Tomato leaflet badly infected with Cladosporium fulvum. Note that the dark colored growth covers nearly the entire lower surface of the leaflet.

THE LEAF MOLD OF TOMATOES, CAUSED BY CLADOSPORIUM FULVUM CKE.

THESIS FOR DEGREE OF M. S.

WALTER KENNETH MAKEMSON

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CONTENTS.

The Leaf Mold of Tomatoes, caused by

Cladosporium fulvum Cke.

XII. Control measures.
  (a) Spraying.
    1. Resistance of the organism to fungicides.
      (1) Germination and growth tests in moist chambers.
      (a) Infection on sprayed and dusted plants.
  (b) Fumigation.
    1. Formaldehyde gas.
    2. Sulfur fumes.
  (c) Additional prophylactic measures.
XIII. Summary.
XIV. Literature cited.

THE LEAF MOLD OF TOMATOES, CAUSED BY CLADOSPORIUM FULVUM COKE.

INTRODUCTION.

Tomato Leaf Mold (caused by Cladosporium fulvum), probably the most serious parasitic disease of greenhouse tomatoes in Michigan, was first described from specimens sent from South Carolina to Prof. M. C. Cooke in England, (1883)*. The article containing this description gave the diagnoses of the new species contained in Ravenel's distributed sets of specimens entitled "North American Fungu". **

Since 1883 the fungus causing this leaf mold has been mentioned in various experiment station reports, agricultural publications and texts on Plant Pathology, but these reports, other than giving a description of the appearance of the fungus on the tomato plant, contain very little additional information to that first given in "Grevillea" in 1883. Nor is any efficient method of control given, most authors assuming that Bordeaux spray will effectively check its spread.

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* The date in parenthesis refers to the literature cited at the close of this paper.

** The species in question was described as follows: "Cladosporium fulvum. Effusum, lanosum, fulvum. Hyphis erectis, flexuosis, septatis, nodulosis, parce ramosis, fulvis. Sporis ellipticis, uniseptatis, vix constrictis, pallide fulvis, hyalinis (.01 - .02 x .0045 mm)."

In the United States the leaf mold seems to be almost uniformly distributed over the country, especially where tomatoes are grown under glass. Halsted (1885) reported the disease as occurring on tomatoes grown in the open air in the vicinity of Ames, Iowa. The fungus was described in the Report of the U. S. Department of Agriculture for the year 1888, from material sent to the Department by a Mr. Wilson of Vineland, N. J., where the mold occurred in the greenhouse. Bailey (1893) reported the fungus in greenhouses in New York State where it was not regarded as being a serious pest; Selby (1896) reported its occurrence in Ohio; Rolfs (1898) in Florida; Orton (1904) stated that the disease was serious in Maryland and Ohio; Jones and Morse mentioned the occurrence of the mold in Vermont in a disease survey for the same year; and Barre (1910) reported it as being prevalent in South Carolina.

I have the verbal report of Mr. H. C. Young of the Department of Botany at the Michigan Agricultural College that the leaf mold was a serious pest in greenhouses in the vicinity of Raleigh, N.C. in 1915. The earliest specimens from Michigan in the Agricultural College herbarium date back to 1898, since which time it has been reported from practically every part of the State where tomatoes are grown under glass.

Abroad the fungus seems to have gained as wide a distribution as in America. Plowright (1887) reported the organism in England for the first time; Prillieux and Delacroix (1891) reported it in the Department of the North in France; Traverso (1890), in Italy; Briosi and Cavara (1898) at Pavia, Italy; Froggatt (1906) in New South Wales, Australia; Bos (1901) in Holland; Lind (1907-'09) In Denmark; Sorauer (1908) in Germany; M. T. Cooke and Horne (1908) in Cuba; Schechner (1910) in Austria.

Just where the fungus originated is hard to determine but it evidently came from some part of the New World, probably the western side of South America to which the tomato plant is indigenous and where it has been in cultivation for more than three hundred years. At any rate, it is significant that the disease was first observed in a southern state of North America and when we consider its ease of dissemination and the rapidity with which it has spread to all parts of the world, it does not require a great deal of imagination to see how it could have developed along with the tomato plant in its uncultivated state and finally become a serious parasite of cultivated tomatoes, especially on the foliage of tender greenhouse plants.

THE DISEASE.

Economic Importance.

An accurate estimate of the loss caused by this tomato disease is difficult to make because the infection, under different conditions, is so variable. In one greenhouse the disease may get an early start before the tomato vines have yet begun to bear and in such a case, the loss may be the entire tomato crop, due to the exceedingly rapid spread of the fungus. Again, the fungus may not get a good start until the vines have set most of the fruit and in this condition, the plants may be able to withstand the fungus and mature the majority of the fruits. In either case, the loss is brought about by two factors: the failure of the new fruit to set because the blossoms are blasted by the fungous attack and the failure of green fruit to grow to marketable size.

Prillieux and Delacroix (1891) stated that to tell which plants are diseased it is "only necessary to count the fruits, whose number is greatly diminished". One of the largest growers of greenhouse tomatoes in Michigan states that "if he could hold the vines below, there would be some size to the fruit above", meaning that if the lower leaves, which are the first out and consequently first subject to the attack of the fungus, could be kept green, there would not be much third class or cull fruit.

These cull fruits, borne on the upper fruit branches, were about the size of small walnuts when the author visited the infected greenhouses and it is unlikely that they became larger as the foliage of the plants bearing them was nearly dead. In another part of the greenhouses, where younger plants were growing, the other factor which causes even a greater loss of fruit could be observed. In this case, the last fruit branch had in almost every instance failed to set any fruit, due to blasting of the blossoms; the calyces, corollas and even young ovaries being infected. (See Fig. 1) This was on the 10th day of August, before the field grown tomatoes were ripe and while there was yet a good demand for the hothouse fruit.

Some idea of the seriousness of the disease may be had when one considers the capital invested by the grower mentioned above. These greenhouses were covered by 180,000 sq. ft. of overhead glass, under which were set 35,000 tomato plants. Not one plant of the 35,000 was found to be free from the disease and most of them were very badly infected, the older plants having at least 75 percent of the foliage killed at the date of visit. The younger plants still retained their badly infected foliage but all of the later blossoms were blasted. The plants were headed back at the third wire, or about six feet from the ground which gave on an average about five fruit bearing branches per plant.

Fig. 1 - Blasted tomato blossoms. Only one fruit has set on this fruit branch.

Leaving out of consideration the stunting effect upon all developing fruits and considering only the loss from blasted blossoms and undersized fruit on the last fruit-bearing branch, a conservative estimate of the loss involved would be about 30 percent of the total crop. This is a considerable amount as the owner of these greenhouses estimated that the crop, altho cut short, would yield a ten thousand dollar return. Growers over the state report infections of varying seriousness but, as before stated, it is difficult to estimate the percentage of loss which indirectly affects the fruit by consuming the plant's food supply, destroying the leaf tissue, and blasting the blossoms. Members of the Office of Cotton and Truck Investigations report in the Plant Disease Survey Bulletin No. 1, August 15, 1917, "Cladosporium fulvum observed in one large field in Southern Florida, causing about 30 percent injury, leaves turning yellow and dropping. Yield from this field as compared with adjoining fields was reduced about one third".

Description.

Common terms which have been applied to this disease are "Tomato Leaf Mold", "Tomato Leaf Rust", "Leaf Spot", "Common Blight" and inaptly, "Soft Rot", or "Tomato Rot". Tomato Leaf Mold seems to be the most fitting term used to designate the disease since the fungus presents a

characteristic moldy appearance on the tomato leaves. This name is also less apt to be confused with the other diseases of the tomato. The term "Blight" (preferably Southern Blight) is usually associated with a disease of the tomato caused by Bacillus solanacearum Erw. Smith, or with a disease of the tomato caused by Fusarium lycopersici and often called blight, especially in the South. "Leaf Spot" is usually applied to the disease caused by Septoria lycopersici and the fungus can hardly be said to produce a rusty appearance on the host, hence the unsuitability of the term "Rust".

Appearance on the Leaves.

This is essentially a disease of the tomato foliage. On the under side of the tomato leaf newly infected areas show at first a whitish downy growth which, compared to Ridgeway's color charts, is a pale olive-buff color. Figure 2 shows a leaflet greatly magnified, with the younger downy growth of the fungus at the margins of the leaflet. The growth below is usually accompanied by a yellowing of the cells on the upper surface opposite the infection. This at first appears as a rather indefinite spot whose pale yellow color gradually merges into the chlorophyll green of the leaf but it later turns a deep ochre-yellow color. When the leaf tissue is finally killed, it becomes a red-brown color

Fig. 2 - Leaflet magnified eight times to show the younger, downy, fungous growth at the margins of the infected area.

above. As the infection develops, the fungous growth on the lower surface becomes a characteristic tawny-olive color and the infected area increases rapidly in size. Infected leaves usually show a great many infected areas (See Fig. 3) so that in a very short time the entire lower surface of the leaf may be covered with the tawny-olive colored growth. (See Fig. 4). The leaf may, however, show only a few large infected spots or areas (See Fig. 5). Under certain conditions, the upper surface of the leaf may show a scattering growth of the fungus but this never becomes as heavy as on the lower side. (See Fig. 6, which shows infection on the lower side of the two leaflets shown above; the two lower leaflets were photographed from their upper surface, which is yellowed in the infected areas and bears numerous tufts of fungous growth).

The infected leaves soon commence to die, due to the growth of the fungus. Areas which were first infected are killed first and the fungous growth in these dead areas sometimes becomes a beautiful purple color on the lower surface. Thus the lower surface of a partly killed leaf may show a great many violet-purple colored areas, surrounded by the tawny-olive color of the later infections.

Fig. 3 - Infected leaflet with many infected areas somewhat evenly spaced over the surface.

Fig. 4 - The entire lower surface of this tomato leaflet is covered with fungous growth composed of spores and conidiophores.

Fig. 5 - Tomato leaf infected in large separated areas with Cladosporium fulvum Cke.

Fig. 6 - Conidiophores on the upper surface of the two leaflets shown below. Note that the growth is scattered. Stomata are fewer on the upper epidermis of the tomato leaflet.

Plowright (1887) described this color production, which Cooke (1883) did not mention in the original description, and suggested that the fungus be known as "Cladosporium fulvum, Cooke, var. violaceum; Hyphis conidiisque violaceis".

Since physiological studies,(see section on Physiology), have shown that the color is not due to a different variety of Cladosporium but to the action of certain factors upon the fungus itself, the writer does not believe the erection of a separate variety for the violet colored fungus justifiable.

Leaves are infected as they develop on the plant, consequently the lower leaves are the first to be killed. The fungus attacks the new growth as it is produced so that a diseased plant characteristically has killed and infected leaves on the lower portion of the stem with the newly produced foliage above as the only part remaining green and uninjured. Growers of greenhouse plants "top" them or "head them back" when they become five or six feet tall; therefore, we may find that no part of the plant is free from disease after a short time, since good greenhouse practice favors the removal of all new growth subsequent to the "heading back" process.

Where White Fly is present in a greenhouse, a saprophytic mold known as Cladosporium herbarum may often be found. This mold is not a parasite on the

tomato plant but obtains its subsistance from the honey-like exudate deposited upon the tomato leaves, stems and fruit by the White Fly. Cladosporium herbarum may be easily differentiated from the parasitic Tomato Leaf Mold in four ways: (1) Cladosporium herbarum usually occurs upon the upper surface of the tomato leaf, Cladosporium fulvum is found in greater abundance on the lower side. (2) Cladosporium herbarum is altogether superficial, i.e. the entire growth may be scraped off of the foliage or fruit and the latter found to be uninjured while Cladosporium fulvum mycelium is found to be inside the leaves or foliage and cannot be removed. (3) Cladosporium herbarum does not cause a discoloration of the leaf tissue; Cladosporium fulvum does. (4) Cladosporium herbarum presents a dirty, greyish-black appearance on the plant; Cladosporium fulvum is a tawny-olive color (see Figs. 7 and 8.. The under side of the leaf showing C. herbarum is infected in three small areas by C. fulvum but shows none of the C. herbarum growth).

Appearance on The Stems.

Plowright (1887) described the fungus attack on the stems in the following way: "Not only are the leaves covered by the Cladosporium but the stems have also succumbed, long brown lines and elongated patches being present upon them".

Fig. 7 - Cladosporium fulvum on leaflet at left, Cladosporium herbarum on leaflet to the right. The Cladosporium herbarum growth is seen to be greater on the upper surface of the leaflet than the growth of Cladosporium fulvum at the left, also upon the upper surface.

Fig. 8 - Cladosporium fulvum shown on under side of leaflet at left, also in two small patches on leaflet at right which is infected with C. herbarum on the upper surface. Note that Cladosporium herbarum does not show in under surface of leaf where honey dew exudate of the white fly is not deposited.

Massee (1910) described the fungus as attacking the stems, and Leaflet #262 of the Board of Agriculture and Fisheries (1912) described it upon the tomato stem as follows: "The fungus forms long, rust colored afterwards blackish streaks on the stem and more or less circular, scattered patches on the fruit".

The writer has found the fungus present abundantly on the young stems of the fruit branches, also on young side branches where it apparently had spread from an infection starting on the foliage at the end of the branch but no case has been observed where main stems of the plants were infected. Certainly main stem infection, if it does occur, is rare in Michigan as no infection of this kind was observed on over 50,000 infected plants in greenhouses visited. Furthermore, many attempts to infect artificially large tomato stems, both by sowing spores on cut and on uninjured areas, resulted negatively.

Appearance on Fruit.

Halsted (1885) reported a destructive fruit rot of the tomato at Ames, Iowa, ascribed by him to Cladosporium fulvum. He performed numerous inoculation experiments upon the tomato fruit with spores taken from the leaves. These inoculations were reported as being successful in part but from the description, it seems doubtful that the rot was caused by Cladosporium fulvum.

Halsted himself was somewhat doubtful as to this point.

Orton (1903) called the disease "Scab" and Ferraris (1915) stated that "on fruits appear very similar little scabs but only on green fruit is there much change".

According to Plowright (1887), "the fruit itself once set, seems generally to escape".

Numerous attempts by the writer to produce an infection on tomato fruits, ranging from the size of a pea upward, have failed wherever the skin was uninjured. Various methods of inoculating the fruit were tried.

Thirty inoculations were made by sowing fungus spores on the skin of the fruit in a small drop of water, afterwards covering the inoculation with a fleck of wet cotton. This method never failed to produce infection upon leaves but failed in every case with the fruit, superficial mycelium only being produced which finally died. Fruit was also bagged after being heavily inoculated with Cladosporium spores but no infection occurred.

On fruit injured by cutting with a scalpel and then inoculating with spores, but one case of infection occurred and this was accompanied by an Alternaria growth so the result was considered negative. Green fruit when injured and inoculated with spores, showed great power of resistance; the injury was generally healed by the formation of callus or scar tissue and

the fungus seemed to be unable to obtain a foothold.

In infected greenhouses, only one case was found of a fruit of any size being infected (see Fig. 9). This fruit was about one-fourth grown and a microscopical examination showed the presence of Cladosporium fulvum conidiophores and conidia intermingled with those of a species of Botrytis. Fruits from the size of a pea downward and ovaries were unquestionably infected with Cladosporium fulvum as spores and conidiophores were found in abundance upon them. Although Cladosporium fulvum may infect fruit through an injury or in connection with injury from other fungi, it cannot be said to be a disease of the sound fruit under ordinary Michigan conditions and is of minor importance in this regard.

Appearance on the Blossoms.

Blossom infection is equally as serious and important as the infection on the leaves. Here the fungus obtains a hold on the calyces, the petals, stamens and pistil, and the developing ovary itself becomes infected from the other parts so that blasting of the blossoms is of common and widespread occurrence. Figure 9 shows young infected ovaries, the infected areas of which have become browned and dark colored. Figure 10 shows four young infected ovaries removed from the infected branch shown in Figure 1. Figure 11 shows one of these ovaries with the attached calyx considerably enlarged.

Fig. 9 - Young tomato fruits infected with Tomato Leaf Mold. Healthy fruit shown at left, diseased fruit at right.

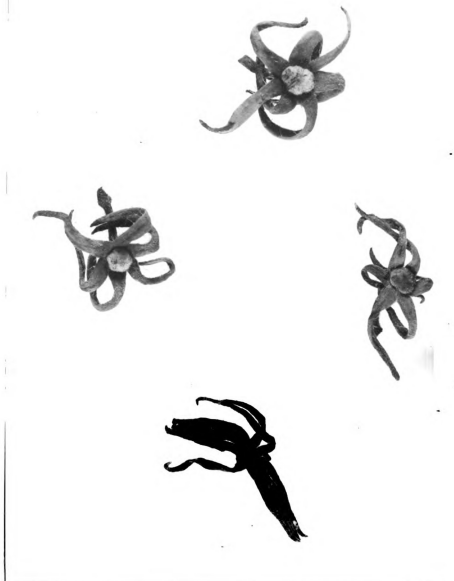

Fig. 10 - Infected calyces and ovaries of tomato. Ovaries are browned and calyces covered with masses of spores.

Fig. 11 - Young tomato fruit (shown natural size in Fig.10), greatly enlarged to show fungous growth on calyx. Growth on ovary itself are not conidiophores but glandular plant hairs.

The fungus growth can be easily seen upon the calyx lobes and the darkened area shows the infected part of the ovary. Such fruits never develop but soon fall from the vine. Microscopic examination of these blasted blossoms showed conidiophores and conidia upon the calyx and in one case, upon the style and stigma of the flower. No conidia could be detected upon the ovary itself but it was abundantly infected in the interior with the fungous mycelium.

That the fungus will attack the blossoms and young ovaries and not the more mature fruit may be explained by the fact that the calyx has stomata and is similar in structure to a leaf, so that the fungus can find ready entrance. The young ovary of the flower also has stomata and lenticels which may afford an entrance for the parasite. Blasting of the ovary was obtained on two different occasions by placing conidia upon the stigma. On the other hand, the stomata occurring on the very young fruit are rapidly transformed into lenticels and by the time fruit has reached the size of a pea no more stomatal openings are to be found but numerous lenticels have taken their place. The skin of the tomato is naturally very tough, consequently after the stomata disappear no openings are left by which the fungus can enter the fruit except by going directly through the epidermis. Infection studies have

shown the method of infection of Cladosporium fulvum to be stomatal hence its inability to infect the tomato fruit after the fruit has attained a certain size.

ETIOLOGY OF THE DISEASE.

Previous Work.

Infection experiments reported for Cladosporium fulvum are scarce; most authors have been content to describe the fungus which is so plainly associated with the host that infection experiments seemed needless. Halsted (1883) did the first work of this kind but, as stated above, it is doubtful whether the organism observed was Cladosporium fulvum. Prillieux and Delacroix (1890) reported that infection experiments succeeded perfectly; at the end of three weeks fructification of the fungus took place and the leaves began to die. That pure cultures of the fungus were used is not mentioned, however, so the presumption is that the inoculations were made from spores on infected leaves directly to other plants.

Formal Proof of Causation.

Three separate single spore isolations of the organism were made. The first isolation was made from suspected tomato leaves sent in by Mr. C. W. Waid of

the Department of Horticulture, from the greenhouse of Melvin Lennon of Ann Arbor, Michigan, on June 29th, 1916. The infected leaves compared favorably with the descriptions of the disease given in Saccardo and standard texts on Pathology and a single spore isolation was made by means of the "loop dilution" and "poured plate" method, using a corn meal agar medium.

Pure Culture.

Cultures obtained from this isolation made on July 1, 1916 were used for observation of the fungus in pure culture on different media until November 5, 1916 when artificial inoculations were tried upon tomato plants but these resulted negatively. Accordingly on November 23, 1916 a new single spore inoculation was made from infected leaves sent in from the greenhouse of a Mr. Dudley of Redford, Michigan. Cultures were made from the spores produced by the growth from this single spore inoculation and on December 21, 1916 eighteen inoculations were made upon six young tomato plants. The spores were sown in small drops of water placed on the leaves, then covered by small flecks of wet cotton which served to maintain a moist condition and also retain the spores in a definite place, a method used by Levin (1916) in studying the tomato leaf spot disease.

Production of the Disease on Healthy Plants by Inoculation from Pure Culture.

The above inoculations were uniformly successful and infection on all plants was found to be present on January 10, 1917. In three weeks spores were being produced freely and the characteristic yellowing of the leaves was noted. Many of these infected leaves were wrapped in paper, placed in flower pots and covered with soil. Some of the pots were placed out doors, others left in the greenhouse and others put in different parts of the Botanical Building where conditions of temperature and moisture varied. This was done to see if possible what the effect of "wintering" or a "rest period" would have upon the spores.

Reisolation and Reinoculation.

On April 24, 1917, nearly four months after placing the infected leaves in flower pots, they were examined and a single spore isolation made from spores on the leaves. The isolation was successful and after growth in culture on corn meal agar plants were again inoculated with spores from the isolation. Infection was positive and characteristic of the organism.

The rest period did not seem to have lessened the viability of the spores in the least and the plants were successfully inoculated with spores directly from these

leaves on June 21, 1917, which shows that the spores may cause infection after a considerable time.

TAXONOMY OF CLADOSPORIUM FULVUM.

Cladosporium fulvum Cke., was first described by Cooke in 1883 as an imperfect form belonging to the family Dematiaceae of the group of "Imperfect Fungi" and it still remains in this group as no perfect stage has been reported for it. The fungus may some time be found to have a perfect stage, presumably in the Mycosphaerellaceae, since Janczewski (1893), established the connection of what he called Cladosporium herbarum, causing an injurious disease of cereals, with the Ascomycetous germ Mycosphaerella.

Infected tomato leaves wintered over in the soil of flower pots, gave no indication of having a perfect stage formed upon them but inoculations of spores from pure culture upon autoclaved corn grains in test tubes on June 19, 1917 showed on August 10, 1917 rather thick mats of fungus hyphae distributed through which were numerous spherical bodies ranging from 50 to 120 microns in diameter. These bodies presented a structure typically perithecial in appearance with a thin, pseudoparenchymatous wall but although partially hollow contained neither asci nor spores, and could not be made to form spores by ordinary variations in the cultural technique.

MORPHOLOGY OF THE PARASITE.

Mycelium.

The mycelium of the organism varies considerably according to the culture media used, age of culture, etc. The young hyphae from germinated spores are about 2 microns in diameter, delicate, hyaline and septate, at first unbranched but soon sending out numerous lateral branches at right angles to the main strand. These hyphae grow very well in Van Tieghem moist chambers or upon the tomato leaf surface (see Figure 14), where they may attain a length of several hundred microns. Some have been observed in moist chambers which were 400 microns long.

On nutrient media, such as corn meal agar, the mycelium averages about 3.5 microns in diameter, is much longer, more numerously branched than in the moist chamber and presents a granular rather than a hyaline appearance. Septa are here more numerous and constrictions often occur between the cells which sometimes become greatly swollen.

Older mycelium grown in a sterilized extract expressed from tomato foliage is a dark brown in color, frequently septate, granular and averages 5 microns in diameter. On autoclaved corn kernels the mycelium produces dark colored, stromatoid masses, composed of

hyphae possessing greatly swollen and globular shaped cells which are easily broken apart; cells of this kind often measure 10 microns in diameter.

Conidiophores.

Conidiophores are four to eight septate, nodulose, sparingly branched and dark brown in color. Their length and width varies from 75 to 140 x 3 to 5 microns with an average of 80 to 90 x 4 to 5 microns.

The conidiophores project from the leaf surface in clusters which emerge from the stomata. Consequently it usually occurs that the conidiophores are of greater diameter as the top than at the base where they are densely crowded and form a stroma-like mass. (See Figs. 12 and 13). Branches usually occur below the septa, often at right angles to the conidiophore and may be long or mere wart-like processes from which the conidia are produced. A single branch usually occurs below a septum but sometimes a whorl of them is found. The conidiophore generally ends in a chain of conidia but often tapers to a narrow tip or forms a "boot-like" process. (See Fig. 13).

Conidia.

Conidia are produced acropetally either at the tip of the conidiophore or laterally from wart-like projections which arise immediately below the septa or sometimes laterally from other conidia. They begin to be formed.

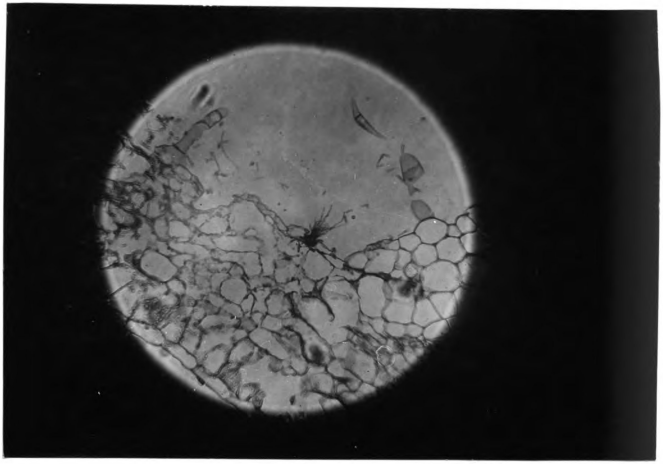

Fig. 12 - Cross section of tomato leaflet near midrib (on left), which shows a cluster of conidiophores emerging from a stoma. Note a few darkly stained hyphal branches extending from the cluster into the leaf tissue and also inter-cellular mycelium.

Fig. 13 - Cluster of conidiophores photographed from section cut from a badly infected leaflet. Note the peculiar "Boot" shape of the conidiophore tip in several cases. Spores are mostly broken from the conidiphores but many are shown in the field.

when the conidiophore is comparatively short (40 - 50 microns) and continue to be produced in chains as the conidiophores elongate. The youngest conidium of each chain is the terminal one. As many as six conidia are often found composing one chain.

Conidia are ovoid to elliptical in outline, apiculate at one end, usually uniseptate, pale brown in color and measure 10 - 35 x 5 - 7 microns. The average length and width is 6 x 18 microns.

RELATION OF PARASITE TO HOST.
Infection and Conidiophore Production.

Type of Infection.

To determine the type of infection two methods were employed: (1) Microscopes were set up with moisture supplied slides (see Fig. 14). Spores from pure culture were sown in a droplet of water on the leaflet of one plant and the leaflet then placed between the cover glass and slide of the microscope shown on the right in Fig. 14 A long narrow strip of filter paper, having a small square opening cut from one end, was placed under the cover glass and over the leaf in such a way that the square opening lay opposite the inoculated area on the leaf. This permitted observation of the inoculated surface through the microscope, held the cover slip away from the inoculated area and supplied moisture from a tall vial containing water. The other microscope was used in the same way, with the exception

Fig. 14 - Apparatus used in preliminary work to determine method of fungous infection.

that spores taken directly from an infected leaf were sown on the leaflet. (2) Inoculations of spores were made on a number of tomato leaflets and the epidermis stripped from the inoculated areas after 12, 24, 36, 60 and 72 hours.

By means of the microscope observation method germ tubes of various lengths were observed after six hours. At the end of twelve hours some of these were quite long, producing a superficial mycelium on the leaf surface but no infection was found until the next day, when the germ tubes had become very long and much branched. (See Fig. 15). Several cases of stomatal entrance by the hyphae were noted after thirty-six hours but penetration directly through the epidermis was not observed.

That the type of infection is stomatal was proven beyond a doubt by the second method of stripping the epidermis from inoculated areas.* Epidermis from twenty-four hour inoculations showed no entrance of the germ tubes but the ends of many were turned toward stomata, in some cases reversing their original direction of growth. This seemed to show the existance of a chemotactic influence exerted through the stomata upon the germ tube. Epidermis stripped from leaves after

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* Pieces of epidermis stripped from the leaf were fixed in hot 85% alcohol, then run down to 50% alcohol and stained with an Erlich's Haematoxylon- clove-oil-eosin stain. This stained the epidermal cells a deep pink and the hyphal threads on their surface blue.

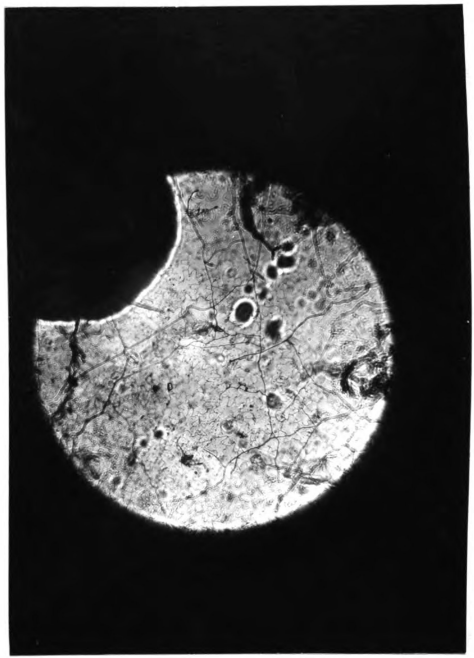

Fig. 15 - Photomicrograph taken from upper surface of epidermis stripped from inoculated tomato leaf. Note fungous hyphae overrunning the surface, three of which penetrate stomata (in circles).

thirty-six hours showed numbers of germ tubes entering stomata. The germ tube may immediately enter a stoma but more cases were observed where it had reached considerable length before doing so and lateral branches were observed to enter as often as main branches. (See Fig. 15). Sometimes two branches, given off at approximately the same place on the germ tube, enter the stoma.

A pronounced enlargement in the diameter of the germ tube occurs immediately upon its entrance through the stoma and the protoplasmic contents become more granular in appearance.

Inoculations made upon both lower and upper surfaces of leaves have given positive results, showing that infection does occur upon either side of the leaf which is what would be expected, as stomata are present upon both leaf surfaces, in greater numbers, however, on the lower side.

Conidiophores.

Numerous hyphal branches pass to the stomata (see Fig. 1), where a stomatoid structure is formed. Conidiophores arise in clumps through the stomata from these stromatic masses of cells, which often measure twenty to thirty microns in diameter and must rupture the stomatal opening during their growth, since the latter does not measure over thirty microns in length and is only about four microns in width. The conidiophores spread outward as they grow, the top portion of the cluster often reaching a diameter of one hundred microns (see Fig. 13).

As the stomata are spaced from thirty to eighty microns apart on the under side of the tomato leaf, it is no surprising thing that badly infected leaves seem to be a solid mass of spores on the under side when viewed by the naked eye. Viewed by means of the low power of the compound microscope, the conidiophores appear like little clumps of shrubs thickly and evenly planted over a level field, with their branches interlaced at the top.

Morbid Anatomy of the Host.*

Sections cut from newly infected leaves show mostly intercellular mycelium but with growth of the fungus, the hyphae are found to be both inter- and intra- cellular. Haustoria are not produced, the hyphae ramifying through all parts of the leaf tissue, particularly around the tracheary tubes. Tracheary tubes cut in cross section show a ring of fungous hyphae around them (see Figs. 16 and 17), and in longitudinal section the hyphae are

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* Histological Technique.
The fixing agents used were Flemming's fixative medium formula, alcohol-formaldehyde and Gilson's Fixative. Flemming's triple stain and Durand's stain were used in staining sections but no success was had with the latter. The alcohol-formaldehyde fixative seemed the better where sections were stained but specimens fixed in Flemming's fixative, sectioned by hand and mounted without staining gave better sections and more detail than those cut by the microtome and stained. By this method the fungous hyphae showed up very black with the septa clear and the host cells became greenish brown, giving plenty of differentiation for histological study. Flemming's triple stain gave better results than the Durand stain but the results were far from satisfactory.

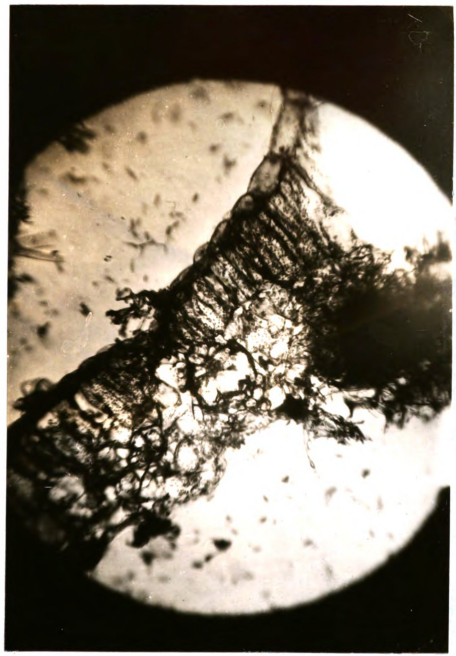

Fig. 16 - Photomicrograph of cross section of tomato leaflet, showing ring of fungous hyphae around the badly disorganized tracheary tissue. Note also the badly disorganized appearance of the infected host's leaf tissue.

Fig. 17 - Photomicrograph showing ring of hyphae in Fig. 16 under greater magnification. The mycelial septa may be seen in the lower portion of the circle.

found parallel to the sides of the tubes and closely appressed to them, (see Fig. 18) which indicates that the parasite is a strong contender for the water supply of the host.

The mycelium at first does not greatly affect the host cells but its growth is very rapid and the host tissue and cells soon become disorganized and broken apart. The spongy parenchyma and tracheary tissue seem to be more susceptible tothe fungous attack than the pallisade parenchyma and is considerably more disorganized. A greater abundance of fungous mycelium is also found in the spongy parenchyma region.

PHYSIOLOGICAL STUDIES.

In order to reach a better understanding of the fungus in relation to its environment on the host, several experiments were conducted under controlled conditions.

Cultural Studies.

At first some difficulty was encountered in obtaining single spore isolations of Cladosporium fulvum by means of the loop dilution and poured plate method. The first plate poured was found to contain all the spores in too great a number for isolation while plates number two and three had no spores at all. This was found to be due to the tendency of the spores to "ball up" or remain in clumps in the drops of sterile water used for the spore

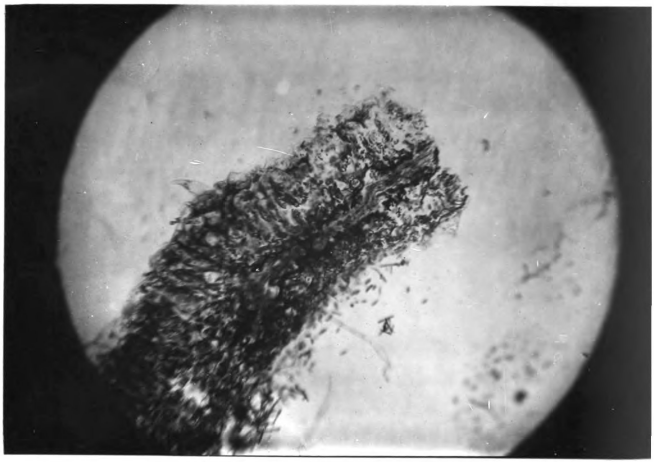

Fig. 18 - Tracheary tissue cut in longitudinal section, showing fungous tissue (black colored lines) closely parallel to the sides of the water tubes.

suspension and also to their remaining on the surface of the water for which they have a strong affinity. When spores were placed in a drop of 95% alcohol they did not remain upon the surface but immediately became distributed throughout it. This indicates that the spore wall may contain minute ridges or irregularities which hold some air and thus prevent the wetting of the spore and its submersion, or it is possible that some fatty substance not easily wetted by water is present in the spore wall.

The difficulty was removed by thoroughly mixing the spores in the droplet of water with a sterile scalpel, using some pressure. This gave a fairly even spore suspension which could be transferred from one dilution tube to another by platinum needle loops. Single spores were marked by means of India ink circles around them on the petri dish bottoms and as soon as germination took place they were transferred to corn-meal agar slants in test tubes. Here the fungus develops slowly at first, the small clump of hyphae soon showing microscopically as a pearly white spot. Spores are formed shortly after the mycelium reaches the surface of the agar and spreading takes place slowly.

Conidia are formed freely on culture media, the conidiophores arising from stroma-like formations which are quite evenly distributed over the surface of the medium. Viewed in mass, the conidia and conidiophores present a velvety, olive-green color.

Growth on Various Media.

This fungus does not show great mycelial growth. On agar slants the hyphae do not penetrate deeply into the agar nor does the mycelium grow to all parts of a liquid medium. In describing the fungus on the foliage, it was mentioned that certain infected, dead areas were purplish in color. On certain media, notably corn meal and oat meal agar, a beautiful purple color is produced. Time was not available to study the nature of this coloring matter but it may be mentioned that on infected leaves the color is located in the conidiophores and conidia themselves, where, under the microscope, it appears as a light violet blue. The infected area itself, after having the fungous growth removed from its surface, shows none of this coloration whereas in cultures the color is a secretion product into the agar.

As will be noted later (see effect of light on growth of fungus) color production seems to occur best in diffused light. The fungous growth on infected leaves of plants placed in a dark room very soon becomes a light pink in color and when compared with check plants left in the light showed a striking difference in color. Dryness seemed also to stimulate color production in the mycelium which grew on the sides of culture flasks containing tomato extract media. Adhereing in clumps to the flask, this mycelium receives no direct moisture

from the flask and it becomes a pink color. Dying of the infected leaf tissue removes the customary moisture from the fungous growth and is probably the cause of the color production here.

Shear's corn meal agar: Fungous growth moderate, submerged mycelium dirty white colored. Spore production profuse, olive green in color. Purple color production in medium very profuse in diffuse light or darkness.

Rolled oat agar: Growth moderate, spore production fair. Similar to growth on corn meal but spore and purple color production not so heavy..

Standard Dextrose agar: Growth heavy, submerged mycelium fuliginous. Spore production was greater than on corn meal agar. Purple color production absent.

Tomato leaf agar: Growth moderate, spore production profuse, olive green, mycelium fuliginous. No purple color production.

Autoclaved corn grains: Growth moderate, submerged growth black, superficial growth fulvous. No spore production on submerged mycelium, moderate spore production on spperficial. No purple color production.

Autoclaved bean seed: Growth similar to that on corn.

Filtered tomato extract: (Prepared by filtering extracted juice of tomato leaves and stems through a Chamberlain "F" filter). Growth slow, finally moderate in clumps, black. Spore production absent, except on

clumps of fungous hyphae growing on the sides of the flasks. Purple color production by submerged growth absent. A peculiarity was noticed in the growth of the fungus in liquid media, i.e., spores when inoculated into the media grew and produced separate clumps of mycelium which remain attached rather firmly to the sides and bottom of the culture tube; or float on the surface of the liquid. These clumps send out branches a very short distance and secondary growth clumps are not produced except by the germination of spores which come from superficial clumps of mycelium. Mycelial growth, compared to that of most fungi, is thus very sparse and it was found impossible to grow enough of it to do enzyme work with the organism.

Sterilized tomato extract: Growth very similar to that in the filtered tomato extract.

Duggar's (1917) Synthetic corn meal medium: Growth fair, and very similar to above. Spore production abundant on superficial mycelium.

Coon's (1916) cheap synthetic medium: Growth average, olive green color.

Effect of Humidity.

To study the relation of moisture to the growth of the fungus, the sulphuric acid method described by Stevens (1916) was used. Five large battery jars were inverted on a large glass plate,the outside air being excluded

by sealing with vaseline.

Small tomato plants (averaging 2½ inches) of the Livingston Globe variety were selected and planted in glass tumblers which were sealed with paraffin to prevent evaporation of the moisture from the soil. The plants were run in duplicate and one leaf of each was inoculated with a heavy suspension of spores. The acid, contained in beakers, was changed every three days and used in 200 c.c. amounts in each chamber. Lambrecht polymeters were placed in the chambers to check the moisture content (see Fig. 19).

It is realized that absolute moisture content values could not be maintained in such large chambers, containing transpiring plants and subject to a considerable temperature range, but the polymeters showed that a good range of humidity content was obtained in the different chambers and approximate values were all that could be hoped for. The following table gives the results of the experiment:

Table I.

Battery Jar No.	Sp.Gr.of H_2SO_4 used	Approx.Theoretical relative humidity	Polymeter readings.	Fungous growth	Plant growth
1	H_2O	100%	97%	Good	Fair
2	1.215	78.7+%	*88%	Excellent	Excellent
3	1.275	65.5+%	*75%	Poor	Fair
4	1.398	38 %	*60%	None	Poor
5	1.835 dish of $CaCl_2$	0%	*25%	None	Poor

* These readings, which are higher than the theoretical values may be somewhat in error on account of inaccuracies in the polymeter used but at any rate, a higher humidity is to be expected since the plants are constantly giving off water.

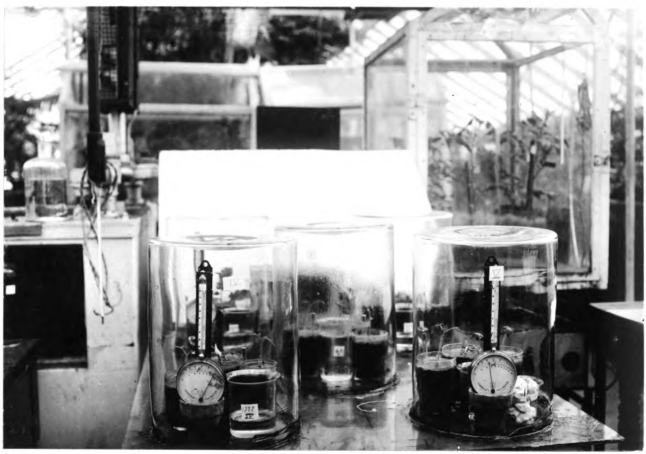

Fig. 19 - Apparatus used for conducting experiment on relation of fungous growth to humidity.

Germination and superficial growth was noted on plants in Chambers 1, 2 and 3, three days after inoculation. Eleven days after inoculation conidiophores, with conidia, were scraped from infected leaves of plants in Chambers 1, 2 and 3, but the conidiophore growth on plants in Chamber 3 was scanty. The infected leaves of this plant were yellowed, however, and soon fell off. Microscopic examination showed the leaf tissue to be badly infected with fungous mycelium. Infected leaves on plants in Chambers 1 and 2 remained on the plant much longer altho producing many conidiophores and spores. The appearance of the fungus in Chamber No. 1 was not quite typical when compared to check plants since the atmosphere was so humid that the spores germinated while yet attached to the conidiophore and this gave a somewhat "woolly" appearance to the growth.

The above results show quite strikingly the relation of moisture to the rapid development of this fungus which grows best in a humid atmosphere but withstands a considerable range of humidity.

An interesting coordination of fungous and plant growth was also given by the experiment. Plants in Chamber 2 were far larger than those grown in the other chambers, in fact were several inches taller than check plants grown in the greenhouse (see Fig. 20).

Fig. 20 - Photograph taken of plants grown in humidity chamber shown in Fig. 19.

Effect of temperature.

The apparatus used for temperature relation studies consisted of a metal box with a compartment at one end filled with a mixture of salt and ice. The other end had a compartment to which water was continually supplied and kept boiling by means of a Hot-point immersion electric heater. Between these two ends were seven compartments which gave temperatures ranging from 9°C to 47° C. Some daily variations occurred so readings were taken twice each day for thirty days, added and then averaged. The differential chamber was made to serve a three-fold purpose, i.e., to determine the relation of temperature to the growth of the fungus on the host plant; to the growth of the fungus in vitro; and to spore germination. Results are given in the following tables:

Table II.
Growth of fungus on host plant.

Plant	Temperature	Amount and character of growth of the fungus.
1	9° C	Growth poor. Spores produced but hyaline in color. Not typical.
2	20	Growth good, light colored, spore production good. Spread slow.
3	24	Growth excellent, typical, spread good, spore production profuse.
4	27	Growth good, conidiophores long but spore production scanty.
5	30	Growth poor, mostly superficial.
6	34	Growth negative. Host plant killed two weeks after start of experiment.
7	47	Host plant killed.

These data show the minimum temperature for fungous development on the plant to be about 9°C, the optimum 20 to 25° C. and the maximum 30° C. The optimum for fungous growth is again found to be closely correlated with the host plant's growth (see Fig. 21). These plants were inoculated as soon as the first true leaves had formed and were in the differential chamber for thirty-eight days or long enough for good development and growth of the fungus. Moisture was present on the glass top of the differential chamber at all times because of the differences in external and internal temperature. Therefore the experiment is considered to have been conducted in a constant, saturated atmosphere.

Table III.
Growth of Fungus in Vitro.

Tube.	Temp.	Amount and characteristic of growth.
1	9° C	Growth poor, spores produced but colorless.
2	20	Growth excellent, spore production profuse, typical.
3	24	Growth excellent, spore production profuse, typical
4	27	Growth fair, spore production average.
5	30	Growth poor, whitish, similar to #1 in quantity, few spores.
6	34	Growth negative.
7	47	Growth negative.

Fig. 31 - Photograph taken of plants grown in differential chamber at different temperatures, twenty-eight days after start of experiment.

Little need be said regarding the growth of the fungus in vitro except to note its correlation of growth with growth on the host plant, the optimum for both fungus and host again, as in the experiment on humidity, being the same.

Table IV.

Relation of Temperature to spore germination.

Moist chambers placed in differential chamber at 9 A.M. on August 15, 1917.

Chamber	Temp.	11 A.M.	1 P.M.	3 P.M.	8 P.M.	10 P.M.	8-16-17 8 P.M.
1	8°C	-	-	-	-	-	- *
2	18	-	+	+	+	+	+
3	24	-	+	+	+	+	+
4	30	-	-	-	-	-	- ‡
5	37	-	-	-	-	-	-
6	50	-	-	-	-	-	-

\+ indicates germination; - indicates no germination.

* Slight germination after four days.

‡ Germinated after being taken from chamber.

For the experiment on spore germination spore suspensions were made on cover glasses which were then inverted to serve as tops of Van Tieghem cells. This method supplies plenty of moisture to spores for germination and enables observation by means of the microscope.

Effect of light on spore germination.

To determine the effect of light on growing germ tubes, ten spore suspensions were made in Van Tieghem cells, five of which were placed in a box lined with black paper, five exposed to strong diffuse light. During the night the latter were exposed to the light coming from blue or "daylight" glass in a Bausch and Lomb microscope lamp. Temperature was practically constant at 78° F. Results given are average lengths of the longest germ tube which could be found in each culture.

Table V.

Time (started 10:45 A.M.)	Av. Length of germ tube. Light	Dark
12:45 P. M.	-	-
3:45 P. M.	10 microns	15 microns
4:45 P. M.	15-30 microns	25-30 microns
10 P. M.	50-60 microns	70-90 microns
3 P. M. Following day	100 microns	100 microns

This experiment was repeated on the following day with the examinations made at longer intervals. The following results were obtained:

Table VI.

Time (started 9 A. M.	Average length of germ tube	
	Light	Dark
7 P. M.	Average 25-30 microns Longest 40-50	Average 40-50 microns Longest 60-70
12 M. (next day)	Average 100	Average 100

Light seems to have a retarding effect upon the growing germ tube at first but the final length attained in the distilled water hanging drop suspension is practically the same. Both of these experiments appear to show that retarding influence of light on growth of the germ tubes. The fact that the ultimate growth is the same in light and darkness is doubtless due to the exhaustion of the food supply in the spores, so that the total possible growth is the same, being reached sooner in darkness than in light.

Relation of light to fungous growth.

Cultures on autoclaved corn grains and upon corn meal agar were incubated side by side in strong diffuse light and in darkness, at temperatures ranging from 20° to 26° C. A striking difference was noted in the two sets of corn

meal agar cultures a month after the experiment was started. (See Fig. 22). Little difference could be seen on the autoclaved corn cultures except a greater production of spores on the cultures grown in the dark and a darker colored mycelium.

Results of Experiment on Corn Meal Agar.

Dark	Light
Mycelium profuse, dark colored. Spore production very profuse. Purple color produced in medium deep and abundant. Medium all somewhat darkened.	Mycelial growth moderate. Light in color. Spore production scanty. Purple color of light shade and not abundant. Medium not darkened.

Light appears to be unfavorable to the best development of Cladosporium fulvum in culture, a fact which goes to substantiate observations made of the fungus development upon the host in the greenhouse. Tomato plants which were growing in the greenhouse during the spring were inoculated with the mold fungus in May and positive infection resulted in every case. Excessive spread and development did not take place, however, until the early part of July, at which time the greenhouse roof was painted white. After this time all new foliage became badly infected and no new fruit set. On the 8th of August a heavy rain washed the paint from the glass roof with the result that a

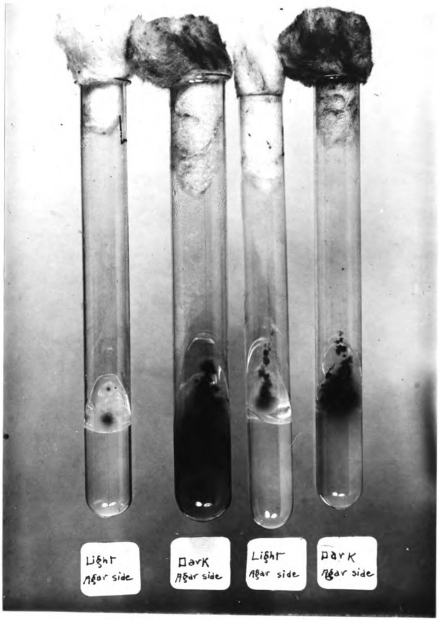

Fig. 22 - Corn meal agar tubes grown in light and dark. Note abundance of fungous growth and amount of purple color produced in tubes of agar grown in dark.

greater amount of light was admitted. New foliage produced by the plants after this date was not badly infected nor were the blossoms badly blasted altho the infected plants were still in the house and spores were present in profusion upon their leaves. Temperature and humidity undoubtedly play a part in preventing the spread of the fungus but from the results obtained in the above experiment, it seems that light also may exert a noticeable influence.

Growers almost unanimously report that severe attacks of the fungus follow cloudy or rainy weather.

Galloway (1888) quotes from the letter of a tomato grower as follows: "A few weeks ago, during a light fall of snow, which lasted about twenty-four hours, the fungus spread rapidly altho the houses were kept about as warm as usual".

At Lake Odessa, Michigan, the owner of a greenhouse which had badly infected plants stated that the fungus had been troublesome in the houses for four years and that its attack always became most severe after cloudy or rainy weather.

Cloudy weather was also blamed for a bad infection of greenhouse plants at West Raleigh, N. C. in the spring of 1915.

Since physiological studies dealing with humidity and light have shown both to be important factors governing the growth of the fungus, the above reports receive additional support. Dark, cloudy days not only decrease

the amount of light in the greenhouse but also tend to increase the amount of moisture present which furnishes ideal conditions for the development of the organism.

Effect of reaction of culture medium.

This experiment was run in duplicate, using different liquid synthetic media. One set of cultures was run on Coon's (1916) synthetic medium, the other was run on Duggar's (1917) liquid synthetic cornmeal medium. Ten tubes were inoculated in each set, ranging in reaction from -15 Fuller's scale to +30 .

Table VI.

Effect of reaction of culture medium.

Tube.	Reaction.	Coon's synthetic medium. Growth.	Duggar's synthetic medium. Growth.
1	-15 Fuller's scale	Poor	Poor
2	-10	Poor	Poor
3	-5	Poor	Poor
4	0	Poor	Poor
5	5	Fair	Fair
6	10	Good	Good
7	15	Good	Fair
8	20	Fair	Fair
9	25	Poor	Poor
10	30	Poor	Poor

These data show that the fungus prefers a reaction of medium ranging from +10 to +15 Fuller's scale, but can withstand a considerable range in the reaction. Little growth occurred in the two tubes at either extreme of the reaction.

Effect of air exclusion.

Growth in cultures sealed with paraffin was good at first but ceased in about ten days and no spores were produced. No color developed in the medium (see Fig. 23).

Translocation of starch.

In its attack on the tomato plant, the causal organism blasts blossoms which is an immediate and very apparent loss. Its influence on the plant and indirectly on the fruit, is less apparent but nevertheless serious and evidently due to two factors; the consumption of plant food by the fungus and the killing of the foliage which removes the plant's food producing apparatus. The close proximity of the fungous hyphae to the host's water tubes indicates that it is a contender for this source of food but its action on the plant food itself presents a different problem. It seemed possible that the organism might possess some power of withholding starch from the plant and to determine whether or not translocation of starch from infected leaves occurred, the following experiment was performed.

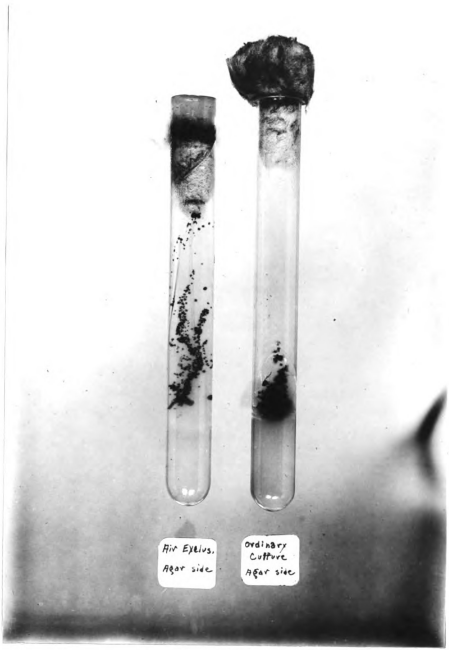

Fig. 23 - Effect of exclusion of air upon the growth of Cladosporium fulvum in vitro.

Circular sections were cut from healthy, also infected, leaves of plants which had been in the light for twelve hours. These sections were immediately killed by immersing them in hot 85% alcohol, after which they were placed in 70% alcohol to remain until the completion of the experiment. The plants were then placed in a dark room and sections cut from diseased and healthy leaves at the end of twelve, twenty-four and thirty-six hours. These sections, cut at different times, were put in small Esmarch dishes, set on a white sheet of paper and tested with an iodine solution containing one gram of potassium iodide, 100 c.c. of water and .3 gram of sublimed iodine. No difference could be seen between diseased and healthy leaf sections cut from the plants before they were placed in darkness but a remarkable difference occurred between those cut later. In sections cut twelve hours later the sections from the healthy leaf gave no starch test whatever; the diseased leaf section, however, gave a starch reaction nearly one-half as strong as the reaction on the leaf sections first cut. Subsequent diseased leaf cuttings all gave a positive starch test, indicating its presence in the leaf tissue after thirty-six hours. A microscopic examination of this leaf tissue showed the presence of dark blue starch grains in great numbers. Whether this failure of starch to be translocated is due to enzymatic action or to disorganization of the leaf tissue is not known but the former seems more probable as the leaves selected were not badly diseased.

DISSEMINATION OF THE FUNGUS.

Spores of Cladosporium fulvum are dislodged in a dust-like cloud when a badly infected plant is ever so slightly touched. Air currents or any mechanical disturbance are thus thought to cause them to leave the conidiophore and be distributed in all directions. That they are distributed in quantities is evident from the rapidity with which an infection spreads and the even growth of the fungus upon the plant leaf (see Fig. 3). An appearance resembling the natural infection on the leaf may be produced experimentally by placing a heavy spore suspension in a de Vilbiss atomizer and spraying the plant heavily. This gives infected areas, thickly and evenly spaced on the leaf, a characteristic of the early stages of infection on the naturally infected plant (see Fig. 24).

Air current dissemination of the spores was demonstrated experimentally with a number of diseased plants in a large moist chamber. Fifteen healthy plants were sprinkled with water and set upon boards in the top of the chamber, after which the door at one end was opened part way and an air current from an electric fan intermittently blown over the infected and healthy plants for five minutes. The healthy plants were then removed and placed in a closed moist chamber to await results. Within ten days every plant of the entire fifteen showed a heavy, typical infection.

Fig. 24 - Photograph of artificially inoculated tomato leaflet. Compare with Fig. 3.

It does not appear that the Cladosporium spore is "popped" or "shot" from the conidiophore, since with inoculations made on agar slants in tubes containing considerable condensation water, the fungus developed above the water only where inoculated, the water at the bottom of the slant remaining free from infection. Furthermore, where the spores are undisturbed, the chains of spores reach a considerable length.

PATHOGENICITY.

Artificial Inoculation of blossoms.

Inoculations of tomato flowers by placing fungous spores on the floral parts, either dry or in a drop of water, invariably gave positive results. The anther tube turns a dark brown color and has a water logged appearance; the style and stigma become blackened and infected petals become browned and shrunken. Fig. 35 shows two artifically inoculated flowers at the left numbered 1 and 2. The flowers at the right are of practically the same age but not inoculated. Fig. 36 shows the four flowers removed from the plant, the infected blossoms below having the anther tube split open and laid back to show the ovaries, which are browned and water logged in appearance. Note that ovary of the check blossoms above is not discolored but normal in appearance.

Fig. 25.- To the left are shown two artifically inoculated tomato blossoms which are withered and blackened. Two healthy check flowers are shown at right.

Fig. 26 - Flowers shown in Fig. 25 were removed from the plant and the floral parts dissected out to expose the ovaries and styles, which on the diseased blossoms are discolored and waterlogged in appearance.

Infected blossoms generally fall from the plant but may dry up and remain for a considerable time. A diseased flower may be easily differentiated from one which blasts because of unfavorable conditions such as failure to become fertilized, etc. In the latter case, the petals are light yellow in color and simply dry up. Neither do the anthers and ovary present the peculiar browned, water-logged appearance of the fungus infected flower.

Field Inoculations.

Barre (1910) says the disease in South Carolina is "common in some localities where tomatoes are grown in moist situations and on plants which are not in a thrifty condition but it is not usually considered very destructive. Where some of the plants are unhealthy and the fungus gets a start on these, it will frequently spread to the healthy plants and do considerable damage".

Dr. E. A. Bessey, of the Department of Botany at the Michigan Agricultural College, tells me that the fungus, during a very rainy period, was prevalent on field grown plants near Miami, Florida, about ten years ago. The fungus is also reported in the Plant Disease Survey Bulletin as causing 30% damage in a Florida field this year (1917).

The fungus is also reported by Halsted (1885) on plants grown outdoors in Iowa; in Ohio by Orton (1904) and in the South outdoors by Stevens and Hall (1913).

Eleven plants, each of a different variety, were artificially inoculated in the field with Cladosporium fulvum spores by means of a de Vilbiss atomizer on the evening of July 17, 1917. An unusual amount of hot, rainy weather occurred during the latter part of July and on the 27th abundant infection was observed. Infected leaves were later found to be entirely covered on the lower surface by Cladosporium spores but the fungus spread slowly and caused very little general injury.

The disease is seldom reported outdoors in Northern states and it seems doubtful that it will ever become a serious pest here. In the South, however, where atmospheric conditions are different, it seems to be a serious trouble and causes considerable loss.

Period of Incubation.

The incubation period varies with conditions, such as temperature, moisture and light. In weak, diffuse light, plenty of moisture and a temperature of 20 to 25 C. the fungus conidiophores may be seen microscopically on inoculated leaves after six days. At a lower temperature, it may be ten days or even longer before growth can be detected. In a dry atmosphere infection may take place and yellowing of leaf tissue occur but no conidiophores or outside growth can be detected. Thus an entire plant is sometimes found to

have numerous yellow, infected areas on its leaves with no spore formation whatever occurring. Plants artificially inoculated outdoors during a dry period were observed to show the above appearance and in such cases, killing of leaf tissue seems to be more rapid than where much outside growth occurs. This apparently is one reason why infections are confined to local areas on outside grown plants in the North.

In the greenhouse, however, almost ideal conditions of moisture, temperature and light are provided for the growth of the fungus, for its optimum requirements for growth very closely parallel those for the growth of the host plant (see Temperature and Humidity Experiments).

LIFE HISTORY OF THE ORGANISM.

Aside from the postulated possibility of the organism possessing a perfect stage in the Mycosphaerellaceae, there remain two possible methods by which it may exist and infect the tomato plants the succeeding year. Since all plants are removed from the greenhouse after the fruit is harvested, it hardly seems probable that spores are carried over to the following crop on the diseased plants but it is possible for spores to fall on side walls, window sash and other parts of the greenhouse, where they may remain until the following tomato crop is set. This possibility is supported by the fact that plants inoculated on April 21, 1917, with spores taken from infected leaves sent in from

a greenhouse at Redford, Michigan, on November 5, 1916, were infected with the fungus. The leaves from which the spores were taken had been pressed and dried thoroughly between blotting paper and kept in an envelope for nearly six months, yet the viability of the spores and their capacity for causing infection seemed unimpaired.

For the early spring crop of tomatoes, seed is planted about December 1st. Large growers in Michigan sometimes plant seed on successive dates to give a continuous supply of tomatoes during the season preceding the field grown crop, in which case the period between crops is shorter. The young plants are set in the benches about March, at which time they are good sized plants but since the seed bed is usually located in the greenhouse where the plants are finally set, it is entirely possible that infection occurs before transplanting time.

The tomato crop is generally harvested by the first of September which gives a period of seven months between crops if we assume that infection does not occur before transplanting time. Spore germination tests have shown some spores to be capable of germination even after a period of a year, which strongly indicates the ability to produce an infection after a seven months rest period. In some houses, two tomato crops are grown in the year, a fall and a spring

crop, so there is practically no gap between crops. Growers report the fall crop to be fully as badly diseased as the spring crop.

Another method by which the organism may be carried over is its ability to exist as a saprophyte. Its growth on culture media is easily obtained even on sterilized filter paper where it grows and produces spores. The organic material found in most greenhouses, along with the abundant moisture usually present, must furnish good conditions for saprophytic growth.

Greenhouse men say the fungus "just comes" anywhere from the middle of June up to the first of July on the spring crop. It undoubtedly is present long before this date but not on all plants. A few infected leaves could have enough spores produced to infect an entire greenhouse in a very short time, especially under certain atmospheric conditions and so the fungus attack apparently comes at a certain date.

IMMUNITY PHENOMENA.

Over twenty of the best and most commonly grown varieties of tomatoes were tested in triplicate for resistance or susceptibility of varieties but no case of varietal resistance was found. All apparently were susceptible to such a degree that serious injury resulted from the attack of the fungus. The varieties tested were the following:

Acme	Ponderosa
Beauty	Stone
Bonny Best	Early Jewel
Earliana	Chalks Early Jewel
Enormous	Potato Leaf var.
Livingstons Globe	Earliest of All
Grand Rapids Forcing	Comet
Trophy	Chalks Jewel
Perfection	Suttons Best of All
Chalks Early	Hummer

Cream City

Judging by the title of an article by J. Lind (1909), this investigator found a variety of tomato resistant to this disease but it has been impossible to obtain the article for consultation.

CONTROL MEASURES.

Spraying.

An effective control measure for this fungus is a mooted question among authorities who describe the disease and offer suggestions as to its control.

Galloway (1888) advised the use of sulphuret of potassium, one-half ounce of the salt to a gallon of water; Prillieux and Delacroix (1870) stated that "sulphuring experimented with in one greenhouse appears efficient and to arrest the spread of the disease.

Spraying with Bordeaux mixture, 3% copper and 3% lime has not seemed to modify the growth of the fungus sensibly". Bailey (1893) advised the use of ammoniacal copper carbonate solution to check the spread of the fungus but Rolfs (1898), Barre (1910), Stevens and Hall (1910) and Massee (1910) advised the use of Bordeaux mixture.

Lindan stated in Soraner (1908) that repeated sulfuring and use of Bordeaux have been recommended but so far no results seem to have been attained by either means.

Bourcart (1913) gives the following quotations in regard to the control of Cladosporium fulvum: "Mohr and Nijpels found that sulphur acts more effectively than copper salts to arrest this disease". "Mohr particularly advises the use of polysulphides against this disease which act in a more efficient manner than copper salts".

"Selby, Halsted, Nijpels and Earle point out the good effects of preventative treatment with bouillie bordelaise".

"Jenkins and Britton found that modified eau Celeste had no good effect, whilst bouillie bordelaise entirely removed this disease."

From such a mass of diverse opinions, it seems impossible to pick any one effective control remedy without trying all, but the prophylactic measures

advised by Stevens and Hall (1913) seem at least to be in harmony with the theory of disease control as well as the results arrived at in humidity experiments.

Resistance of the organism to fungicides.

Two experimental methods for testing the efficacy of fungicides were employed, one a test for spore germination and growth in the presence of the fungicide, the other a test of fungous infection on sprayed plants.

Germination and growth tests in moist chambers. For the first method Van Tieghem cells were employed. Chemically clean cover glasses were sprayed with each dilution of fungicide used and were then allowed to dry for at least six hours at room temperature. After drying, a very small drop of distilled water was placed on the center of each cover glass by means of a drawn glass capillary tube as it was necessary to have this moisture present to hold the spores to the glass. By means of a needle, spores of C. fulvum were introduced into this drop. The cover clip was then used as the top of a Van Tieghem cell. It is realised that with this method conditions may be somewhat different than found on a sprayed plant but they approximate the conditions under which the spores germinate and infect.

A second set of experiments was run, in which a small drop of the fungicide itself was placed in the center of the cover glasses in place of the drop of distilled water. The results of these experiments were identical with the one in which only distilled water was used and are given in table VII.

Formulae for strengths of Bordeaux mixture used: The Bordeaux mixture used in these experiments was made up from two stock solutions, one of which contained one gram of chemically pure Kahlbaum) CaO to 50 c.c. of water, the other contained one gram of chemically pure $CuSO_4$ to 50 c.c. of water. This strength of stock solutions is equivalent to solutions containing 8 lbs. of CaO, or $CuSO_4$ per 50 gallons of water, and if equal parts of each are mixed, will give a 4-4-50 Bordeaux mixture. (Doolittle, 1915). By varying the amount of each stock solution and adding the proper amount of water, Bordeaux mixture of different strengths may be obtained as follows:

6-4-50 Mixture.

Stock solution of $CuSO_4$	60 c. c.
" " " $Ca(OH)_2$	40 c. c. + 10 c.c. H_2O

4-4-50 Mixture.

Stock solution of $CuSO_4$	20 c. c.
" " " $Ca(OH)_2$	20 c. c.

4-3-50 Mixture.

Stock solution of $CuSO_4$ ..... 40 c. c.

" " " $Ca(OH)_2$ ..... 30 c .c. - 10 c.c. H_2O

3-3-50 Mixture.

Stock solution of $CuSO_4$ ..... 30 c.c. - 10 c.c. H_2O

" " " $Ca(OH)_2$..... 30 c.c. - 10 c.c. H_2O

2-2-50 Mixture.

Stock solution of $CuSO_4$ ..... 20 c.c. - 20 c.c. H_2O

" " " $Ca(OH)_2$ ..... 20 c.c. - 20 c.c. H_2O

Table VII.*

Germination experiments with Bordeaux mixture on cover slips.

Moist Chamber	Solution strength used and results.				
	6-4-50	4-4-50	4-3-50	3-3-50	2-2-50
1	+	+	+	+	+
2	+	+	+	+	+
3	-	+	+	+	+
4	+	+	+	+	+
5	+	-	+	+	+
6	+	+	+	+	+
7	+	+	+	+	+
8	+	+	+	+	+
9	+	+	+	+	+
10 Check	+	+	+	+	+

* Germination and growth indicated by a positive (+), or a negative (-), sign for each cell used.

The above experiments demonstrate that copper in Bordeaux mixture solution has little appreciable effect in Van Tieghem cells upon either germination of Cladosporium fulvum spores or upon the growth of the germ tube since that growth appeared normal when compared with the checks and was fully as long. The above experiment was repeated with Bordeaux mixture made from ordinary commercial $CuSO_4$ and CaO but results were practically the same.

The following five experiments were performed by the second method of using fungicide droplet on the cover glass.

Ammoniacal copper carbonate: Formula used was 1 gm. $CuCO_3$, 8 c.c. ammonia and 1600 c.c. of water. $CuCO_3$ was first dissolved in the ammonia and then added to the water. This is equivalent to 5 oz. $CuCO_3$, 3 pints ammonia and 50 gallons water (the usual formula).

Ten moist chambers were used in this test, one as a check and positive germination took place in all, with the production of normal appearing germ tubes.

Potassium sulfid: This has been recommended as a controlling spray for this fungus, to be used at the rate of one-half ounce to the gallon of water. It proved insefficient in moist chamber tests and did not inhibit in the least the germination of spores nor prevent germ tube production.

Formaldehyde: Two tablespoonfuls (1 oz. approximately) of a 40% solution of formaldehyde in two and one-half gallons of water prevented all spore germination entirely.

Commercial Lime-sulfur: Commercial lime-sulfur testing 33° Baume' and diluted 1-40 did not inhibit germination but compared with checks the germ tubes appeared abnormal. Some were greatly swollen and short; many presented a "twisted" appearance and some were greatly enlarged and swollen at the terminal end which presented a knob-like appearance.

Self-boiled lime-sulphur solution: For this solution 3.2 oz. CaO, and 3.2 oz. of flowers of sulfur were used to one gallon of water which is equivalent to a 10-10-50 solution strength. Except the check, germination was in every case negative. Ten spore suspensions were made here, as in the case of all tests conducted and the experiment was repeated with the same results.

Infection on sprayed and dusted plants. A de Vilbiss atomizer was used to apply the fungicides listed in the following table and the plants were covered thoroughly by the spray after which they were permitted to dry for six hours. A heavy spore suspension was then atomized over the entire plant by means of a clean de Vilbiss atomizer and the plants set immediately into a large moist chamber. In the case of the formaldehyde solution, the spores were first applied , permitted to dry upon the plant and the spray then applied.

Five sprayed plants and two checks were used for each fungicide test, the results of which were taken after a fifteen day incubation period and were as follows:

Table VIII.
Experiments with sprayed and dusted plants.

Fungicide	Infection on inoc. plants	Infection on sprayed plants					Infection on check plants Check
	No spray but inoculated	1	2	3	4	5	No treatment or inoc.
Bordeaux 6-4-50	Heavy	Heavy	Heavy	Heavy	Heavy	Heavy	Negative
4-4-50	"	"	"	"	"	"	"
4-3-50	"	Moderate	"	"	Neg.	"	"
3-3-50	"	Heavy	"	"	Heavy	"	"
2-2-50	"	Light	Neg.	"	"	"	"
Formaldehyde	Heavy	Heavy	Heavy	Heavy	Heavy	Heavy	Negative
Commercial Lime-sulfur	Heavy	Neg.	One Spot	Two Spots	Neg.	Four Spots	Negative
Self-boiled Lime-sulfur	Heavy	Two Spots	Three Spots	Neg.	Neg.	One Spot	Negative
Sulfur * Dust	Heavy	Heavy	Heavy	Heavy	Heavy	Heavy	Negative

* Sulfur dust fungicide put out by Niagara Spray Co., and containing 65% sulfur, 15% arsenate of lead.

Contrary to indications from moist chamber tests, self-boiled lime-sulfur did not control the fungus on the sprayed plants but its action is decidedly better than Bordeaux mixture. General infection resulted on plants sprayed with the latter, in most cases the entire leaf area was infected. The formaldehyde spray appeared just as ineffective and sulfur dust proved valueless.

Plants sprayed with commercial lime sulfur were lightly infected and it seems that this fungicide may prove to be a valuable spray to use in combating the fungus as it is easier to obtain and does not leave a heavy deposit of material upon the leaf.

None of the fungicides used above appeared to have injurious effects upon the plants but there is a possibility that self-boiled lime-sulfur, if used often, would so encrust the leaf that the chlorophyll apparatus of the plant might be seriously interfered with.

Fumigation.

The writer has been frequently informed of tomato growers whose houses have never been troubled with Cladosporium fulvum on the vines and has himself seen houses which have grown tomatoes for years but which are entirely free from this fungus. This fact suggested the possibility that a greenhouse might be fumigated between crops and thus freed of the disease. Formaldehyde

gas and sulfur fumes are often used in fumigation because of their fungicidal nature but it should be remembered that both are fatal to plant life and must be used at a time when the hothouse is empty.

Formaldehyde gas.

Morse (1907) recommended the use of 23 ounces of potassium permanganate with three pints of 40% formaldehyde per 1000 cubic feet for treating diseased seed potatoes. The potassium permanganate is spread evenly over the bottom of a large shallow pan and the formaldehyde poured over it to liberate formaldehyde gas. In the experiment, amounts of potassium permanganate and formaldehyde equivalent to the above were used in a large culture room which was found, by measurement to have a capacity of 147 cubic feet. Diseased plants with both dead and living infected leaves were set on the floor of this room, on shelves half-way to the ceiling and fastened near the ceiling.

After twenty-four hours in the fumigation chamber, hanging drop spore suspensions of spores were made in Van Tieghem cells to note germination which occurred in about half of the cells. The germ tubes, however, were abnormal in appearance when compared with those in check cells. The germ tubes remained short, swollen and often irregular in outline. It is not known whether these spores were capable of producing infection on tomato leaves or not, as the fumigation was done so

near the end of the experiment that time was not available in which to make the test.

Sulfur fumes.

Rosenau (1913) advocates the use of five pounds of sulfur per 1000 cubic feet of air space for disinfection purposes. The sulfur is placed in an iron container which is set in a pan of water to provide moisture necessary (1/5 pound per pound of sulfur used), for the effectiveness of the gas produced. Ignition of the sulfur is accomplished easily by making a little crater in the sulfur into which is poured some alcohol. This is lighted and it ignites the sulfur.

Plants were placed in the same culture room which was used for the above experiment and in approximately the same position, after which the pot of sulfur was set on a box about three feet from the floor (to prevent smothering of the flame) and lighted. Three-fourths of a pound of flowers of fulfur was used and the room was not opened for twenty-four hours. Some sulfur in the bottom of the pot was found unburned at the end of the experiment, therefore, the amount used was somewhat smaller in quantity than recommended.

Van Tieghem cells were again employed to determine the effect of the sulfur fumes upon spore germination and twelve spore suspensions were made on the cover slips which formed the moist chambers. Each spore suspension

was composed of spores taken from a leaf in a different part of the room and from both dried and green leaves. By this method it was hoped to get the result of the action of the gas in the top and bottom of the room and upon both the mature dry spores and young spores with a greater moisture content. Infected leaves were numbered when spores were taken from them, each number corresponding to a Van Tieghem cell.

After eight hours, the Van Tieghem cells were examined. All check cells containing spores from untreated leaves were found to contain germinating spores with well developed germ tubes. Not a Cladosporium spore had germinated in any one of twelve spore suspensions made from leaves which were exposed to the action of the sulfur fumes. Cladosporium fulvum spores appear to be especially susceptible to the action of fungicides containing sulfur, as spores belonging to some species of Alternaria or Macrosporium were often observed to have germinated in the cells containing the spores exposed to the sulfur fumes. The same occurrence was also noticed in the Van Tieghem cells used to conduct the experiment with self-boiled lime-sulfur. Here again it was found that large spores of a Macrosporium type had germinated while all Cladosporium spores had failed to do so.

Sulfur fumigation as given above, offers the best method, available to the grower of greenhouse tomatoes, for ridding the houses of the fungous pest causing

tomato leaf mold. This method is not expensive nor difficult to apply and it appears that a house once rid of the disease may not be infected for a considerable time, especially if the location of the greenhouse is not near other infected plants. In the latter case, it often happens that tomato growing houses in cities are located comparatively near each other. This increases the possibility of infection. Since the spores are air borne and distributed in great numbers, the greater the distance between infected and uninfected plants, the less likely the possibility of infection on the healthy plants.

Additional Prophylactic Measures.

In addition to the use of sulfur fumigation and spraying infected plants with a dilute commercial lime-sulfur solution or with self-boiled lime-sulfur, clean cultivation and control of ventilation are essential.

Temperature control seems to be out of the question as the temperature best suited to the tomato plant growth is also optimum for the growth of the fungus but by proper control of ventilation, the grower may prevent excessive conditions of humidity and so control the spread of the disease to a certain extent.

Clean cultivation, or the thorough removal of all diseased material and infected plants from the house at the end of the tomato season is also essential to eradication of the pest.

SUMMARY.

Tomato Leaf Mold (Cladosporium fulvum), a serious disease of tomato foliage, was first described by Cooke (1883) from North Carolina. The fungus is widely distributed over the United States and foreign countries, occurring as a bad pest on tomato plants grown in the open in Southern climates and on plants grown under glass in Northern latitudes.

Blasting of blossoms, killing of vines and undersized fruit result from the attack of the mold and cause losses of 20 to 30 percent of the total crop.

The fungus may be recognized by the velvety, tawny-olive colored patches of growth which it produces on the under side of the host plant's leaves and by the yellow spots produced in the leaf tissue above. Purple patches of fungous growth may also be found scattered over a diseased leaf after killing of tissue begins. This fungus is easily distinguished from Cladosporium herbarum, which is a saprophyte and produces a black colored fungus growth, usually upon the upper surface of the leaf and does not enter healthy tomato leaf tissue.

Fruit once set escapes the disease and main stems of the vines are not often attacked. Blossoms are especially susceptible.

Cladosporium fulvum was isolated and grown in pure culture. Typical infections followed the inoculation of tomato leaves from pure culture and the fungus was re-isolated from those infected leaves. A peculiarity of the fungus when grown in pure culture is the formation of a purple color in certain media.

Infection is stomatal. The mycelium is both inter- and intra- cellular and is found in greatest abundance around the tracheary tissue. Conidiophores arise from a stroma-like formation through the stomata.

Moisture favors growth.

Minimum temperature for growth of the fungus is below 9 C. The optimum temperature for growth is 30 to 35 C and the maximum is below 34 C.

Strong, diffuse light is detrimental to spore and color formation. Darkness or cloudy weather favor spore production and spread of the fungus.

The organism prefers a reaction of medium varying from +10° to +15° Fuller's scale, but withstands a considerable range in the reaction.

Translocation of starch in infected plant leaves is interfered with.

The organism is disseminated by air currents and draughts. The conidia apparently are not "shot" from their attachment to the conidiophore.

Field inoculations were entirely successful but the fungus does not spread badly under conditions in the North.

The inoculation period is short, varying usually from six to ten days but may require a longer time under conditions of low humidity or temperature.

Growth as a saprophyte may enable the fungus to exist between crops but the longevity of the conidia probably accounts for its survival.

Bordeaux mixture has been proved beyond question to be insfficient in the control of Leaf Mold. Ammoniacal copper carbonate, sulfide of potassium and sulfur dusting seem also valueless.

Sprays containing sulfur, such as self-boiled lime-sulfur and concentrated lime-sulfur appear to check the fungous growth but the data obtained are too meager to make an accurate estimate of their value. Commercial lime-sulfur appeared to prevent plant infection more effectively than self-boiled lime-sulfur, although it was less effective in moist chamber experiments.

Fumigation with Formaldehyde gas as a prophylactic measure apparently has an injurious effect upon spore germination but the quantity necessary to be used for effectiveness makes the process expensive.

Sulfur fumigation, as conducted experimentally, is successful as a means of killing the spores of Cladosporium fulvum on trash and leaves and so provides a means of eradicating or at least lessening the sources of infection in the greenhouse.

Ventilation control and clean culture methods are to be recommended as prophylactic measures.

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PLATE I.

A. - Cross section of an infected tomato leaflet, showing the inter- and intra-cellular mycelium, also conidiophores emerging through the stoma from the stroma-like structure of fungous tissue.

B. - Conidia, highly magnified.

C. - Conidiophore with conidia attached, highly magnified.

PLATE I

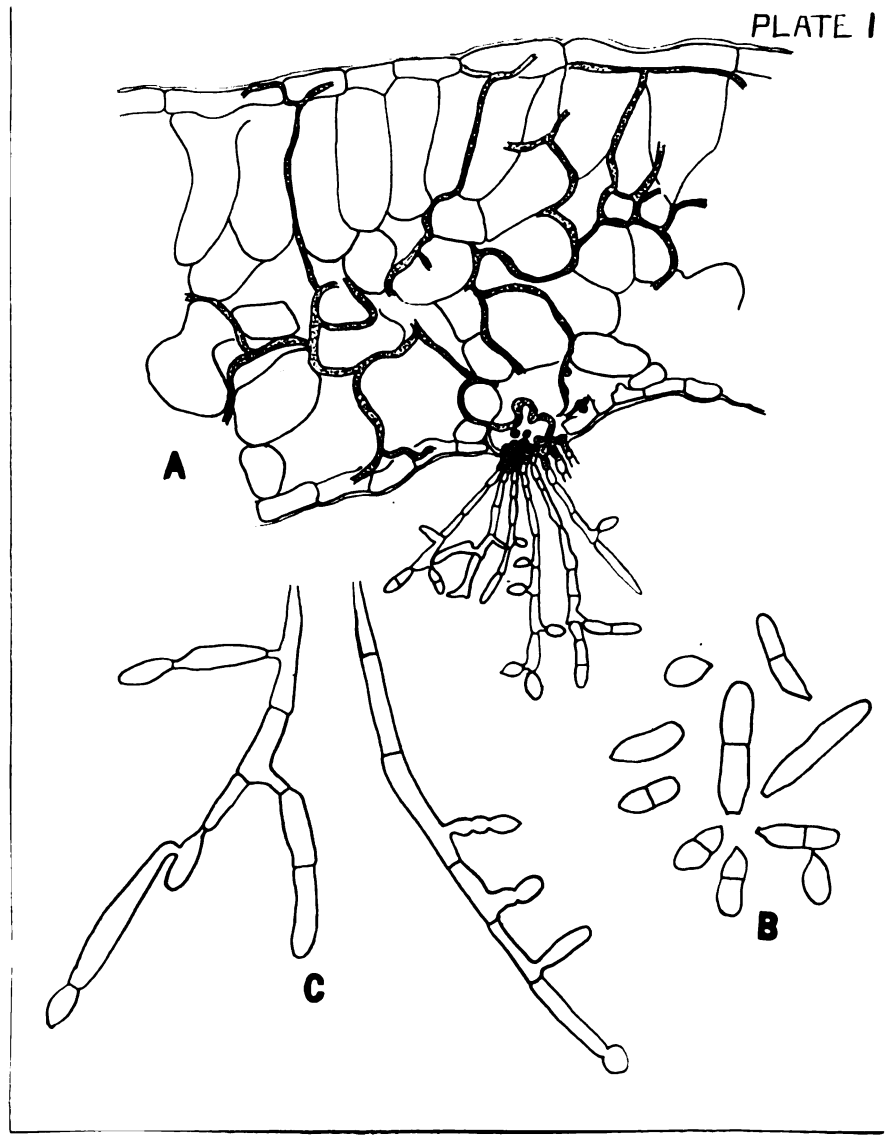

PLATE II.

A. - Conidia in various stages of germination.

B. - Typical Cladosporium fulvum mycelium from ten day old moist chamber culture.

C. - Spore which was kept dry for a year and then germinated in a moist chamber and produced a peculiar, many septate germ tube. Some cells appear similar to secondary spores.

PLATE II