PHYSIOLOGICAL STUDIES ON PARASITIC FUSARIA WITH SPECIAL REFERENCE TO FUSARIUM LYCOPERSICI SACCARDO

by

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INTRODUCTION

Tomato growing as an industry in the United States has in the last few years come to be of great importance. This is indicated by the following statement from the 13th census report. (25): "Judged by value, tomatoes were the most important vegetable, the value exceeding 13,700,000 dollars in 1909." Rogers (44), giving a history of the tomato industry, says, in 1911 9,850,000 cases were packed in the United States and the fresh market was also kept well supplied. Stuckey (53) says that in 1915 it was estimated that from 3,000 to 6,000 acres were planted to tomatoes annually.

Tomatoes are grown in many of the states. The acreage planted to tomatoes is especially large in the Southern Gulf States, where environmental conditions favor their growth. Among the northern states Delaware, New Jersey, and Maryland rank first in tomato growing. Exclusive of Delaware, Maryland packs more tomatoes than all the rest of the United States (34). In the West, California leads in the production of tomatoes. Tennessee, South Carolina, Idaho, Washington, Indiana, Missouri, Illinois, and Michigan all grow tomatoes in large quantities both

for the canneries and for the fresh market. Tomato growing under glass is common in some of the northern states.

TOMATO DISEASES.

A large number of diseases on tomatoes have been reported, among the more serious from the economic standpoint are the following:

Anthracnose (Gloesporium fructigenum or Colletotrichum phomides);

Blossom-end Rot or Point Rot (may be due to Fusarium solani);

Early Blight (Alternaria solani);

Late Blight (Phytophthora infestans);

Leaf Mold or Blight (Cladosporium fulvum);

Leaf Spot Disease (Septoria lycopersici);

Sclerotium Wilt or Blight (Sclerotium Rolfsii);

Tomato Wilt or Blight, or"Sleepy Disease" (Fusarium lycopersici Sacc.);

Western Blight or Yellows (Fusarium species);

Black Rot of fruit (Macrosporium solani);

Chlorosis of tomato (Cause unknown);

Mosaic disease (Cause unknown).

Tomato Wilt, Bacterial Wilt, and Septoria Leaf Spot are the most important diseases of this plant.

My studies have been with the organism causing tomato wilt, Fussarium lycopersici Sacc. Massee (29) reports this organism to have first been described by Saccardo in 1882 from Italian specimens growing on tomatoes, but it was not at that time connected with the disease. The first description of the disease was made by Massee (29) in 1895. The disease is now known to be widespread. It has been reported from Guernsey (30). In 1910 it was found in widely distributed districts of England (30). It was discovered in New South Wales in 1906 (14). A description of the wilt as occurring in India was given in 1913 (55). Its occurrence has been reported in the United States from the following states: Massachusetts (51), New Jersey (6), Maryland (34), Delaware (5), Virginia (7), South Carolina (2), Tennessee (13), Florida (45), Louisiana (12), Georgia (53), Arkansas (19), Missouri, Illinois (27), Colorado (35), Arizona (31), and New Mexico (33).

This wilt, in certain sections of the country, has been the most economically important tomato disease. There is no disease of the tomato which causes more destruction than the fusarial wilt. Whole fields may be devastated before, or by the time of bearing fruit, thus causing the total failure of the crop. In 1904 it was the cause of a half million dollar loss in Florida (35). In Illinois it has been the cause of the loss of many a promising crop (27). Twenty-five percent of the tomato crop of Arizona was

destroyed by this fungus in 1906 (36). In 1907 the loss in Louisiana was fifty percent of the total crop (37). The wilt was still the disease causing the most damage in Louisiana during the year 1913 (12). It was common in New Jersey in 1915 and resulting losses were severe in places. (18). From these few specific instances we readily see why the fusarial wilt of tomato is regarded as a disease of great economic importance.

FUSARIAL DISEASES.

The genus Fusarium contains a large number of species which are the cause of serious diseases. The diseases are of two types: the wilts and blights, in which the fungus lives in the vascular tissue; and the rots, the fungus here attacking the parenchyma.

The wilts and blights first become apparent in the leaves, in the former, a yellowing and wilting occurs, in the latter, a yellowing followed by fall of leaves. The vascular tissue in roots and stems is darkened, quite frequently the whole root becomes dark. The fungus by growing in the xylem, thus clogging the vessels, and by causing death of many roots, cuts off the water supply of the plant and brings about its death. It is thought by some that the fungus also has a toxic effect which is effective in killing the plant.

The rots, attacking the parenchyma bring about

local destruction of the tissue causing dry rots, powdery rots, or wet rots. If rots occur in stems, they cause yellowing and fall of leaves.

The diseases caused by these fungi are among the most destructive of plant diseases. Stevens (49) makes this statement; "Taken as a whole, the genus is one of the most serious with which Plant Pathology has to do". Wollenweber (58) says that the wilt diseases are common and destructive to various crops in this country, the most of them are caused by Fusaria and hence, the wilt problem is chiefly a Fusarium problem. Orton (38) states that the Fusarial wilt of potato is a nation wide problem. The losses annually from potato wilt run up into the millions of dollars. The flax wilt Fusarium (Fusarium lini) has become so prevalent in the soil in some sections of the country that the culture of flax as a crop has been abandoned. McCollum (31) says that the Fusarial diseases are by far the most destructive class of diseases to be dealt with in Arizona. The cotton wilt caused by Fusarium vasinfectum Atk. is widely distributed in cotton growing sections, and is extremely destructive not only, as is the case with all these soil organisms, causing the loss of the crop, but also destroying the usefulness of the soil for some time (50). Some additional Fusarial diseases are: Cabbage yellows (Fusarium conglutians),

Cowpea wilt (Fusarium vasinfectum Atk.) , Watermelon wilt (Fusarium niveum) , Dry rot of potatoes (Fusarium coeruleum), Dry powdery rot of potatoes (Fusarium trichotheciodies), and Carnation stem rot (Fusarium species).

INFLUENCE OF ENVIRONMENT ON DISEASE.

Observation and experiment have shown that plant diseases are markedly influenced by weather conditions. Reed (41) mentions that epidemics of plant diseases have been noticed many times to be associated with certain weather conditions. By weather conditions we usually mean temperature, moisture, and light, and wind might also be included. Duggar (8) says these conditions may effect host or parasite independently, or the inter-relation of the two, also that it is hard to determine just which factor is "finally operative" and what are the direct and indirect effects.

The moisture factor is one of great importance. Reed (41) points out the difficulty of distinguishing between the effects of this factor and temperature, but states that in certain cases the water factor is the most important, for example, the asparagus rust, whose water relation has been worked out by Stone (52), Smith (52), and Sirrine (48). The case of the asparagus rust illustrates both the direct and the indirect effects of moisture. A bountiful supply of soil water causes abundant growth, resulting in a strong healthy plant which resists invasion

by the rust much better that the small weak plant struggling along in a dry habitat, and thus indirectly affects the spread of the rust. But moisture is needed for germination of the spores and hence directly aids infection. The moisture needed here must cling to the foliage of the plant and thus, as would be expected, dew favors infection. Moisture also aids the formation of spores.

Many diseases are influenced directly because of the necessity of moisture for germination of their spores. And so the spread or check of disease by moisture becomes quite evident. Lutman (28) finds from observations for twenty years that the worst epidemics of Potato blight caused by Phytophthora infestans have always occurred in years which have had excessive rainfall. Reed finds Sclerotinia fructigena, the fungus causing brown rot of stone fruits, to be always present in fruit orchards, but that it only becomes destructive when excessive moisture is present.

Examples might be multiplied but these show sufficiently the dependence of disease on the moisture factor of its environment.

Light seems to play its greatest role indirectly. It is necessary for the development of normal healthy plants, and thus will have an indirect influence. In certain cases, etiolated plants develop diseases which do not occur

on normal green plants, as shown by Brooks (4), etiolated lettuce plants being attacked by Botrytis cinera. Reed (41) finds the mildew of wheat will not fully develop on etiolated plants, but when these infected etiolated plants are brought into sunlight, full infection takes place.

Temperature is a factor of great importance in the occurrence of diseases. It is the controlling factor in many cases. The influence of temperature has long been recognized, but of late it has received more and more attention, and various studies have been made for the purpose of finding for specific organisms just what relation temperature bears to infection. Hitherto most of the data on this subject has been obtained from casual observation in the field. Gilman (17) in 1915 remarks that the knowledge in this field is "very limited and fragmentary although the importance is usually recognized". Evidence of this increasing attention to the factor of temperature are such articles as that by Jones (23) on "Soil Temperature as a factor in Phytopathology". Attention is called to various studies to show the "lively interest" which Phytopathologists are taking in soil temperature problems. Soil temperature is, of course, only one phase of the temperature problem, but a most important one because of the large number of our root invading fungi.

The geographic distribution of diseases shows the influence of temperature. Miss Westerdijke (56), in

discussing phytophthology of the tropics, says that compared with temperate regions few diseases exist. Her explanation is that the constant high temperature does not allow them to develop , having found in her laboratory that for 600 fungi, the optimum temperature was beneath 30 degrees C., often under 20 degrees C. Again Edgerton (10) groups the diseases occurring in Louisiana according tothe temperature at which they develop. There are those which develop during hattest weather of summer, as various wilts, root rots, and leaf spot diseases. Then there are those diseases common to more northern districts, such as onion mildew and bean anthracnose. There are also those diseases common in more northern states which do not appear in Louisiana, or only sparingly.

Reed (41) mentions several examples showing the part temperature plays in spread of disease, as Bitter Rot of apples caused by Glomerella ruformaculans whose spread as Scott (46) worked out, is favored by hot weather and checked by a period of cool weather. Other examples were Peach leaf curl, caused by Exosacus deformans, which Pierce (40) finds is more prevalent after a period of hot weather, and strawberry mildew, favored by alterations of temperature--cool nights followed by sunny days.

Controlling environmental factors and making temperature the varying factor, Balls (1) found that the sore

shin fungus of cotton can attack cotton at 30 degrees C., but not at 33 degrees C.

Edgerton (10) finds optimum temperature for growth of bean anthracnose fungus, Colletotrichum lindemuthianum, to be 21-23 degrees C. and the maximum temperature 30-31 degrees C. By these results he explains why the disease develops on the spring crop but not on the summer crop.

The smuts of grain are other examples where the temperature factor is important. Humphery (23) states that soil temperature of 0-5 degrees are unfavorable to infection as are temperatures higher than 23 degrees C., while 15-23 degrees C. are the optimum.

In a similar manner, work with the Fusaria has shown temperature to be a controlling factor in their production of disease. Jones (22) says "Wollenweber makes the generalization that the root invading Fusariums are warm soil organisms". The following summary of work dealing with Fusarial diseases and their temperature relations seems to justify this. The fact that Fusarial diseases are most common in the southern states lends support to this view. McCallum says in making a report of the plant diseases of Arizona that Fusarial diseases are the most destructive class to be dealt with. We also find that cotton, watermelons, and bananas have a serious

wilt disease and all of these are southern crops.

Jones (21) and Gifford (16) find that a high soil temperature following germination is one of the controlling factors in the development of the damping off of coniferous seedlings. It is caused by a species of Fusarium.

Wolf (57) noticed that the Fusarium disease of pansies occurred in those beds where manures were not well mixed with the soil, but where the fertilizers were well decomposed and mixed, no wilt was found. This seems to me that the high temperature caused by the decomposition of organic material was probably a factor influencing infection.

Humphrey (20), working in Washington with the Fusarium tomato blight of the Pacific Northwest, finds that when the soil temperature rose to near the optimum (30 degrees C.) for the growth of the fungus, the symptoms of the blight appear and with this rise in temperature, the virulence of the parasite increases and the blight becomes general.

Orton (38), in discussing the diseases of the potato, states that the Fusarium wilt is a disease of warm climates. Arizona, California, Ohio, Missouri, and Nebraska suffer more from this wilt than the states on the northern border where it is practically unknown.

Gilman (17), working with Fusarium conglutians, which causes cabbage yellows, finds by experimenting under controlled conditions that a temperature of 17 degrees or

above is necessary for infection and that at all temperatures below this, the fungus is not capable of producing the disease.

Tisdale (54), by controlling the soil temperature, found the critical temperature for infection of flax by Fusarium lini to be about 15-16 degrees C. By holding the temperature at 13-14 degrees C., the flax plants grew well in inoculated soil and no signs of wilting occurred, but when the temperature was allowed to run above16 degrees C. for a single day, the plants wilted although the temperature was again lowered.

Link (26), working on two strains of Fusaria, one a rot producer, the other the cause of a wilt in potato, finds that the latter (Fusarium oxysporium) has an optimum temperature of 30 degrees C., while the other (Fusarium trichotheciodies) has an optimum of 20-22 degrees C. By these facts Link explains the reason for Fusarium trichotheciodies producing the rots in storage where the cooler temperature prevails, and Fusarium oxysporium producing the wilt in the field where the higher temperatures are found.

Reddick (43) undertook to find the part soil temperature plays in infection using the bean and its parasite, Fusarium martii phaseoli. The optimum temperature for the fungus was found to be between 27 and 31.5 degrees C. The loss in dry weight of infected plants grown at 34 degrees C. as compared with healthy plants, was less than those grown at 22 degrees C., which shows that the temperature above the optimum favored infection less than that below.

The present studies are mainly concerned with a study of tomato wilt and its causual organism, Fusarium lycopersici Sacc. The work was carried on with reference to the culture characteristics of the fungus, the influence of environmental conditions on its growth and development, its capacity for infecting tomato plants, and its pathological effects on the host.

Three strains of Fusarium lycopersici Sacc. were used in these studies. Two were obtained from C. W. Edgerton of Louisiana, and are designated by their culture number of his laboratory, namely 1707 and 1814. The other strain was received from L. C. Kunkel of the Bureau of Plant Industry.

CULTURAL CHARACTERISTICS OF FUSARIUM LYCOPERSICI SACC.

According to Wollenweber (58) Fusarium lycopersici Sacc. belongs to the section Elegans of the Fusaria. The characteristics of this section may be briefly summarized as follows: All species produce scattered ellipsoidal unicellular conidia which average 5-12 u. by 2-3.5 u. in size. The sickle shaped conidia are mostly 3-septate, but 4- and 5-septate ones also occur. Their average size differs with the species, the majority of 3-septate ones being 25-40 u. by 3-4.5 u. The 4-septate ones are somewhat larger and the 5-septate average 40-50 u. Conidia in masses are mostly salmon colored, sometimes brownish, white, or orange. Conidiophores are verticillately

branched in sporodochia. Chlamydospores are ellipsoidal, terminal, and intercalated; unicellular ones being 5-10 u., 1-septate ones up to 12 u. Ascigerous stage is unknown. Wollenweber gives the following description of Fusarium lycopersici Sacc.: "Differs from Fusarium oxysporum Schlect. in having conidia of a little larger average size, a perfect pionnotes, colorless sclerotia on steamed potatoes, and no odor. Vascular parasité, cause of wilt disease of Solanum lycopersici in all parts of the United States, except most northern; probably also in Southern Europe."

3-septate conidia of F. oxysporum are 25-45 u. by 3.25-4.5 u.

4- and 5-septate are 40-50 u. by 3.5-4.75 u.

INFLUENCE OF MEDIA.

The organism was grown on potato dextrose agar, prune agar, corn meal agar, lima bean juice agar, potato tuber plugs, bean pod tissue, rice, bread, and tomato stem decoction. The media was prepared as follows:

Potato Dextrose Agar:-Potatoes were sliced and 200 g. of them were cooked in 1000 cc. distilled H_2O in an autoclave at 5-7 lb. pressure for 25 min. The liquid was filtered thru absorbent cotton and made up to 1000 cc. by adding distilled water. To this liquid was added 20 g. of agar and 20 g. of dextrose and the mixture heated in the autoclave for 30 min. at 4-6 lb. pressure. This was then filtered thru absorbent cotton, tubed, and sterilized at 4-6 lb. pressure for 25 min.

Corn Meal Juice Agar:-Corn meal, of which 100 g. were taken, was mixed with 1000 cc. H_2O and heated in a cooker over a gas flame and boiled for 15 min., then strained thru a fine cloth, made up to 1000 cc., 20 g. agar added, and the whole heated in an autoclave at 5-7 lb. pressure for 25 min. The mixture was then strained thru cotton, tubed, and sterilized at 5-7 lb. pressure for 30 min.

Lima Bean Juice Agar:-Dried lima beans were ground and 50 g. were cooked 1-2 hour in 500 cc. H_2O, then simmered slightly for another half hour. The liquid was drained off thru a fine wire strainer, and made up to 500 cc., 10 g. of agar were added, and the whole heated in an autoclave for 20 min. at 7-8 lb. pressure. The mixture was then filtered thru cotton, tubed and sterilized in an autoclave at 7-8 lb. pressure for 25 min.

Prune Agar:-Dried prunes (50g.) were cooked in 700cc. of H_2O in the autoclave at 105 degrees C. for 15 min. Prunes were macerated, liquid filtered off thru absorbent cotton, and restored to 700 cc. 14 g. of agar was added and dissolved by heating in an autoclave at 105 degrees C. for 15-30 min., filtered thru absorbent cotton tubed, and sterilized.

Rice:-The rice was placed in test tubes to the depth of an inch and covered with water until it stood

twice as high in the tube as the rice. The tubes were then sterilized at 108-110 lb. pressure for 30 min.

Bread:-The bread was broken into bits, placed in the tubes, moistened with water, and sterilized in an autoclave at 108-110 lb. pressure for 30 min.

Potatoes:-Cylinders of potato tubes cut in two diagonally were placed in test tubes, a few cc. of water added, and sterilized at 108-110 lb. for 30 min.

Beans:-String beans were placed in a test tube, moistened, and sterilized.

Tomato Stem Decoction:-The leaves and stems were chopped fine and 25 g. dry weight were heated in 500 g. of water in a cooker over a gas burner, boiled for 15 min.(stirring sufficiently to prevent burning) then filtered and made up to 500 cc., 10 g. of agar added, and the whole heated in an autoclave at 8-10 lb. pressure for 30 min., then filtered thru absorbent cotton, tubed, and sterilized.

The cultures were grown in test tubes and incubated at 26 degrees C. Ridgeway's color guide was used in determining the color. Observations were made for over a month and the main cultural characteristics are indicated in the following tabulations.

MEDIA	CHARACTERISTICS OF MYCELIAL GROWTH	COLOR PRODUCTION
Potato Dextrose Agar	Abundant growth of white mycelial threads covering nearly whole of slope in about a week. Colony grows radially, the mycelium being high in center and depressed on edges.	Colors of lavender and violet hues, varying from purplish lilac to dull bluish violet.
Bean Agar	Growth abundant, strands loose, and clinging together, giving surface a somewhat reticulated appearance.	No color produced.
Prune Agar	Growth not abundant, no erect aerial threads. A mat of mycelium covering surface of slope.	Colors of lavender and violet hues.
Corn Meal Agar	Growth not abundant, flat mat of mycelium covering surface of slope with here and there a few bunches of erect threads.	Colors of lilac and purple hues.
Rice	Abundant growth, mycelial threads high, not dense. Shrinking of rice occurs after about 6 days.	Colors of vinaceous hues and darker reds. Also salmon color.
Bread	Abundant growth, somewhat compact.	Lilac and violet hues.
Potato	Abundant, dense, white aerial threads	Lavender and violet hues.
Beans	Patches of long, white aerial threads clinging together, moist appearance.	No color usually, rarely a vinaceous drab.
Tomato Stem Decoction	Aerial threads around edges. Thin mat of mycelium covering surface.	No color.

The above results indicate that the most abundant growth is formed on the starchy media.

Microconidia are produced abundantly on all the above media, the least so on bean agar. They are hyaline and ovate to ellipsoidal. The non-septate vary in size from 3-4.6 u. in diameter and from 2.6 to 30 u. in length. The 2- and 1-septate conidia average 2-4 u. by 12-30 u. in size. The conidia are generally 3-septate, a 5-septate one being observed occasionally. The 3-septate

conidia were produced most abundantly on the bean pod tissue on all the media used. They are hyaline, sickle shaped, and do not have a strongly developed foot. Figures give the variations of the conidia formed on the different media. They vary from 3 to 5 u. in diameter and from 24-50 u. in length.

Chlamydospored are formed on all the above media. They may be found in various stages of development when the culture is about a week old. They are more or less perfect spheres in shape, except those that are septate. They have a thick heavy wall, are usually of a yellowish color and are both terminal and intercalary. They average in diameter 5-13 u.

INFLUENCE OF ENVIRONMENT ON COLOR PRODUCTION.

Color is produced by many of the Fusaria. It may be found in the conidia, in the mycelium, or in the substratum on which the Fusarium is growing. The mycelium of Fusarium lycopersici and its substratum have been seen colored, but never the conidia. Sherbakoff (47) says color varies considerably but on the whole is stable enough to be used in dividing Fusaria into sections, and sharp contrasts might even be used for specific differentiations. Various workers have noted the influence of certain factors of environment on color production, but just what chemical substance this color is, what it is formed from, why temperature and light should

influence its formation, are questions yet to be answered. Lathrop (34) indirectly suggests aldehydes as a cause.

Media:-The color varies somewhat on the different media but is mostly of the lavender and violet hues with reds also. The most brilliant color is produced on rice and also the greatest amount. The starchy media give the most color. Color was no more intense on 2 per cent dextrose potato agar than on the 4 per cent. On potato dextrose agar no color is produced. This together with the fact that no color is formed on bean pod tissue or on bean agar seems to indicate that an abundance of carbohydrate material is necessary for color production.

Temperature:-The following observations were made from cultures growing on plates of 2 per cent dextrose potato agar. At 12-15 degrees C. only a faint color is produced. At 33-34° C. no color is formed though a small amount of growth takes place. The temperature of the incubator dropped to 30-31 degrees C. and color appeared. Temperatures from 20-28 degrees C. are favorable for production of color.

Light:-Plates of dextrose potato agar were inoculated and incubated at 20-22 degrees C., some being placed in the light, others in the dark. The rate of growth in both cases was approximately the same, but

the color was different. The color of the culture developed in the dark was of the lavender hues, while the culture in the light was salmon color. One plate left in the dark for a couple of days formed a lavender color and was then placed in the light, and a salmon colored ring was formed. It was again placed in the dark and then the light, so that alternating rings of lavender and salmon color were obtained.

ELEMENTS NECESSARY FOR GROWTH.

Disregarding specific variations it is generally conceded that fungi require the following nine elements: carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, potassium, magnesium, and iron. The fungi growing on organic material, living or dead, obtain these elements. In synthetic media the carbon is supplied in the form of carbohydrates, most often as glucose or sucrose; nitrogen is supplied in the form of nitrates or ammonia compounds. The other elements are added in the form of inorganic salts, such as $MgSO_4$ or $FeCl_3$.

In all the following experiments the measure of growth was the dry weight formed. In each case Richards' synthetic media was used. This nutrient solution contains the following materials: KNO_3, 1g; KH_2PO_4, 5g; $MgSO_4$, 2.5 g; $FeCl_3$, trace; and sucrose, 5g. per 100 cc. of water. Erlenmeyer flasks of 150 cc. capacity were used with 50 cc. of solution to a flask. The media was sterilized at

100 degrees C. on three successive days for 20, 15, and 15 min. respectively, 24 hours elapsing between each sterilization. Materials to be added to the media were placed in the flasks after sterilization under aseptic conditions. The length of incubation was 12 days at 28 degrees C. in every case. After incubation the culture were filtered off on Gooch crucibles which had been prepared with asbestos mats and dried to constant weight at 105 degrees C. These crucibles were again dried to constant weight at 105 degrees C., and the dry weight formed determined. In all cases before weighing, the crucibles were cooled in a dessicator. The dry weight in the following tables is given in milligrams. The cultures were always run in duplicates.

Dry weight formed in nutrient solution minus various elements

Media	Culture		
	a	b	Average
Full Nutrient Solution	237.0	253.0	240.0
Nutrient Solution-K	109.8	----	109.8
" " -Mg	63.7	13.3	38.5
" " -S	52.9	21.7	37.25
" " -P	13.0	8.7	10.85
" " -N	3.5	5.1	4.3
" " -all minerals	3.6	2.5	3.05
" " -sugar	5.0	2.3	3.65

From the above results we see that the ommission of any of the elements results in a marked decrease in dry weight formed. Of the elements making up the inorganic salts the absence of N. produces the most marked decrease in

growth. Without any of the mineral elements growth can not continue, thus showing them to be necessary to the fungus. Carbohydrates are also shown to be essential for growth and development.

INFLUENCE OF ALKALINE AND ACID MEDIA AND CERTAIN SALTS ON GROWTH.

Influence of $ZnSO_4$:-

Dry Weight formed in $ZnSO_4$ of different normalities.

Normality	Culture a	b	Average
N/1	270.3	275.4	272.8
N/.075	273.0	258.4	265.7
N/.05	257.4	223.9	240.65
N/.025	258.5	264.2	261.63
N/.0125	215.7	250.2	232.9
N/.00625	240.0	257.0	248.5
N/.003125	277.4	252.6	265.2
N/.0015625	226.6	226.8	226.7
Full Nutrient	237.6	-----	237.6

In the above experiment the concentration was 1 cc. of the $ZnSO_4$ solution to 50 cc. of the nutrient solution. From the experiment it is evident for all strengths used that $ZnSO_4$ does not produce a retarding effect on growth, but in most cases a slightly stimulating effect, the more so the higher the strength.

Influence of KBr;-

Dry weight formedin KBr of different normalities.

Normality	Culture a	b	Average
N/1	315.9	258.9	287.4
N/.075	257.2	254.0	250.6
N/.05	235.4	287.0	261.2
N/.025	308.8	240.5	274.6
N/.0125	248.1	210.2	229.6
N/.00625	282.0	237.6	259.8
N/.003125	289.5	246.1	267.8
N/.0015625	233.2	237.2	235.2
Full Nutrient	253.0	210.0	231.5

In this experiment a somewhat stimulating effect on the growth of Fusarium lycopersici was produced by KBr. The amount of stimulation this salt has on growth is similar to that of $ZnSO_4$. (Concentration =KBr 1 cc.+50 cc.Sol.)

Because of lack of time further normalities were not tested, but it would be interesting to find what normality would retard growth.

Influence of acid and alkaline media:-

Richards' solution has a slightly acid reaction, so in the following work the solution was made neutral by the addition of normal NaOH before the addition of the acid or alkali.

Dry weight formed in Acid Media:-

Amount of N HCl .8800 to 100 cc. of Nutrient Solution	Culture a	b	Average
10cc.	0	0	0
5 cc.	79.5	44.7	61.6
1 cc.	163.9	202.0	182.95
Neutral Solution	228.	158.4	193.1

Dry weight formed in Alkaline Medium

Amount of N NaOH .9600 to 100 cc. of Media	Culture a	b	Average
10 cc.	266.4	206.8	236.6
5 cc.	238.1	190.2	214.15
1 cc.	212.0	170.0	191.0
Neutral Solution	228.0	158.4	193.1

The acid shows a retarding effect on growth, even preventing growth with the strongest concentration. The growth in alkaline medium seems to be less affected with the stronger concentration than with the weaker. Comparing these results in the neutral solution with those in Richards' Solution which is slightly acid, it appears that the neutral solution is not so favorable to growth as the slightly acid solution, still the neutral solution + 1 cc. acid does not bear this out.

TEMPERATURE RELATIONS

We have seen that temperature is one of the controlling factors in infection. Experiments were carried out to determine the rate of growth of the organism in pure culture, for this would be an important factor to know in finding the relation of temperature to infection.

Diameter of colonies in centimeters.

Fusarium lycopersici strain 65.

Age of Colony in days.	6-10°C.		10-15°C.		17-20°C.		18-22°C.		25-26°C.		28°C.		31-32°C.		32-34°C.		38°C.	
	Culture a	b	Culture a	b	Culture a	b	Culture a	b	Culture a	b	Culture a	b	Culture a	b	Culture a	b	Culture a	b
1.	0	0	0	0	.3	.4	.1	.1	.9	.9	1.2	1.1	.8	.8	.4	.4	0	0
2.	0	0	.1	.1	.9	1.2	.7	.7	2.1	2.0	2.5	2.4	1.2	1.2	.5	.5	0	0
3.	0	0	.3	.3	1.7	2.0	1.6	1.5	3.3	3.0	3.7	3.6	1.7	1.6	.6	.6	0	0
4.	0	0	.3	.3	2.3	2.5	2.5	2.5	4.4	4.1	5.0	4.7	2.5	2.4	.6	.6	0	0
5.	0	0	.44	.4	2.9	3.0	3.0	3.3	5.5	5.2	6.3	6.0	3.0	3.4	.6	.6	0	0
6.	0	0	.55	.5	3.6	3.8	4.0	4.2	6.9	6.5	7.9	7.5	4.1	3.9	.65	.7	0	0
7.	0	0	.7	.6	4.6	4.8	5.0	5.2	8.3	7.8	9.8	9.0	4.9	4.8	.9	1.0	0	0
Average per day	0	0	.1	.08	.65	.68	.71	.74	1.18	1.11	1.4	1.2	.7	.68	.12	.14	0	0
Average per day for series	0		.09		.66		.72		1.14		1.3		.69		.13		0	

Fusarium lycopersici strain 1814.

Age of Colony in days.	6-10°C.		15-17°C.		19-20°C.		24-25°C.		25-26°C.		27-28°C.		31-33°C.		38°C.	
	Culture a	b	Culture a	b	Culture a	b	Culture a	b	Culture a	b	Culture a	b	Culture a	b	Culture a	b
1.	0	0	0	.05	.2	.3	.7	.5	.6	.5	1.0	.5	.2	.3	0	0
2.	0	0	.05	.2	.6	.7	2.2	1.8	2.0	1.6	2.0	1.8	.9	.8	0	0
3.	0	0	.15	.5	1.3	1.4	3.5	3.0	3.1	2.7	3.3	2.8	1.2	1.4	0	0
4.	0	0	.6	1.5	2.0	2.2	5.0	4.2	4.0	3.9	4.2	4.1	1.7	1.7	0	0
5.	0	0	1.5	2.1	3.2	3.0	6.0	5.3	5.0	5.0	5.4	5.1	2.3	2.7	0	0
6.	0	0	2.1	2.6	3.8	3.6	6.9	6.3	6.6	6.0	6.4	6.2	3.0	3.2	0	0
7.	0	0	2.8	3.5	4.5	4.6	7.9	7.3	7.5	7.5	7.6	7.5	3.5	3.6	0	0
Average per day.	0	0	.4	.5	.64	.64	1.12	1.04	1.09	1.07	1.08	1.09	.5	.51	0	0
Average per day for series.	0		.45		.64		1.08		1.07		1.07		.5		0	

Fusarium lycopersici strain 1707.

Age of Colony in days.	6-10°C.		15-17°C.		19-20°C.		24-25°C.		25-26°C.		27-28°C.		31-33°C.		38°C.	
	Culture a	b	Culture a	b	Culture a	b	Culture a	b	Culture a	b	Culture a	b	Culture a	b	Culture a	b
1.	0	0	.0	.0	.3	.2	.8	.9	.5	.6	.5	.6	.3	.3	0	0
2.	0	0	.2	.3	.7	.6	2.0	2.1	1.2	2.0	1.7	1.8	.9	.9	0	0
3.	0	0	.6	.7	1.3	1.2	3.2	3.2	2.6	2.8	2.8	3.0	1.3	1.5	0	0
4.	0	0	1.1	1.2	1.8	1.7	3.7	4.0	3.7	3.9	3.7	4.0	1.8	2.0	0	0
5.	0	0	1.5	1.6	2.5	2.3	5.2	5.2	5.0	5.1	5.0	5.2	2.6	2.7	0	0
6.	0	0	2.0	2.0	3.2	3.0	6.3	6.5	6.4	6.3	6.0	6.2	3.1	3.0	0	0
7.	0	0	2.4	2.5	4.0	3.7	7.4	7.6	7.6	7.6	7.5	7.7	3.8	3.5	0	0
Average per day	0	0	.34	.35	.57	.53	1.05	1.08	1.08	1.08	1.07	1.1	.52	.5	0	0
Average per day for series.	0		.345		.55		1.07		1.08		1.08		.51		0	

The organism was grown at temperatures from 6-38 degrees C., on 2 per cent Dextrose Potato Agar in petri dishes. A bit of mycelium was transferred to the center of each plate and the plates incubated at the various temperatures for several days. The diameter of the colony was measured every 24 hours. The table on page 25 gives the results. The average growth per day is obtained by dividing the diameter of the colony at the end of the seven days by the number of days.

Results given in the table show that the optimum temperature varies a little with the strain, but can be said to be between 25-28 degrees C. The maximum for growth is about 34 degrees C., for it was noted that when the temperature ran up to 34 degrees C. the diameter of the colony remained stationary. The plate kept at 38 degrees C. for seven days was removed, placed at a favorable temperature, and growth took place thus showing that growth was only inhibited at the higher temperature.

INOCULATION EXPERIMENTS

The object in view in the beginning of these experiments was, first, to perfect a method of inoculation whereby the plants might be readily wilted, and then to find the relation of temperature to infection, and to test the resistance of varieties to infection. None of these aims were satisfactorily accomplished.

SYMPTOMS.

Small plants with 2 or 3 pairs of leaves, set into soil, heavily inoculated with a pure culture of Fusarium lycopersici Sacc., may show signs of the disease within three weeks after transplanting. The first outward change is the shrivelling and yellowing of the first pair of leaves, beginning at the tips. These leaves soon die and drop off, while the corresponding leaves on the control plant at this time are still in good condition. At this stage the vascular bundles appear as darkened strands running thru the stem, which may be seen plainly by holding the plant to the light. Often these darkened areas at this time cease at the petiole of the dead leaf. The upper leaves now soon yellow and wilt, but before they are completely dead, the stem just below the tip bends, and the upper part of the plant hangs limp and soon dies. The roots of such a plant are short and brown at the tips. The stems and roots when cut across show the vascular regions to be darkened.

The symptoms as noted in the field by various workers may be summarized as follows: The disease usually becomes apparent when the plant has attained full growth, about the time of flowering or setting of the fruits. The first symptom is the yellowing of the lower leaves, followed by wilting, and then death. The drying begins at the tips of the leaves. More leaves above gradually sicken and die, as the disease travels up the stem. The whole plant has a stunted and sickly

appearance. During the last stages the plants hang limp on the stalks, the leaves are almost gone, stems are black, and stunted fruits, if any, are rotting on the vines. Cross-sections of the stems of the diseased plants show darkened areas in the vascular region, or they may be entirely dark. Isolated plants, only, may show the disease, or it may spread from these until whole fields are blighted. The best method of diagnosing the disease is an examination of the interior of the stems.

HISTOLOGY OF INFECTED PLANT.

The distribution of the fungus within the tissues of the stem is limited to the xylem. In the root the fungus may be found in epidermis, cortex, and vascular tissue. These observations were made from sections cut from stems and roots of wilted plants. These stems and roots were fixed in chromo-acetic solution, imbedded in parafine in the usual manner, and stained with Flemming's Triple Stain. The fungus, staining orange, showed up nicely within the ducts.

The hyphae were found to fill almost completely some of the smaller vessels. In the larger ducts the hyphae were not seen so densely massed. Cross-sections show a preponderance of cut ends of mycelial threads. Longitudinal sections showed the hyphae running longitu-

dinally thru the ducts. In roots that were dark at the time of fixing, the epidermis is gone, the cortex is mostly replaced by a mass of hyphae, and the vascular tissue is filled with mycelium. In a root which was apparently in good condition when fixed except for a darkened center, the hyphae were found permeating all epidermis and cortex cells and also in the vascular bundle in the center, and as yet no cells were broken down.

Apparently wounds are not necessary for infection. Seeds were germinated and grown on Pfeffer's Solution to which agar had been added, under sterile conditions, in a large test tube. Later the tube was inoculated and hyphae grew up to the root. The plant after a time wilted. As there was no chance for wounds in these roots the fungus must have pierced the epidermis.

Why the fungus should prefer the xylem in the stem for its habitat has not been explained. It seems strange it should grow here in view of the fact that the xylem transports no foods. It has been suggested that branches from hyphae in ducts may enter other cells of stems for nourishment but this was not observed in the sections, nor was anything concerning this found in the literature. The oxygen supply has also been mentioned as a reason for growth here.

The browning of the vascular bundles is another

problem to be solved. Just what changes take place are not known. It would be interesting to know how long fungus grows within bundles before it is darkened or if the darkening ever precedes the fungus. Lathrop has proved that aldehydes are produced by Fusarium cubeuse in synthetic media and suggests the darkening may be due to the action of aldehydes.

GREENHOUSE EXPERIMENTS.

The strains of Fusarium lycopersici used in this first experiment were 1707 and 1814 and a strain of a Fusarium isolated from stems obtained in the vicinity of St. Louis, Missouri. The latter is designated as A.

Seeds were germinated in a sandy soil and allowed to grow until the second pair of leaves were partially developed. The plants were then transplanted to 6 inch pots, three to a pot. At the same time the plants were set into this soil, it was inoculated, one of two methods being used; a portion of the media the culture was growing in (rice, potato dextrose agar, or beans) was placed along side the roots, or the roots were watered with a solution containing spores and bits of mycelium. For each variety of tomato used, a control pot was run in which plants were transplanted but soil was not inoculated. Stone, Beauty, Livingstone Globe, and Red Rock were the varieties used.

After six weeks, wilting had occurred in only one pot. Two plants of the Livingstone Globe variety, inoculated with strain 1707 developed the symptoms of the disease, and the organism was reisolated from the stems. Cross-sections of the stems showed the presence of the hyphae in the xylem. One plant of the Stone variety, inoculated with strain A, having only the first pair of leaves when transplanted, never developed a second pair and finally died. A Fusarium was isolated from this stem. The remaining plants at the end of two months were all in good condition and no difference was apparent between them and the control plants. The plants soon after this were unfortunately subjected to freezing temperature so no more data was obtained.

Another experiment similar to the above was started using the following varieties of tomato, Beauty, June Pink, Livingstone Globe, Earliana, and Stone. The strain received from Kunkel was used in inoculation and is designated as 65. The method followed was similar to that used above except that cultures growing on beans were used in all cases for it had been found that macroconidia were produced most abundantly on beans. A few days after inoculation all plants excepting those in one pot were frozen. The plants in this pot were of the Beauty variety, and 26 days after inoculation, they showed the first symptoms of the disease after which they rapidly wilted, all three plants dying. The organism was reisolated from these stems and later produced the disease in other plants.

Another set of experiments were tried using June Pink, Beauty, Red Rock, Earliana, and Livingstone Globe varieties, inoculating with strain 62. Of these only two pots showed wilting, one of June Pinks, another of Red Rock. None of the others wilted within 8 weeks., when the experiment was discontinued.

Method in the following experiment was the same as that used above except the soil contained leaf mold and was sterilized before inoculation. The results are given in the following table.
Time of inoculation February 14.

Strain of Fusarium lycopersici	No. of plants	Date of Observation	General Condition of Plant.
65	3 Earliana	March 2	1 plant wilted, leaves drying.
		" 4	Above plant dead A second plant leaves drooping and yellow.
		" 7	2nd plant dead.
		" 11	Remaining plant darkened in vascular bundles, all leaves fallen except a small pair of 3rd leaves.
Strain 65 reisolated from wilted Beauty plant.	2 Earliana 1 Beauty	March 7	1 Earliana plant showing symptoms.
		" 11	Above Earliana plant dying and 2nd Earliana plant's leaves yellowing and drying at tips. Darkened areas reaching to upper leaves. Beauty plant fairly good appearance. Darkened vascular bundles.
		" 14	Second Earliana plant dying. Beauty plant all leaves gone except upper pair.
Control Plants	2 Earliana 1 Beauty	April 1	Plants in good condition.

TESTING OF VARIETIES FOR RESISTANCE.

Edgerton's method (11) for testing varieties resistant to wilt was followed. Four inch pots were filled with soil, rich in leaf mold, and sterilized. The soil was then inoculated with a pure culture of the fungus, strain 65 being used. This was done by watering the soil with a solution containing spores and mycelium from cultures on beans, and also adding to the soil some of the bean tissue with its mat of mycelium. After inoculation, the seeds were planted. Seeds of all the varieties were also planted in sterile soil which was not inoculated. At the time of planting the seed the soil was watered with sterile water, afterwards with tap water. At the end of 76 days no symptoms of the disease had occurred in any of the following varieties of tomatoes: Red Currant, Red Plum, Red Rock, Thornburn's Improved Terracotta, Atlantic Prize, Michigan Early, Dwarf Station Upright, Earliana, Golden Queen, Norton forcing, Buckeye State, and Livingstone's Coreless.

The following table gives the results for the rest of the varieties tested.

Variety of Tomato	No. of Plants	No. of Plants showing symptoms	Percent of Affected Plants
Red Cherry	19	7	38.8
Red Peach	26	1	3.9
Red Pear	25	3	12.0
Yellow Peach	12	1	8.3
Yellow Pear	15	4	26.6
June Pink	18	4	22.3
Early Ruby	22	1	4.5
Yellow Cherry	38	9	23.4
Thornburn's Lemon Queen	9	2	22.2
Imperial	21	2	9.5
Cushion	30	1	3.3
Livingstone's Globe	23	4	10.7
Stone	24	3	12.5
Trucker's Favorite	30	1	3.3
Sterling Castle	31	1	3.2
Beauty	20	6	33.3
Optimus	18	2	11.1
Freedom	24	2	8.3
Lorillard	33	2	5.7
Perfection	27	1	3.7
Paragon	24	1	4.2
Matchless	30	6	20.0
King of the Earlies	28	1	3.5
Ponderosa	31	4	1.3
Frogmore Selected	15	3	20.0
Thornburn's Long Keeper	30	2	6.6
Trophy	24	3	1.4
Mikado	26	1	3.7
Bonny Best	28	4	14.3
Acme	32	1	3.1
Carter's Sunrise	25	3	12.0
Ford Hook's First	18	3	16.6
Dwarf Champion	29	4	13.7
Enormous	25	5	20.0
Success	32	2	9.0
Chalks Early Jewel	18	6	33.3

No diseases occurred in any of the control plants.

As the percentage of diseased plants is so small in

every case, the fact that several varieties were not infected does not prove anything as to their resistance. Other workers have obtained quite commonly from 75 to 90 percent of infection in the plants. It seems that wilting should occur in at least 85 percent of the plants before conclusions can be drawn. As the disease developed in so few of the plants grown in the inoculated soil in any of the experiments, and as it developed so slowly in pure culture where all conditions for infection seemed favorable, it is probable that the culture is no longer virulent. The symptoms in the field occur at about the time of flowering or later so it may be that more of the plants will show the diseases later.

INOCULATION EXPERIMENTS IN PURE CULTURES

Pfeffer's Solution, to which was added 2 percent of Dextrose and 2 percent of Agar, was prepared, placed in a large test tube to the depth of 2 in. and sterilized. Earliana tomato seeds were sterilized in a calcium hypochlorite solution for 2 hours. This seed sterilizer is made by mixing 10 g. of commercial $CaCl_2$ (titrating 28% chlorine) with 140 cc. H_2O, letting this stand for 10-15 min., decanting off the liquid, and filtering. The sterile seeds were then transferred under aseptic conditions to the tubes of Pfeffer's Sol. The seeds were then allowed to germinate and when the cotyledons had unfolded and

the roots were growing into the medium, one tube was inoculated with a pure culture of the Fusarium. The date of inoculation was 4/6, by 4/24 the plant showed no signs of wilting, though it had not developed any leaves beyond its cotyledons though these had developed somewhat after inoculation. The whole surface of the tube was covered with mycelial growth so that the stem was partially buried in the hyphae. The tips of the leaves began to dry up about 4/28 and the plant was dead by 5/1.

Following the above method 8 tubes were prepared, seeds germinated, and seedlings allowed to grow until about an inch high, when four of them were inoculated with strain 65 of the Fusarium. They were placed in pairs, one control and one inoculated tube. Two pairs were placed at room temperature, one in the light, and one in the dark. The other two were placed in incubators, one at 28 degrees C. and the other at 31-32 degrees C.

Date of Observations	Room Temp.	28C.	32C.
4/20	Plants of all tubes showed no signs of the disease.	Controls about 3 in. high, plants of inoculated tube about 1½ in., tips of leaves drying, heavy mycelial growth over surface of medium. Vascular region dark.	No symptoms
4/21		Further drying of leaves.	
4/24	Very slight drying at tips of cotyledons. 2nd leaves not developed. Roots short as compared to controls, dark at tips.	Plants of inoculated tubes dead or dying. Controls still in good condition.	Slight drying at tips. Root system short.
5/3	Control plants in light still in good condition. Inoculated plant developed no further, leaves drying still more.		

From the above data we see that the plants succumbed to the disease at 28 degrees C., the optimum for the Fusaria, in shorter time than at the other temperatures. But this one experiment does not warrant the drawing of any conclusions.

CONTROL OF TOMATO WILT.

Fusarium lycopersici is a soil organism. The fungus gains entrance into the host thru the root system. It pierces the epidermal cells of the host, probably by secretion of enzyme which dissolves the cell walls. Inside the host the mycelium grows and increases, and conidia and chlamydospores are formed. With the dying of the plant the fungus is returned to the soil. Here it may continue growth if organic material is present. The spores will exist for sometime within soil without losing their capacity for growth.

The soil may become contaminated in various ways. Bits of infected soil from one field may cling to implements or the feet of animals and be carried to other fields. Drainage water may also spread the fungus. Seeds from plants grown in diseased fields may also carry the organism to new areas.

Knowing the method of infection, we have a basis for establishing the control of the disease. Workers dealing with the problem of the control of tomato wilt usually give three methods; the removal and destruction of diseased plants from the fields, the rotation of crops, and the growth of varieties resistant to the

disease. The addition of substances to the soil such as lime has been used and does decrease wilt, but is hardly practical.

The first method combined with the second proves to be quite effective in the control of the disease. The principle of the first method is plain, simply the removal of the cause of infection. But as many roots remain in the ground when the plant is pulled, and as the fungus is already in the soil to begin with, this method alone would not check the disease.

The principle underlying the second method is the fact that the fungus is a soil organism and by leaving tomatoes off the fields for several years, the amount of the fungus in the soil will not be increased, but will more probably die out.

The method of control offering the most hope to the tomato grower is the develpoment of varieties resistant to the wilt. The knowledge of disease resistance is not extensive. Orton (39) tells us that the need for research is great. The problems to be dealt with are problems of heredity and of pathology. The basis of disease resistance in case of physiological diseases is the development of strains adapted to the environment, and in case of

diseases due to parasites, the development of the strains found in nature to be resistant. Reed (42) gives the methods of attack which have been used in the past, in developing resistant strains. One is the development of strains from plants surviving unattacked in a badly diseased field, and the other the development of resistance by crossing a susceptible variety with a resistant one and thereby adding to the good qualities of the susceptible plant the quality of resistance.

The following summaries give the most important results obtained so far on the development of strains of tomato resistant to the wilt.

Edgerton (12) in 1913 says that the most satisfactory method of control is by the development of resistant strains. At that time the tomato varieties on the market were susceptible, though in varying degrees. He expressed the belief that resistant strains could be developed, in fact, had been. In a field planted to the same variety, a plant was found which had withstood the wilt while all others in the field had succumbed, and from this a wilt resistant strain was developed. Later in 1918 Edgerton (11) reports that this strain proved to have undesirable marketable qualities, but experiments were being carried out on crossing it with plants

having desirable qualities, and the results seem hopeful.

Experiments were carried out in Florida in 1907, but as all varieties proved susceptible, this line of investigation was discontinued. (45)

Durst (9) in 1918 reports the results of five years experiments on wilt resistant varieties of tomatoes. Large numbers of varieties have been tested on infested soils. It has been found that susceptibility varies widely, and that those plants possessing the greatest resistance usually have possessed the undesirable marketable qualities, but by repeated selection strains have been developed from those varieties possessing desirable marketable characteristics which are capable of living thru the season in infected soil. This improved stock has produced over twice as much marketable fruit as that produced by varieties on the market..

Experiments have been carried out in Tennessee on the selection of tomatoes for resistance to tomato wilt. (13). Seeds were obtained from plants free from disease, but growing in badly wilted fields, and from those on the market. These seeds were planted in fields which had previously suffered almost total loss from wilt and in fields where the wilt was unknown. In the latter no wilt occurred in any plants, in the former field all the commercial varieties

succumbed to the wilt and some of the selected seeds. One strain of the "Beauty" type showed remarkable resistance and offers a hope that in the future a blight resistant type satisfactory in all respects may be developed from this. The Globe tomato was found to have natural resistance, but no desirable qualities for culture.

The growth of resistant varieties is the best method of control for all fusarial diseases. From various plants subject to fusarial diseases varieties have been produced which are resistant. This work does not progress rapidly because often the plant which possesses the quality of resistance does not possess qualities desired in a plant for culture. Thus comes the necessity of crossing with plants of more desirable characters.

Bolley (3) has developed a variety of flax resistant to the wilt. Jones and Gilman (23) have found strains of cabbage which resist the yellows. Fulton (15) reports the production of a watermelon highly resistant to the wilt. Resistant strains of cotton have also been developed.

These studies were made under the direction and at the suggestion of Dr.G.M.Reed of the Botany Department of the University of Missouri. His many suggestions and criticisms have been most helpful and instructive.

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EXPLANATION OF PLATES.

Plate I

Fig.1. Conidia from a 20-day old culture on potato dextrose agar.

Fig.2. Conidia from a 20-day old culture on potato tuber plugs.

Plate II

Fig.1. Conidia from a 6-day old culture on bean agar.

Fig.2. Conidia from a 15-day old culture on bean agar.

Plate III

Fig.1. Conidia from a 7-day old culture on corn meal agar.

Fig.2. Conidia from a 15-day old culture on corn meal agar.

Plate IV

Fig.1. Conidia from a 7-day old culture on prune agar.

Fig.2. Conidia from a 15-day old culture on prune agar.

Plate V

Fig.1. Conidia from a 7-day old culture on bean pod tissue.

Fig.2. Conidia from a 16-day old culture on bean pod tissue.

Plate VI

Fig.1. Conidia from a 7-day old culture on rice.

Fig.2. Conidia from a 20-day old culture on rice.

Plate VII

Fig.1. Conidia from a 7-day old culture on bread.

Fig.2. Conidia from a 30-day old culture on bread.

Plate VIII

Fig.1. Chlamydospores from a 39-day old culture on potato dextrose agar.

(All the above drawings are camera lucida sketches X750)

Plate IX

Fig.1. Culture growing on potato dextrose agar at 30-32 degrees C.,2 weeks old.

Fig.2. Culture growing on potato dextrose agar at 32 degrees C.,2 weeks old.

Plate X

Fig.1. Shadowgraph of culture growing on potato dextrose agar at 20-22 degrees C., $4\frac{1}{2}$ days old.

Fig.2. Shadowgraph of culture growing on potato dextrose agar at 28 degrees C.,$4\frac{1}{2}$ days old.

Plate XI

Fig.1. Tomato plants growing on Pfeffer's nutrient media plus 2 per cent of agar and 3 per cent of dextrose.

Plate I.

Fig. 1.

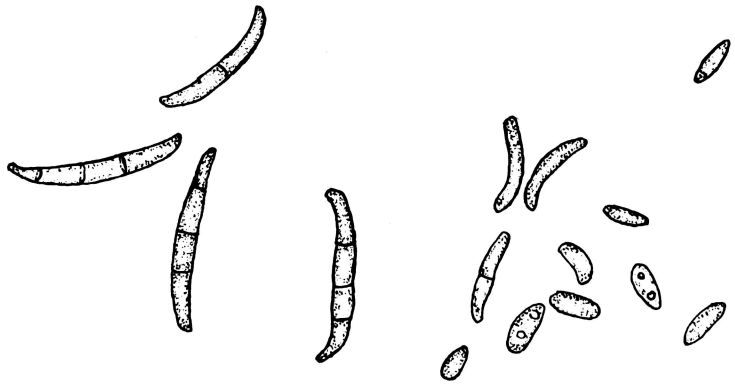

Fig. 2.

Plate II.

Fig. 1.

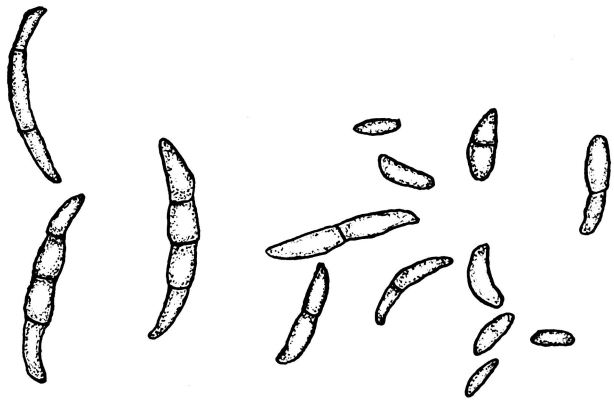

Fig. 2.

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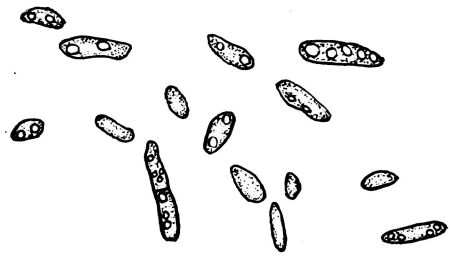

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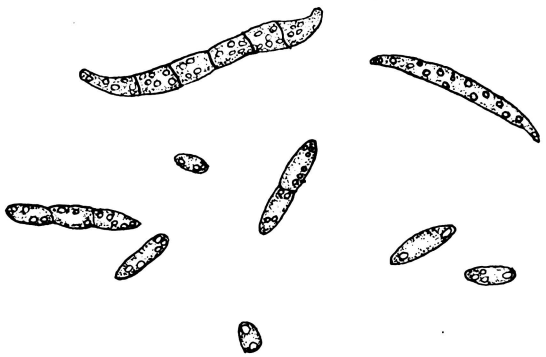

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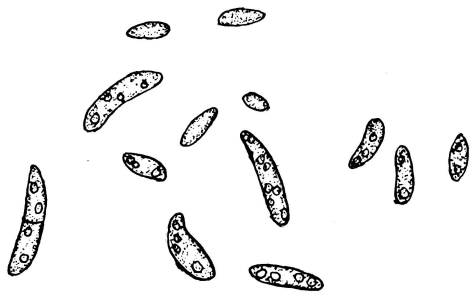
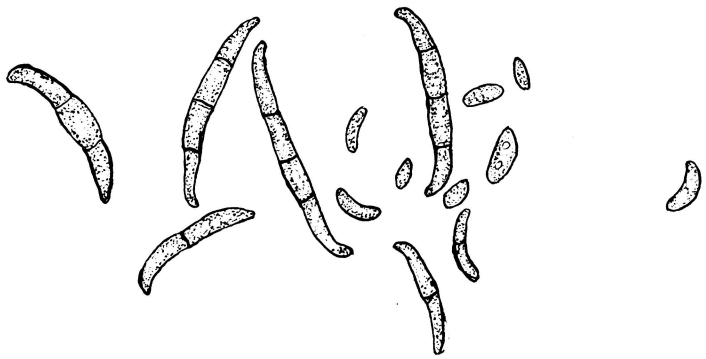


Fig. 1.

Fig. 2.

Plate V.



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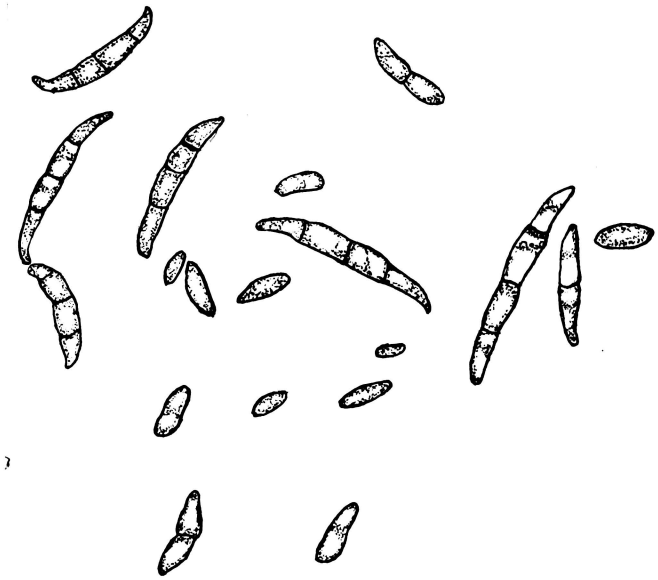

Fig. 2.

Plate VI.

Fig. 1.

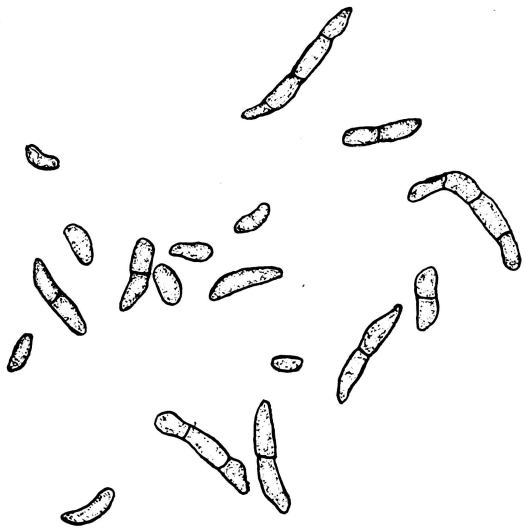

Fig. 2.

Plate VII.

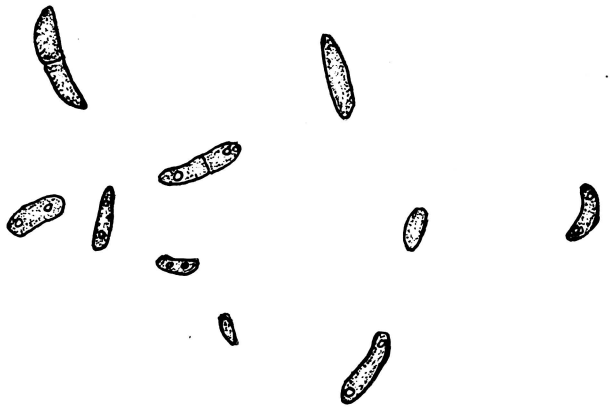

Fig. 1.

Fig. 2.

Plate VIII.

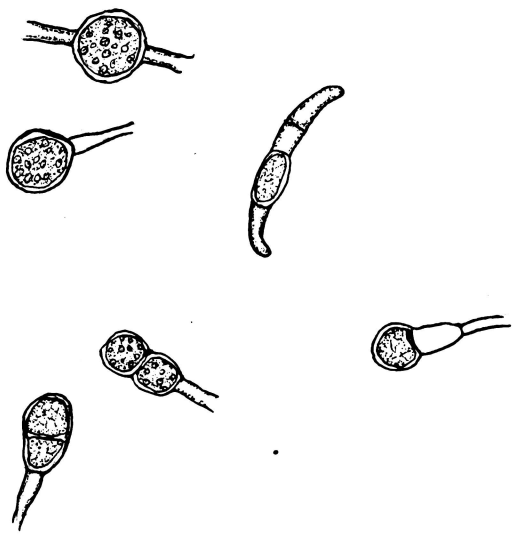

Fig. 1.

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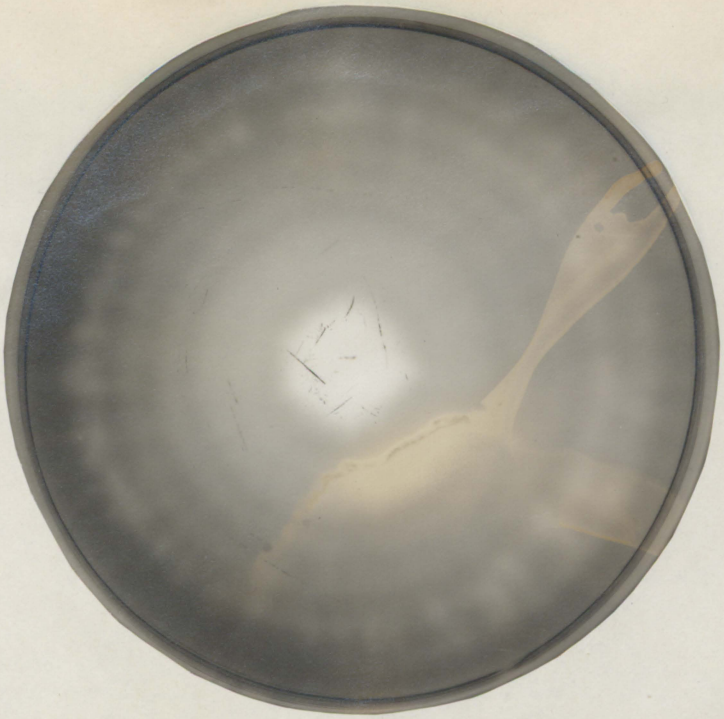
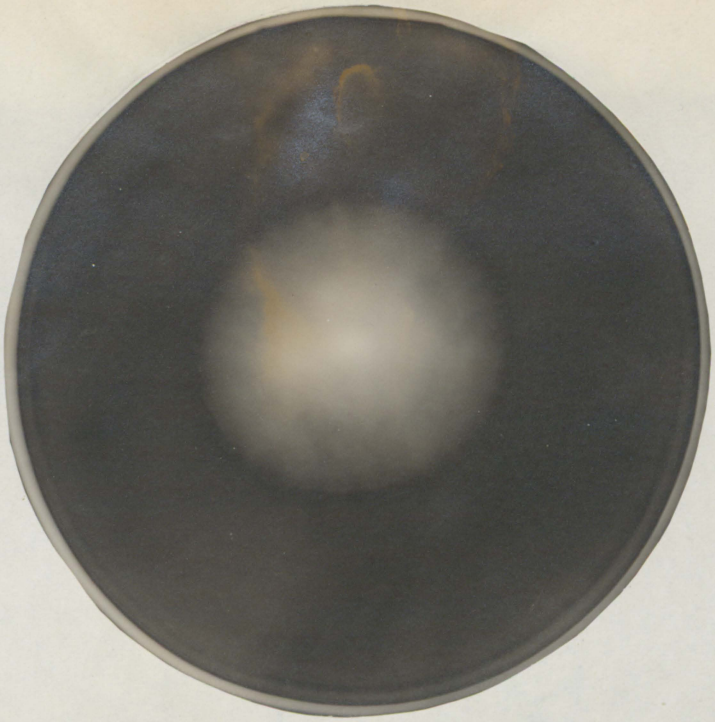


Fig. 1.

Fig. 2.

Plate X.



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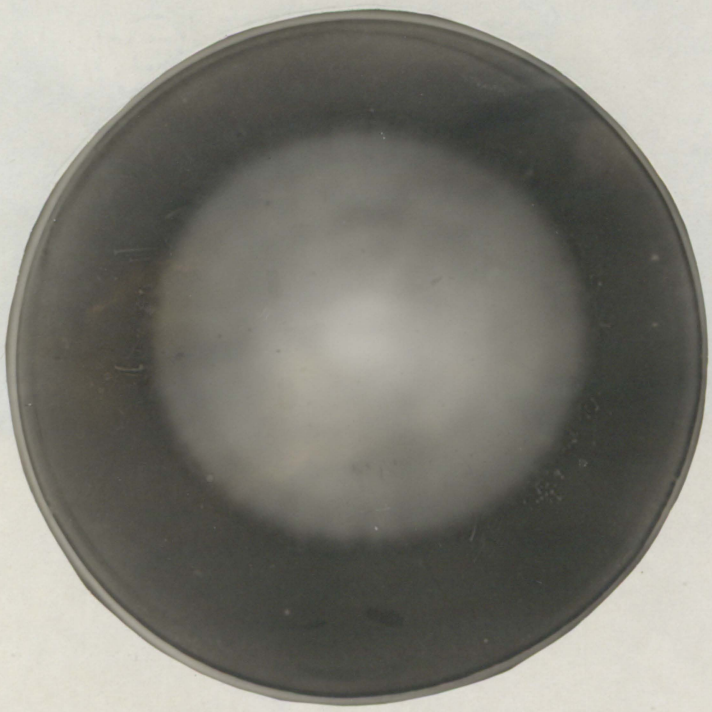
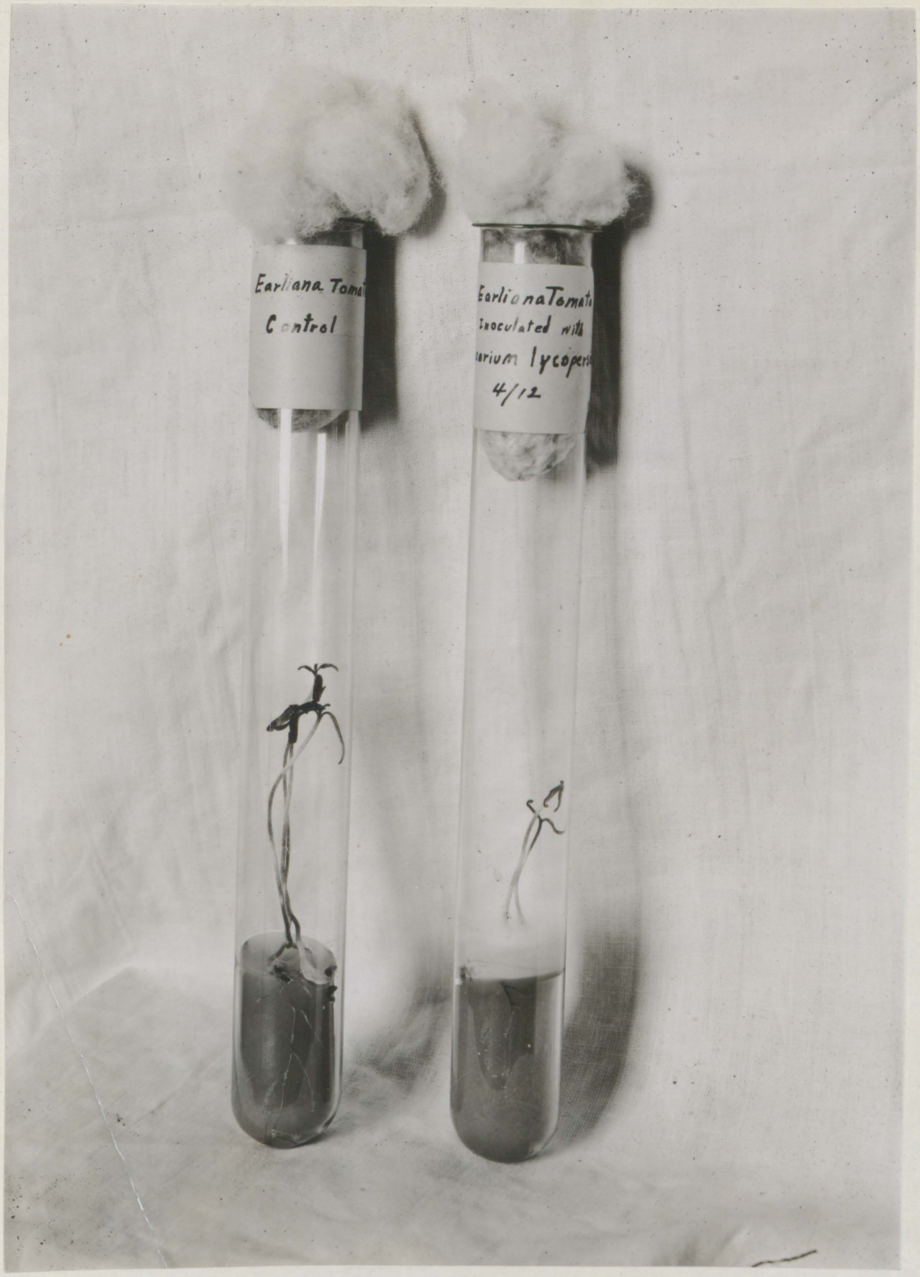


Fig. 2.

Plate XI.



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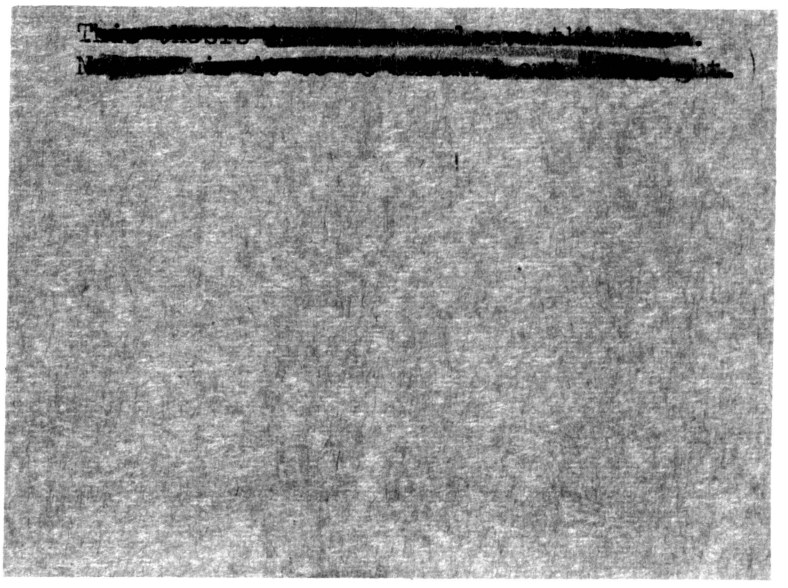

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