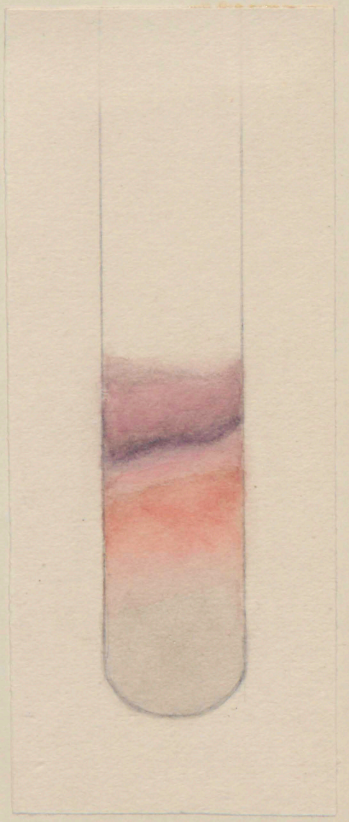

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Plate III

Strains Isolated From Cowpeas

1. Bc, on rice, 6 days old culture.
2. Bc, on rice, 9 days old culture.
3. Bc, on rice, 18 days old culture.
4. Bc, on rice, 32 days old culture.
5. Bc, on rye bread, 21 days old culture.
6. Bc, on tapioca, 28 days old culture.

PLATE III

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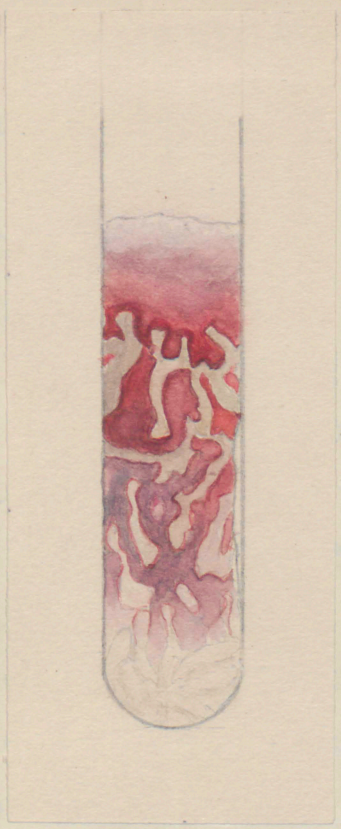

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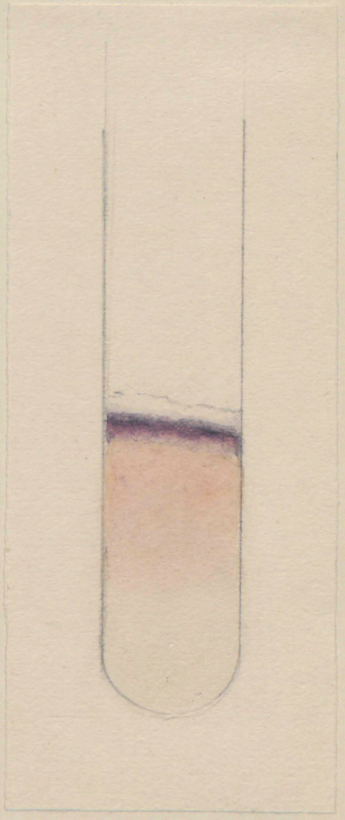

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Plate IV

Figs. 1-4 from cowpeas.

Figs. 5-6 from carnation.

1. Bc, on potato tuber plug, transfer of mycelium, 30 days old culture.
2. Bc, on potato tuber plug, from a macroconidium, culture 15 days old.
3. B, on rice, 12 days old culture.
4. Bz, on rice, 12 days old culture.
5. A, on tapioca, 31 days old culture.
6. An, on rice, 10 days old culture.

PLATE IV

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Plate V

Strains Isolated From Carnation

1. A, on rye bread, 8 days old.
2. A, from carnation, on rye bread, 17 days old.
3. A, on rice, 13 days old.
4. A, on potato tuber plug, 13 days old.
5. A, on potato tuber plug, 50 days old.
6. A, on rice, 19 days old.

PLATE V

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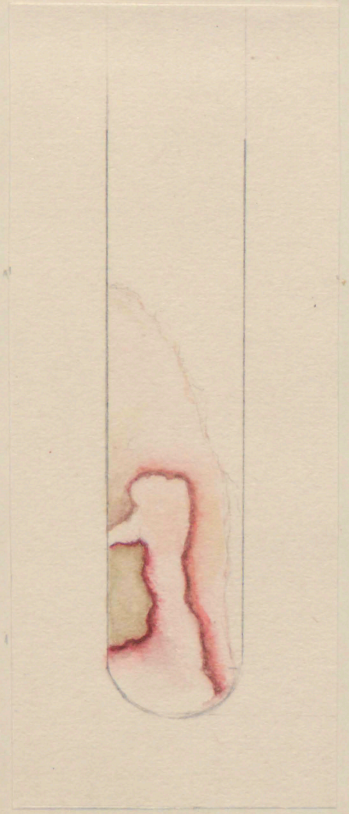

4

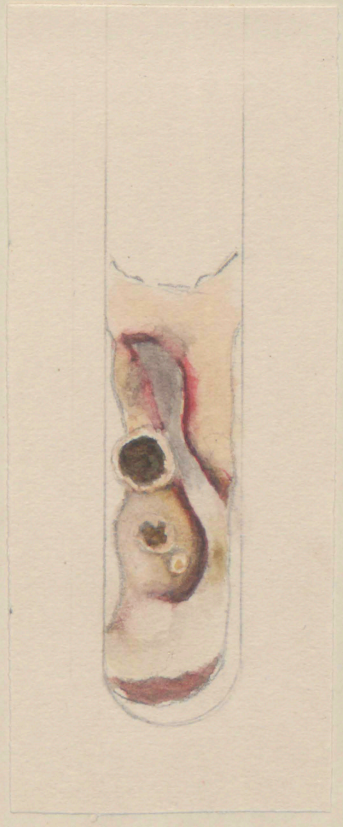

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Plate VI

1-5. Strains Isolated From Watermelon

1. Wk, on rice, 9 days old culture.
2. Wk, on rice, 47 days old culture.
3. Wf, on potato tuber plug, 18 days old culture.
4. Wc, on potato tuber plug, 55 days old culture.
5. Wc, on rice, 55 days old culture.
6. *Fusarium discolor*, var. *sulphureum*, isolated from a rotted potato, 15 days old culture on potato tuber plug.

PLATE VI

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