A STUDY OF THE CELERY EARLY BLIGHT FUNGUS, CERCOSPORA APII FRES.

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A Study of the Celery Early Blight Fungus, *Cercospora apii*, Fres.

INTRODUCTION.

The importance of celery as a truck crop in Michigan, Florida, New York, California, Colorado, New Jersey, and several other states is well recognized. The peat lands of Orange County, California were set to over three thousand acres of celery in 1910[70]. Throughout this country the words "Kalamazoo" and "celery" have been closely associated for the past twenty-five or thirty years. Ottawa, Kalamazoo, Kent, and Muskegon are the counties in which the industry is most developed in this state. Considerable celery is also grown in truck gardens near Bay City, Detroit, Newberry, and in smaller quantities by truck growers throughout the state. The possibility of the further development of the industry is indicated by the fact that there are in Michigan alone some three million or more acres of adaptable muck and other lands. The obtaining of seed from many sources, domestic and foreign, in which the natural enemies of the plant have long been known, together with the long continued raising of celery year after year without rotation, have led to the establishment in this state of several serious fungous diseases to which celery is subject. Among these is the disease known as Early Blight, the importance of which, in hot seasons, makes desirable its further investigation.

Name of Disease.

The disease has been called by several common names among which are "celery rust"[36], "celery blight"[1,36,54], "celery leaf blight"[4,78], "sun blight of celery"[15], "early blight of celery"[19,20], "celery leaf spot"[24], and "celery spot mould"[11]. The name "rust" has long been discarded due to the fact that at least two true rust fungi have been recorded as attacking celery[79]. The word "spot" is not characteristically descriptive because of the spotting of celery leaves caused by several other fungi. The disease would best be popularly known as the "Early Blight of Celery" because of its early appearance during the growth period of the plant, that is, during the hot months of July and August. It should never be designated as simply "blight" due to the easy confusion with another destructive celery disease known as "Late Blight" which appears the later, cooler part of the growing season[12,70,9,10].

History and Distribution.

The first report of the disease in the United States which the writer has been able to discover was made in 1881 by Galloway[30], in which he has said that practically all of a lot of 10,000 plants were destroyed. At this time he also observed that shaded plants escaped destruction. In his 1888 report he stated that his observations were that "the disease occasions greatest injury in sections where

the summers are long, hot, and dry", and that the disease was unknown where the soil remained cool and moist. He recommended shading with lath screens and destroying the diseased material in the autumn. Ellis and Everhart (1885) described the fungus from specimens collected in Michigan by Dr. Beal[22]. Scribner in his 1886 report described the disease and its causal organism and stated that no satisfactory fungicide had as yet been found[78]. Brillieux and Delacroix (1890) reported the disease as occurring on celery in the experimental garden of the Agronomy Institute at Joinville le Pont, France. They stated that the fungus had already been reported on various other Umbellifers, particularly parsley. The appearance and cause of the disease were described, as were the fungus spores. Note was made of the fact that as the weather becomes cool the disease becomes less virulent. The authors called attention to the method of attachment of the spores by the larger end, correcting the figure of Scribner which pictured the spores attached by the small ends[67]. Halsted (1891) found that the "fungus spores germinated with remarkable rapidity in water and in celery extract". Copper filings placed in a drop of water with the spores inhibited germination, whereas metallic zinc was apparently non-toxic, not preventing the formation of germ tubes. In a small spray experiment, in which he used ammoniacal copper carbonate, he obtained almost complete control of the disease,

the amount of edible product from the sprayed celery being twice that from the unsprayed plants[36,47].

McCarthy (1892) reiterated Galloway's observations on the relation of Early Blight to soil, shade and moisture and stated that celery cannot be successfully grown on upland soils without irrigation. In his rules for combatting the disease he advised making the seed bed in the shade, planting some shading crop as Russian sunflower in the field, using liver of sulfur spray or sulfur dust throughout the growing season, and carefully destroying all diseased leaves. Galloway (1892) as a result of a number of experiments found that proper cultural methods more readily prevented leaf blight than any or several of the various fungicides used. He did not, in this report, state which fungicides were employed, but judging from his 1888 report they were lime and sulfur, Bordeaux mixture, liver of sulfur, ammoniacal copper carbonate, sodium hypochlorate, and sulfur flour. He emphasized an abundance of water, good drainage, and a heavy application of well-rotted manure[31]. Sturgis (1892, 1893, 1897) conducted control experiments in which he found sulfur dust superior to Bordeaux mixture, liver of sulfur, ammoniacal copper carbonate and dilute solutions of copper sulfate[82,83,84,85]. Davis of the Michigan station (1893) published Galloway's notes on the "Celery Leaf Blight" which stated that complete control was realized

By flooding the ground sufficiently often to keep it always soaked, whereas the plants which received only the water falling naturally as rain blighted badly[15].

Rolfs (1896-1898) found that crowded, poorly nourished plants succumbed very readily to the disease and that the blight spread with alarming rapidity during warm, moist, foggy weather. As sprinkling the plants wets the foliage and supplies the moisture necessary for spore germination he advised flooding and trench irrigation. Liver of sulfur (1 oz. to 2 gal. water) applied twice a week, thoroughly wetting the plants, gave almost complete control, as did freshly prepared Bordeaux mixture applied at 10-day or two-week intervals[71,72]. Duggar (1897) grew the fungus in pure culture, using as media nutrient agar, celery petioles and bean stems[19,20]. Lochhead (1900) found that intense heat accompanied by drought favored the disease. He, as had Galloway, observed that celery on shaded, low, wet ground escaped. Ammoniacal copper carbonate (6 oz. $CuCO_3$ to 1 qt. ammonia water and 45 gal. water) sprayed on at two week intervals was recommended[54]. Hume (1899,1900) by placing celery leaves in moist chamber, sowing the leaf surfaces with conidia, and then after 48 hours stripping off portions of the epidermis and examining under the microscope, demonstrated the entrance of the parasite through the stomata. He recommended 4-4-40 Bordeaux mixture applied twice weekly the first part of the season and later

at one week intervals. He preferred this to liver of sulfur because of Bordeaux being the cheaper; he found Bordeaux to have slightly greater fungicidal value than ammoniacal copper carbonate (39).

Other contributions which do not add particularly to the above, but which should be mentioned, are the following:

Tracey (1884, 1885, 1886) noted "considerable damage" to the celery crop at Columbia, Missouri(78). Chester (1891) told of his mistaking septoria blight lesions for those of cercospora (9,10). Humphrey (1891)(40) and Lindau (1908)(53) believed that septoria and cercospora blights of celery genetically connected. Atkinson (1892) gave a good drawing and description of the fungus and corrected the error of Scribner's figure(1,2). Briosi and Cavara (1895 p.57) accurately described the disease and its causal organism(7). Noack (1898) reported the presence of the disease in Brazil(61). Kellerman (1904) also errored in picturing the spores as attached by their small ends to the conidiophores(48). Freeman (1905) very briefly described the effect of the fungus on its host, saying that it was particularly effective against young plants. He recommended Bordeaux mixture (27). Cooke (1906) recorded the fungus under the common name of "celery spot mould", stated its injurious extent in Germany, Austria and the United States, and that it had not yet developed as

a pest in England(11). Klebahn (1906) mentioned the presence of celery Early Blight in various localities of Germany(50). Townsend (1907) pointed out the seriousness of "celery blight or rust" and claimed complete control by the use of either Bordeaux mixture or ammoniacal copper carbonate(88). Massee (1903,1910) described the disease under the heading "Celery and Parsnip Leaf Blight" and states that spores live through the winter, being capable of infecting a crop the following season and inferred that rain is the active agent of dissemination (56,57). Eriksson (1913) after a discussion of the leaf spot of sugar beets mentioned that similar spotting is caused by Cercospora apii on carrots, parsnips, and other Umbellifers,(24). Stevens (1913) described the diseased host and fungus(80). Ferraris (1915) also described the parasite and morbid host and advised the burning of the diseased leaves(25). Taubenhaus (1918) repeated a description and stated that the "White Plume variety seemed to be resistant"(87). Krout (1919) compared the two celery blights and recommended spraying with Bordeaux mixture for both,(51,52).

From the above contributions coming as they do from England, Germany, Austria, France, Italy, Siberia, Canada, South America, and many states of this country, it is reasonable to infer that the disease Celery Early Blight has been present at some time, in mild or serious form, wherever

celery is grown extensively. It is nearly always possible to find few to many Early Blight spots on celery shipped into Michigan from Florida and California. This generalization is corroborated by the following report.

Economic Importance.

In April 1921, at the request of Dr. G. H. Coons of this station, Dr. G. R. Lyman of the Bureau of Plant Industry, Plant Disease Survey, kindly compiled data as to the occurrence of and losses from the Early Blight of celery. This report, a brief of which is here given, embraces thirty-two different states.

California reported "heavy losses and disastrous presence" in 1907 and 1908, "general occurrence but no damage in 1910", "severe (10% loss) in 1919" and "slight (2% loss) in 1920". Robbins of Colorado stated that losses from Early Blight were "very slight" in 1917 and 1918. Clinton (1913) reported the disease as "common and bad" in certain fields in the state of Connecticut, 2% loss from Early and Late Blights in 1917, common but not very serious for the years 1918, 1919 and 1920. Jackson, Elliott and Manns noted presence in Delaware from 1905 to 1919 but stated that it was not severe. From Florida comes many and important reports: Rolfs reported 20% injury in 1905 and 80% in 1906; Winters noted some damage in 1908 and 1910. H. E. Stevens (1912) recorded the disease as "very bad",

10% to 50% of the crop having been attacked, as "rather severe" in 1913, as "common" in 1914 and 1915, "less prevalent" in 1916, that in 1917 the injury was up to 100% locally. In 1918 Hesler noted whole fields "brown as a result of the blight". In 1919 Link and Jagger observed the effect of a "deluge of Early Blight upon celery", stating that the plants as a result are "short, small, and of much reduced market value". The economic importance of the disease in the state of Florida is very evident from these notations.

Dean of Georgia reported "complete destruction" of certain celery crops in Stewart County in 1904 and 1905; that it was negligible in 1906. Temple of the Idaho station recorded it as very prevalent in 1913, the injury being 1 to 15%. Barrett (1907) stated the disease as having been very bad in one Illinois locality and (1911) as having done 75% injury to the crop in Champaign County. In 1913 the presence of the pest was reported in Elkhart County, Indiana. Jackson of the same state reported 25% injury in Marshall County (1916) and as common in Elkhart, Tippecanoe and Madison Counties in 1917. Gardner noted 10% injury to Marion and Elkhart Counties' crop in 1919 and similar loss in 1920. Kentucky reported the disease as very prevalent about Lexington, injury being 10%. Edgerton of Louisiana recorded the presence of Early Blight from 1908 to 1917, reporting losses and injury ranging from "slight" to "con-

siderable". Morse (1908) noted its presence in Androscoggin County, Maine and Shapovalov recorded a "bad outbreak" in Penobscot County. The reports from Maryland (1903-1917) indicate but slight loss, although severe in some localities. Massachusetts reported considerable loss in 1906, as "present but not serious" 1907 to 1912, as "doing large injury" in 1915, and that the losses ranged (1917-1920) from very slight to 4%. (Davis of the Michigan station (1893) stated that "it took nearly the whole crop at Kalamazoo several years ago".)* The same station reported the common presence of the disease (1903-1918) and stated (1914) that the "damage done was less than from Septoria". Minnesota (1908-1918) reported very little injury by Cercospora and Septoria. Reed of Missouri recorded the disease as being very serious in St. Louis County in 1911. Nebraska noted its presence in Lancaster, Gage, and Buffalo Counties (1905-1917). The New Hampshire station reported 20% injury in 1906, 5% in 1908, "not seen" (1909), 15% injury in 1910, and "practically none" in 1911. New Jersey noted the prevalence of Early Blight (1906-1909), recorded as serious in South Jersey in 1912, as "common" and "abundant" in 1919 and 1920.

Jagger of the New York station stated that "losses from celery blights were at least 5 to 10% in 1917. Other reports of losses from this state range from "no reduction in yield"

*Not in Pathologist's Report.

to "common but not severe". Stevens and Fulton of North Carolina noted its presence (1904-1916), recording "much injury" to crop in 1913. North Dakota recorded Early Blight as "occurring" in 1906, "rare" in 1909, "common" in 1916, and "uncommon" in 1917. Ohio in 1905 reported 10% injury due to the Cercospora blight, 5% to 10% in 1906 due to both blights, as negligible from 1907 to 1911, both blights as causing "considerable injury" (1912) and as "less" (1913-1920). From Roseburg, Oregon, N.D. McCall wrote that the celery disease due to Cercospora apii caused widespread injury in 1908. Pennsylvania reported less than 5% injury in 1903, as "slight" (1906-1912), as "present" and causing but "slight loss" 1915-1919, and as very bad, "causing 50% loss in some fields" in 1920. Stene of Rhode Island reported 5% loss in 1903 and quite common in 1907. The presence of the disease is noted as common in South Carolina for 1909-1919, causing 5% loss in 1917. Tennessee noted the presence of Early Blight in 1915. Utah reported (1918) "no measurable loss". Lutman of Vermont (1915-1920) recorded the disease as being "very common but not serious" and as causing injury ranging from 5 to 75% and losses from "negligible" to 5%. Virginia (1911-1916) reported it as "not common" and causing not more than 2% injury. West Virginia wrote that it had occurred in small amounts only. Jones of Wisconsin recorded it as being "bad in some localities and

entirely absent in others" in 1910, and that it caused a 90% loss to some Milwaukee celeriac growers in 1909.

These reports, particularly the one from Florida, prove that the Celery Early Blight disease is of economic importance. It is evident that at times the fungus can cause slight or serious injury to its host wherever celery is grown. Given an abundance of plant food and plenty of rain celery makes a good vigorous growth in spite of much Early Blight spotting. Under these conditions the principal injury is due to the disfiguration of the leaves which necessitates excessive trimming in the preparation of the plant for market. The spotted leaves, moreover, dry out much more rapidly than sound ones, thus causing greater deterioration in shipment. Under less favorable conditions the Early Blight disease supplements the other factors in rendering the crop of little or no market value.

Hosts.

Although in the above citations there are frequent references which indicate that the same specific fungus is by many investigators considered responsible for the production of similar diseases in other umbelliferous hosts, there are no records of the results of work by which cross-infection was demonstrated. Thus far, I have been able to secure infection by Cercospora apii Fres. on only two hosts, namely celery (Apium graveolens L.) and Celeriac (Apium graveolens L.

var. rapaceum D.C.). Repeated inoculations with pure cultures of this fungus and long continued exposure to very badly blighted celery of carrot,(Daucus carota L.), parsnip (Pastinaca sativa L.), parsley (Petroselinum hortense Hoffm.), dill (Anethum graveolens L.), carroway (Carum carvi L.), Pimpinella (Pimpinella saxifragia L.), coriander (Coriandrum sativum L.), and fennel (Foeniculum vulgare Hill.) gave negative results. Likewise I have been unable thus far to secure infection on celery using the fungus isolated from carrot. It thus appears that the Cercospora apii Fres. of celery is a strain or variety distinct from those forms causing similar spotting on other related hosts.

Signs of the Disease.

General.

The common term "blight" at once brings to mind the picture of an afflicted plant, one whose outer foliage at least has the appearance of being scorched as if by the hot sun. In fact, this is the sight presented by celery plants under the worst conditions of Early Blight (Plate III). With the coming of droughty weather, diseased plants appear to wilt and droop more readily than healthy ones. The older stems soon fall and, coming in contact with the moist earth, become rapidly filled with the fungous threads which spread throughout the tissue in part saprophytically. These fallen

leaves and stalks are virtually so much living fungus and this material soon becomes so thoroughly covered by the large numbers of fruiting structures as to have a dark grayish, velvety appearance. It may be said here that these fallen stems and leaves are a very important source of further infection of the crop.

On the Leaves.

The first macroscopic indication of the fungus within the leaf is revealed by the presence of from few to many small slightly yellowish or pale greenish spots a millimeter or more in diameter. These may be noted in from 5 to 8 days after inoculation, when conditions best favor the fungus. These small spots enlarge and coalesce into a rather indefinite yellow spot, the size of which depends upon the number of initial entrances of germ tubes, the time elapsed since infection, the climatic conditions occurring during the growth of the fungus within the leaf tissue, and the condition of the host plant itself. Under the conditions described as best for the fungus, lesions have been found to attain the diameter of from one to two centimeters within ten days after inoculation. These spots are in general approximately elliptical in shape, and cause a wrinkling or slight folding of the healthy tissue immediately surrounding (Plate I, II.).

The border of the lesion is frequently, but not always, slightly raised, particularly where the edge of the involvment meets the larger veins of the leaves, and at times there is a yellowing of the tissue extending two millimeters or more

beyond the border of the brown spot. More spots are found beginning at the leaf margins than in from the edges. As the nutriment of the affected area is exhausted the color of the spot changes, progressively, to a pale brown, a slightly reddish brown, and ultimately to a dull slate or ashen gray as the fruiting structures appear. As indicated under general signs of the disease, the presence of high temperature and moisture and a cutting short of the food supply are instrumental in bringing about abundant fructification of the fungus. Conidiophores under these conditions are capable of producing a crop of spores in from 6 to 10 hours. In one experiment, at 12:00 P.M. a portion of a fruiting surface was found to have conidiophores which bore no spores, but when examined at 6:00 P.M. the following day was found to have produced an entire new crop of typical conidia. This was observed microscopically by using the procedure described later under the heading "Dissemination".

On the Petioles.

Mention has been made of the presence of the fungus on the fallen stems. Occasionally the active, living petioles are attacked, elliptical spots having a water-soaked appearance being produced. The greatest diameter of the ellipse extends in the direction of the length of the stem. The stems frequently break over at these points of infection leaving the foliage beyond to droop and die (Plate III).

On the Fruit.

I have not, as yet, had the opportunity to examine celery flowers for the presence of the fungus. Repeated examination with a hand lens and with the microscope of the seeds kept in stock at this laboratory has failed to reveal the presence of the Early Blight organism or any of its associated effects. Examination was made microscopically of the sediment obtained by centrifuging water in which celery seeds were suspended. What may have been conidiophores of the parasite were seen on several occasions, but the parts bearing the characteristic scars were missing. Because of the wide-spread occurrence of the disease and because of the probable specific nature of the causal organism it is reasonable to believe the parasite is seed-borne. Undoubtedly bits of blighted material containing viable fungus find their way, in the course of seed collection, into stocks of seeds and are thus distributed. Further search should be made using material from other sources.

Etiology.

Proof of Pathogenicity.

Single spore cultures of the organism (Plate XI), were obtained by the use of the ordinary poured plate procedure, spores being obtained at times from diseased leaves and at other times from artificial cultures. The petri dish was inverted and by means of the low power of the microscope single isolated spores of the fungus were located and marked by a

small ring of ink. These were transferred immediately to a cornmeal agar slant and others allowed to grow for two to four days and then transferred. The cultures thus obtained were used in many subsequent successful infection experiments. A typical infection experiment is here described.

Celery trash that had overwintered near the end of the Botany Building was on April 15th, 1920, shaken over two large healthy plants in the rain chamber, some of the refuse being left in contact with the sound leaves. Seven days after (April 22nd) spots were noted on the leaves (Plate II). These gradually assumed the pale brownish color of typical Cercospora lesions and by April 28th had attained an ashen gray appearance. Characteristic spores were first noted on May 3rd. Using spores from the plants thus infected, plates were poured May 4th, 1920 and the organism isolated on May 10th, 1920. June 12th, 18 celery plants and 4 celeriac plants were inoculated using the pure cultures obtained from this isolation. Thirteen days later, June 25th, all inoculated plants showed typical infection. The checks two weeks later also showed infection which was probably due to the distribution of the spores to them by air currents created by opening and closing the doors of the rain chamber. This experiment has been repeated several times with similar results; the checks, however, being better isolated, showed no infection by the Early Blight fungus. In one infection experiment the organism was reisolated and the single spore culture thus obtained was used with success in two subsequent

inoculations.

Morphology.

(Mycelium)--The mycelium of the fungus as it occurs within the host tissue is composed of irregularly septate, fairly heavily walled threads (Plate VI). These vary in width from 2 to 3.5 microns, attaining a diameter of 4 to 5.5 microns in the case of the threads that make up the stroma-like masses from which the fruiting structures arise. The young hyphae are small tubes nearly hyaline, having relatively thin walls with septa only at long intervals and having a finely granular, homogenous appearing protoplasm. As they grow older they become gradually pale brown in color, gnarly, tortuous and very irregular in shape, form septa at shorter intervals, build heavier walls, and their protoplasm becomes more coarsely granular with a few large (.5 to 1.0μ), highly refractive, globular bodies (Fig. 4-5, Plate VI). The mycelium formed in culture varies greatly according to the food constituents and character of the medium. Figure 5, Plate VI shows its growth on Melilotus stems and Figures 3 and 6 in cornmeal broth of P_H 5 and 8.

(Sclerotium-like Bodies)--Dark brown, sclerotium-like masses of threads, having a diameter of 20 to 50μ, are formed in the substomatal spaces (Plate VI, Fig.6). From these through the stomatal pore are thrust the conidiophores which wedge apart and soon obliterate the guard cells.

(Conidiophores)--The conidiophores are fused together in the region where they emerge from the leaf, forming a distinct fascicle (Plates VII, VIII). They are a decided brown in color, usually with 1 to 2 rather indistinct septa near the base but not infrequently having cross walls at 10 to 15μ intervals throughout their length. They vary from 40 to 180μ in length, the average being 40 to 60μ; their width varies from 3.5 to 5.5μ. At the end of a mature conidiophore is seen a distinct scar, circular in outline and with a small circular dot occupying the center which indicates the point of attachment of a spore previously borne (Plate VIII, Figs. 2,3,4). Older conidiophores have along their surface from 2 to 6 of these scars, with a bend or geniculation at each scar (Plate VIII, Figs. 8, 12). The explanation for the presence of these geniculations is evident. A conidiophore having borne a spore, on being brought into moist surroundings sends out from just beneath the scar a new growth. From this new growth a new conidium is abscissed, at a distance of from 10 to 30μ from the first scar. This sympodial branching may be repeated several times, leaving as many distinct knees. As many as six geniculations have been seen (Plate VIII, Fig.8.)

(Spores)--The spores or conidia which are borne at the apices of the conidiophores are much elongated and slender (Plate IX). They are spoken of as inversely clavate or obclavate in form; that is, the part next the point of attachment is greater in diameter than the free ends. (Several

writers have made the error of considering the spores as attached by their small ends (78,48). A slight tendency toward chain formation was also noted (Plate IX, Fig. 11j,k). Over 200 spores from 10 sources were measured, the dimensions being as follows:

	Minimum	Maximum	Average
Length (microns)	22.0	290.0	55.-100.
Width at base "	3.5	4.5	4.0-4.5
Number of cells	2.	30.	4.-12.

The conidia attained their maximum length in the warm, moist atmosphere of the rain chamber where the humidity was high and prolonged. The spore contents are non-vacuolate, finely granular and subhyaline, the ones formed in pure culture being slightly more transparent than ones collected in the field. They are commonly constricted for the length of a cell, this portion appearing devoid of protoplasm (Plate IX, Fig. 11b). One culture media a few typical obclavate spores are formed and abjointed at first, but later the new conidiophore growth becomes longer and remains attached as hypha-like branch. These when mounted appear as long, slender filaments which may be considered atypical spores? (Plate IX, Fig. 11h).

Name of Causal Organism.

The causal organism was first described by Fresenius in 1863 under the name Cercospora apii (28). Through error the credit for this was assigned by Scribner and some others following him to Fries. To avoid mistaking the

authority for the name the abbreviation should be written Fres. or Fresen, not Fr., thus--Cercospora apii Fres. (or Fresen.)

Life History of Causal Organism.

The writer has twice proved that the fungus will overwinter on celery refuse. Microscopical examination of this refuse reveals the presence of the typical conidiophore fascicles, and leaves containing the overwintered parasite when placed in a moist chamber in the warm laboratory are soon covered by a growth of typical Cercospora spores. Early this spring spores which had overwintered on trash were found. They were so very hyaline as to be difficult to see, were much swollen--5.5 to 6.5μ at the base--and failed to germinate. This tends to discredit Massee's statement[56,57] that spores live through the winter and are capable of infecting a crop the following season. The fact that the fungus makes a good growth on sterile muck (Plate V) may indicate another means of overwintering and a source of infection the following season. (Growth on muck is described under "Cultural Characteristics".) The spore, under the suitable climatic conditions described, on coming in contact with a celery leaf germinates, sending its delicate thread-like germ tubes into the leaf tissue. (See under "Infection Phenomena".) Here it grows absorbing nutriment from the leaf cells and producing a well-defined spot. The nutri-

ment at the center of the lesion being first exhausted the fungus here sends out fruiting hyphae which abscisss spores capable of immediately infecting the crop. This course continues throughout the hot, humid summer months until the coming of the cooler, rainy fall weather. Then the fungus within the fallen leaves and petioles assumes a resting stage in the form of the stroma-like masses of heavy-walled mycelial threads described. It thus continues its existence until the coming of hot humid weather again.

Relation of Parasite to Host.

Mode of Infection.

To demonstrate the mode of entrance of the organism healthy celery leaves were placed in a moist chamber and inoculated with spores and mycelium from diseased material. The points of inoculation were marked by means of small pieces of moist filter paper, each having a 5 mm. circular hole in its center. From time to time bits of the leaf epidermis were stripped off and examined. A mount made after 25 hours showed two distinct germ tubes from a piece of mycelium entering a stroma (Plate VI, Fig.1.) Spore germ tubes were also seen entering the leaf tissue in the same manner, but the view was too indistinct to be traced by means of the camera lucida. The spore shown

in Plate VI, Fig. 1, was not seen in the mount illustrated but was placed there for the purpose of size comparison. Hume (1900) demonstrated stomatal entrance in a similar manner. Although repeatedly tried, I have as yet not been fortunate enough to secure pieces of epidermis from young heart leaves which show this entrance; nor have I observed entrance through wounds. Although it is reasonable to believe that the fungus may enter through wounds. Experiments show no increase in percent of infection where the leaves have been wounded. That the parasite can gain entrance to the interior tissue of the leaf when placed on either the upper or lower leaf surfaces was demonstrated in infection experiments. This is to be expected, the stomata being present in both upper and lower epidermis.

Making use of the method employed by Makemson (1918)[55], germ tubes were observed to enter leaves that were not detached from the plant. In this method the leaf is bent over onto a slide on the microscope stage and held in place by means of rubber bands. To supply moisture to the leaf surface a narrow ribbon of filter paper is employed. The end of this paper, which is placed over the leaf, has a small circular opening which marks the place of inoculation. The other end dips into a beaker of water set near the stem of the potted plant.

Spores of the fungus are placed in the small exposed leaf area and over this is placed a cover slip. Using low power direct microscopic observation is possible. Not finding sufficient moisture to be supplied by the filter paper, a modification was made in the form of capillary glass siphons. These supplied water to a small piece of filter paper strip kept on the leaf, at any rate desired, depending upon the diameter of the tube and height of the source of the water. In some experiments, for the purpose of better viewing the spores and germ tubes, the cover slip was removed after sufficient time had elapsed for entrance, and the fungus stained a minute with a 1% aqueous solution of eosin(34). The stain was removed from the leaf surface with filter paper and water, the paper strip removed, the cover slip replaced ove r the area of inoculation, and direct microscopic observation with both high and low power was possible.

It was noted that the fungus most readily enters the leaf when conditions of high humidity are present in the surrounding air. However, where the water forms a visible film over the leaf surface, the germ tubes do not penetrate the stomata. It was noted with spores germinating upon a glass surface that where a moisture film enveloped the germ tube by capillary action this film was pulled along with the extending tube. This small film enveloping the spores may be significant in the infection process.

It is likely that the tube extends the enveloping water film until this film is brought in contact with a stomatal opening into which the water is quickly drawn by capillary action. Thus the germ tubes would build continuous water passageways from the spore to the leaf interior. The tubes, naturally hydrotropic, continue down along the water paths entering into the minute capillary-sized passageways of the parenchyma. The factors involved in the establishment of the parasite within the host after invasion have not been determined. Entrance may be effected in from 6 hours upwards, depending upon the rapidity of germination and the proximity of the stomata.

Although I have not as yet observed entrance of the fungus into young heart leaves, that such does take place is undoubtedly true. Poole and McKay attempt to correlate age of the leaf and stomatal activity with the susceptibility of beet leaves of different maturity to the leaf spot fungus, Cercospora beticola Sacc. (64,65). The stomata of immature celery leaves being less frequently open and having smaller slits than those of mature leaves are facts which must be taken into account when we consider that heart leaves infrequently show evidences of blight. To note accurately the relative susceptibility of young and old leaves, the heart leaves of four healthy plants were tagged March 31st, last; the plants were placed in the rain chamber and inoculated with spores from blighted

leaves. Ten days later pale yellowish spots 1-3 mm. in diameter appeared at the margins of some of the heart leaves. The spots not appearing to enlarge, some of the heart leaves bearing them were placed in moist chamber on April 16th. April 19th the lesions had enlarged to areas measuring about 5 mm. by 8 mm. Examination April 21st revealed them bearing typical conidiophores and spores. By April 23rd the diseased heart leaves remaining in the rain chamber had borne spores. From this it appears that the young immature leaves of celery are capable of being entered by the organism which, however, is unable to cause a definite, easily recognizable Cercospora lesion within twelve to sixteen days. It seems that after the parasite has gained entrance it makes but little apparent progress while the leaf is actively growing. There may be some correlation between the hydrogen ion concentration of the young tissue and its seeming resistance, while young, to the fungus attack. The P_H of immature foliage has not been determined. It is evident from innumerable observations that the fungus makes its most rapid progress in the old retrograding leaves. On mature leaves spots are produced in from five to eight days, whereas it takes at least 10 to 14 days on the immature heart leaves.

On April 15th the heart leaves of four plants were inoculated with diseased material and tagged for later

identification. Eighteen days later these leaves had attained almost their final mature size and bore the characteristic fruiting bodies of the fungus.

Relation to Light.

To determine whether darkness has any influence on the infective powers of the fungus two potted plants were placed in a dark constant temperature apartment and inoculated with diseased material. One plant was kept moist by inverting over it a battery jar in the bottom of which and down for about half the height of the jar was fastened moist filter paper. Some leaves of the other plant were kept moist at the points of inoculation by means of wet absorbent cotton wisps, while others were irrigated with capillary siphons after the manner described in the study of the mode of infection (pp.22 and 23). The uninoculated leaves were considered as checks. Plants were similarly treated in the light of the laboratory. Six days later some of the inoculated leaves began to show signs of infection. Spotted leaves were placed in moist chamber and four days later were observed to have produced typical conidiophores and spores. These results are in contrast to the observations by Pool and McKay[64,65], who worked with the Cercospora leaf spot of sugar beet. They reported that infection probably takes place only in the day time and that immature leaves are not susceptible.

Water and Temperature Relations.

Once established within the host leaves the fungus makes rapid progress during hot, dry weather when the plant is least resistant. The factors which most favor the fungus, as determined by field observations and by controlled conditions in the greenhouse, are high temperatures (80 to 90°F.) coupled with a droughty condition of the soil when the activities and resistance of the celery plant are at low ebb.

When we consider dissemination of the causal organism we must add to these factors the necessity for air currents of sufficient strength to distribute the fungus conidia and of an adequate amount of moisture being present on the celery leaves to permit of the germination of the spores when they arrive. Dews, a high humidity, and light rains, while not being of much value to the plant, supply this moisture requirement of the fungus. One notes a most rapid spread of the disease and fruiting of the fungus in hot, muggy times following a period of drought. To demonstrate the germination of spores in condensation water the method of Duggar(20) (page 59) was employed. A van Tieghem ring was fastened to a slide with vaseline and paraffin, a few drops of water placed in the ring, and over this was inverted a cover slip on the top of which had been placed a number of conidia. The spores readily germinated in the moisture which condensed on the glass,

germ tubes appearing in from 2 to 5 hours at a room temperature of 28°C. (Plate IX, Figs. 3,4,5.)

In the greenhouse proper conditions for a thorough infection of healthy plants were secured in the rain chamber which is a plate glass cage (Wardian case) approximately cubical, having a capacity of 12 cubic feet. Entering this through a hole in one corner of the bottom and extending upward until it nears the center of the glass top is a half-inch water pipe terminating in a small opening from which water emerges forcibly. By means of this watering system is directed forcibly against the top of the small chamber a small jet of water which, breaking into a fine spray, thoroughly wets the leaves of plants placed therein but does not supply sufficient water to the soil to keep the plants vigorous. Healthy potted plants were placed in this apparatus, the mist turned on for a half minute, and the plants "peppered" with spores by shaking diseased material over them. The leaves were kept moist for a period of 8 to 10 hours by turning on the mist for a few seconds at intervals of about two hours. The chamber was maintained at a high temperature by means of two 16 candle-power carbon filament lights. Making use of this apparatus, conditions were obtained which best simulate those in nature when Early Blight is most prevalent and destructive. Knowing these

conditions we are thus better able to understand and explain the severe losses experienced in Florida and other localities.

Pathological Histology.

The character of the mycelium as it occurs within the leaf was described under "Morphology" (page 18). Mention was not made as to the means by which the parasite obtains its nourishment. The mycelium viewed in mounts of small portions of diseased leaves can, by careful focussing, be seen to possess small knob-like structures 2 to 3.5μ in width (Plate VI, Fig.4). These outgrowths or haustoria of the fungus mycelium extend through the cell wall and lie in close contact with the cell contents therefrom absorbing nutriment for the organism. The hyphae as seen in stained paraffin sections of blighted leaves and stems are exclusively intercellular, although in the very old disintegrated tissue they appear to pass right through, ramifying seemingly unobstructed as in the growth on artificial culture medium. An abundance of hyphae shows evidence of a rapid growth throughout the sponge parenchyma and palisade tissue, here readily wedging the cells apart. Distortion of the host tissue is most evident in the regions where the coarse, heavy-walled threads collect to form spherical, stroma-like masses. Guard cells of the stomata are forced apart as the conidiophore bundles are protruded, are overshadowed by the short immature stalks of the bundle, and soon lose their identity. Plate VI,

Fig. 2, illustrates the beginning of this procedure, and Plate VII and Fig. 1 of Plate VIII the ultimate effect. The bases of the fascicles were found to measure as wide as 50μ whereas the slits of mature stomata are only 10μ to 13μ in length.

Physiological Relations.

Effect of the Early Blight Fungus (Cercospora apii Fres.) on the Nitrogen Constants of Celery (Apium graveolens).

To discover what changes in the nitrogenous compounds of celery are brought about under the pathological workings of the Early Blight fungus, and to note the similarities and differences in the action of this fungus and that of the so-called virus or mosaic diseases[44], the following chemical studies were undertaken during the fall of 1920. The methods used were, in the main, those employed by Jodidi and his collaborators in their spinach and cabbage mosaic investigations[46,47]. Minor modifications and deviations are described in the following.

All the determinations, except water content, were made with diseased and healthy celery of one variety, Easy Bleaching, which was collected from plants of the same age and growing in the same garden plot. Due to the abundance of relatively uniform leaf infection and to the small amount of stem infection the work was done entirely on leaves. As far as practicable, mature leaves of apparently the same age were selected. The lesions on

the blighted leaves involved approximately from one-sixth to one-fourth of the leaf area. The leaves were spread in thin layers on cheese cloth and dried at room temperature (20-25°C.) for three days, and then placed for three days in an electric oven which maintained a temperature of 49-54°C. The dried materials were next rubbed through a 40-mesh sieve, then mixed to assure more uniform sampling, and finally put into mason jars and sealed. The dried, powdered leaves thus made ready for use were about the fineness of table pepper. The color of the powdered blighted material was an ashen grayish green, while that of the healthy material was distinctly chlorophyll green.

The TOTAL NITROGEN was determined by the Kjeldahl and Kjeldahl-Gunning methods, 2g. samples of dried, unpowdered leaves being used in the first method, and 2g. samples of the more representative powdered materials in the second. It may be said here that in all ammonia distillations 4% boric acid solution was used as the receiving liquid, brom-phenol blue indicator being used in the titration[76].

NITRIC NITROGEN was estimated by two different methods, F. M. Scales' zinc-copper couple reduction method[75] and the Schulze-Tiemann nitric oxid gas method[23]. According to Jodidi's procedure, 12g. each of healthy and blighted celery were repeatedly extracted and thoroughly washed with 85% alcohol. Milk of lime

was then added to the combined extracts and washings, which were then evaporated to dryness in a vacuum oven at low temperature. The residue was then taken up with hot water, lead acetate solution added, and the whole filtered, washed, and the combined filtrate and washings made up to 2 liters. In the Scales' method 250 cc. samples of the extract were placed in 500 cc. Kjeldahl flasks containing 80g. of the Zn-Cu couple coils. Five grams of c.p. sodium chloride and 1g. of c.p. magnesium oxid were added and the ammonia from the nitrates thus reduced distilled into boric acid. In the gas method 250 cc. aliquot portions of the same extract were used and the work carried out exactly as described[23].

Distillation with Magnesia not being found to give dependable results in the estimation of AMMONIA NITROGEN, a modification of Grafe's method[33] of distillation <u>in vacuo</u> at low temperature was employed. Ten gram samples were placed in one liter flasks, treated with 25cc. of concentrated sodium chloride solution, 35cc. of water, and 15cc. of alcohol. Then the apparatus was carefully made tight, 15cc. of saturated sodium carbonate added by means of the separatory funnel, and the water pump turned on. The temperature of the water bath was then raised to 25-29°C., at which temperature it was held for three hours and then raised to 40-43°C. The water pump reduced the pressure to 21-52 mm. mercury, the

average being 28 mm. Fifty cc. portions of a 4% boric acid solution were used in the Peligot tubes to receive the ammonia. The Peligot tubes were placed in the same ice bath and attached by a Y to the same water pump. Near the end of the 6-7 hour distillation period 15cc. more of alcohol were cautiously added through the separatory funnels. The distillation was continued 20 to 30 minutes longer, during which time the particles of moisture that collect in the necks of the flasks and the conducting tubes and which contain some ammonia were removed by squirting a stream of boiling hot water against the exterior of the glass. The contents of the Peligot tubes were transferred to Erlenmeyers and titrated against standard sulphuric acid, using brom-phenol blue as an indicator.

NITROGEN of NITRITES was tested for only qualitatively. Portions of the extract used in the Nitric Nitrogen determination were made neutral and to them were added 1 cc. quantities of Sulfanilic acid mixture (59)[12]. A red color in the extract from the diseased leaves indicated the presence of nitrites. A faint pink appearing in the extract from the healthy material indicated a trace of Nitrite nitrogen.

Total HYDROLYZABLE NITROGEN and NITROGEN DISTRIBUTION in the hydrolyzed portions were determined as follows: 8g. samples with 400cc. 20% hydrochloric acid were boiled

for 9 hours under a reflux condenser. The contents of the flasks were then filtered and washed with ammonia-free water until free from chlorine. The filtrate and washings of each 8g. sample were thereupon made to 2 liters. Total nitrogen was determined at 500 cc. aliquots by the Kjeldahl-Gunning method. Other 500 cc. portions were evaporated to dryness on the water bath and the ACID AMIDE, HUMIN, and DIAMINO ACID NITROGEN estimated according to Hausmann's nitrogen distribution method[38,45]. The results for MONOAMINO ACID NITROGEN were arrived at by difference. Because of limited time and because of the doubtful nature of the nitrogen extracted with boiling hot water[46], nitrogen distribution by Hausmann's method was not estimated on the extract containing the so-called non-protein nitrogen.

PROTEIN NITROGEN was determined by Stutzer's method[86] a development of the work of Ritthausen[69]. In this method, 1g. samples of the dry celery powder were treated in the beaker with water (100 cc.) heated to boiling and kept on the water bath for 10-15 minutes. Then 2cc. of concentrated potassium-alum solution and 15cc. (.45 g. $Cu(OH)_2$) of Stutzer's solution were added and the mixture stirred thoroughly. On cooling, it was filtered and thoroughly washed with water. The residue and filter paper were transferred to a Kjeldahl flask and the "protein nitrogen" oxidized by the Kjeldahl-Gunning method.

The nitrogen in the filtrate and washings from the

$Cu(OH)_2$ residue was determined by the Kjeldahl-Gunning method and called NON-PROTEIN NITROGEN. The results for this analysis are nearly the same as those arrived at by subtracting the figures for protein N from those for total N; they are at least within the error due to sampling which must be considered in all the estimations.

MOISTURE CONTENT was found by immediately weighing freshly gathered leaves, drying to a constant weight in an electric oven (temp. 99-103°C.) and reweighing. Being more conveniently located than garden material, greenhouse material was chiefly used in this determination.

A tabulation and brief discussion of results follow:

TABULATION OF RESULTS.

Data expressed in percentage of the oven-dried celery leaves.

DETERMINATIONS	BLIGHTED			AV.	HEALTHY			AV.	AV. DIF
MOISTURE CONTENT									#
Field Material-	79.4	76.3		77.8	80.0	80.16		80.08	2.23
Greenhouse " -	75.1	73.8	77.3		84.15	84.25	83.15		#
	74.2	77.75	77.0	75.86	84.7	84.1	84.4	84.125	8.26
TOTAL NITROGEN									
Kjeldahl method									#
(leaves not ground)	2.95	2.82		2.885	4.33	4.70	4.37	4.433	1.54
Kjeldahl-Gunning meth.									#
(leaves ground)	2.93	2.92	2.98	2.943	4.78	4.71	4.83	4.773	1.83
NITRIC NITROGEN									#
Scales' Zn-Cu couple meth.	.156	.156	.1601	.1574	.421	.417	.421	.4197	.2623
Schulze-Tieman									#
NO gas method.	.118	.121	.1195		.3255	.320	.325	.323	.2045
			.118	.1191					
AMMONIA NITROGEN									-
Grafe's method	.0783	.081	.0769	.07693	.06045	.0632	.0714	.0655	.0114
			.07145				.0632		
NITROGEN OF NITRITES									
Qualitative tests	Present-good test				Faint trace				-
TOTAL HYDROLYZABLE "N"									#
Kjeldahl-Gunning		2.601				4.342			1.7313
NITROGEN DISTRIB.									
Hausmann's method.									#
ACID AMIDE NITROGEN	.4843	.4911		.4877575	.7899	.80725		.7986	.3018
"HUMIN" "	.3160	.3091		.31259	.3229	.30913		.3160	.0034
DIAMINO ACID "	.4602	.4533		.456825	.8107	.797		.8038	.3470
MONOAMINO " "	1.340	1.347		1.3443	2.418	2.428		2.4233	1.079
PROTEIN NITROGEN									#
Ritthausen-Stutzer meth.	2.174	2.215	2.188	2.1923	3.261	3.281	3.268	3.27	1.0777
NONPROTEIN NITROGEN									#
N in filtrate after precipitation with Stutzer's reagent $CuOH_2$	.759	.7383	.7383	.7452	1.422	1.415	1.415	1.4173	.6721

From the above data it is seen that the blighted leaves of celery plants have approximately only 3/5 as much total nitrogen and about 1/3 as much nitrate nitrogen as normal leaves. Further examination shows that the protein nitrogen content of the diseased material is 2/3 that of the healthy, whereas the non-protein and total hydrolyzable nitrogen are slightly over 1/2 as much. The hydrolyzable portion of the unaffected leaves revealed on analysis considerably more acid amide, diamino acid, and monoamino acid nitrogen than the corresponding extract of blighted material. "Humin" nitrogen was found to be about the same in both normal and diseased leaves. On the other hand, blighted material in every instance showed more ammonia nitrogen and a much stronger qualitative test for nitrites than the sound material. The slight trace of nitrite nitrogen in "healthy" leaves may be due to the fact that some of them were possibly not free from the disease, although at the time of collection they showed no outward evidence of infection. (The incubation period was found to be 5-7 days.) One may concede the possibility that part of this denitrification may be due to the action of reducing bacteria coming in as secondary infection(6).

The results of these analyses make it evident that the action of the celery early blight organism on the nitrogen constituents of celery is quite similar to the

workings of the so-called mosaic diseases on the various nitrogen compounds of cabbage and spinach. As in the mosaic diseases, the fungus brings about a process of denitrification in the plant tissues whereby the nitrates are reduced. This accounts for less nitrate nitrogen and the presence of nitrites in the diseased tissue.

The nitrites formed in the denitrification process probably react with the amino acids of the celery tissue, nitrogen being eliminated. For example, note the possible reaction with tyrosin, asparagin or glutamin, all three of which have been found in celery [89].

$$\underset{\text{Tyrosine}}{HOC_6H_4CH_2CHNH_2COOH} \xrightarrow{HOON} \underset{\text{Hydroxy Acid}}{HOC_6H_4CH_2CHOHCOOH} + \underset{\text{Elementary Nitrogen.}}{N_2\uparrow} + H_2O$$

We should, therefore, expect less mono- and diamino acid nitrogen.

It also has been shown [86] that under certain biological conditions the nitrogen of the amide groups is readily split off as ammonia.

$$RCONH_2 + HOH \longrightarrow RCOOH + NH_3$$

Hence, we find less acid amide nitrogen in the diseased tissue. The ammonia formed in this hydrolysis may indicate the reason, entirely or in part, why more nitrogen of ammonia was found in blighted leaves.

From the above it is evident that the total nitrogen

is reduced through the loss of the nitrogen of proteins, nitrates, amino acids, and acid amides, nitrogen being evolved for the most part as elementary nitrogen and ammonia. Closer examination of the data reveals the fact that based on the total nitrogen there is 6% more protein nitrogen in the diseased than in healthy material, but that when calculated to the weight of the oven dried leaves there is only 67% as much protein nitrogen in the blighted as in the sound celery leaves. In accounting for the figures based on total nitrogen, it is known that proteins as such are but slightly attacked by nitrous acid and therefore directly contribute very little to this loss of total nitrogen. Considering the result based on the actual weight of the oven-dried leaves it is reasonable to believe that the proteins of the tissue under the immediate influence of pathological conditions are in part hydrolyzed to peptids and amino acids which are readily attacked by nitrous acid. This would explain the presence of less protein nitrogen in the diseased leaves.

The effect of the fungus on the nitrogen metabolism seems to be primarily denitrification. This causes deaminization of the amino acids and possibly to a small extent, proteins. The loss in total nitrogen appears to be the result of the formation of free nitrogen and ammonia.

Accompanying and following the death of the tissue and progress of the fungus there is, naturally, a drying

of the affected parts. Moisture determinations showed that blighted leaves contained 8% less water than sound material.

In brief, the parasitic organism at the expense of and in competition with its hosts selects and utilizes the forms of food suited to its energy and tissue requirements. The fact that this fungus, in common with the "Mosaic" trouble of other plants, is responsible for a smaller percentage of total protein, nitrate, acid amide, monoamino and diamino nitrogen, for a higher percentage of ammonia, and for the presence of nitrites in its host, may give a sort of picture of the chemical changes that accompany disease in plants.

Cultural Characteristics.

In pure culture the fungus was found to grow well on all media employed. Plantings from cornmeal agar cultures were made on the various media shown in the following record, and the growth observed from day to day. The effects of light and darkness on growth and sporulation were also studied at the same time. For growth in darkness, an Arnold sterilizer was used. The diffused light of the laboratory supplied a second lighting effect, and a 50-watt Mazda bulb in the dark room of the laboratory gave a third condition of illumination. In the second and third cases the cultures were placed in a single row to give the full effect of the light to each

tube. The character of the growth was observed frequently but the results here given are those recorded at the end of the experiment. Not considering the amount of growth, the macroscopic appearance of the fungus varied but little from time to time. The color of the small mycelial threads as they first invade the fresh medium is a translucent gray. Growth progressing, the hyphae as they become older manufacture a pigment which varies in color with the character of the medium. On some media it is a dark gray to black, on others shades of brown, but it is generally a deep chromium or mineral green. The dark green pigment so pronounced on cornmeal agar was found by the microscopic examination of sections to be entirely within the mycelial threads and not in the medium. The mycelium penetrates the agar to a depth of .5 to 1.5cm., the color not being present in the young branches which make the borders of the growth (Plate IV.)

Light Relations.

The following record was made after ten days' growth on the various media. The growth in darkness and diffused light took place between 20°C. and 24°C; that in electric light was necessarily a little higher (23°-26°C.)

1. Turnip plug: Darkness- The superficial growth covered about 1/3 the surface of medium and was a dull mouse gray in color. Prominent folds and bunches of a deep green pigmented growth in the medium. This extended to a depth of .5 cm. No typical spores found.

Diffused light- As in darkness. No typical spores.

Electric light- As in darkness. No typical spores found.

2. Rice: Darkness- Mouse gray as in (1). In medium a chromium green pigment 2 mm. deep. No typical spores found.

Diffused light- Similar to that in darkness but aerial growth slightly lighter colored. Typical spores not found.

Electric light- As above. A few typical spores.

3. Potato plug: Darkness- Aerial growth not distinct. Considerable dull, dirty, yew-green growth covering 1/4 of surface of plug. Less elevated than (1) and (2). Spores-

Diffused light- Slightly more elevated and folded.
Electric light- As in diffused light.

4. Bean pods: Darkness- Considerable dull, intensely black growth with short velvety mouse-grayish aerial growth on the many folds and bunches. No typical spores.

Diffused light- Exactly similar to that in dark.

Electric light- Exactly similar.

5. Corn Meal: Darkness- Considerable, the greater portion being submerged and having a dark chromium green pigment which is most intense near center of growth.

Corn Meal cont'd: Diffused light- As in darkness. A few typical spores found.

Electric light- As in darkness.

6. Oat Meal Agar: Darkness- An abundance of dull black growth, citron green at the border. A few clumps of gray aerial mycelium. No typical spores found.

Diffused light- Similar.

Electric light- Similar.

7. Nutrient Broth: Darkness- Characteristic growth of (5) at the surface of the liquid and on the glass. Many small rainette green submerged colonies against the glass. No spores.

Diffused light- Same.

Electric light- Same.

8. Carrot plug: Darkness- Tomentose mouse-gray aerial growth and a tough folded black growth in substrata. Spores abundant.

Diffused light- More abundant growth and spores.

Electric light- As in diffused light.

9. Nutrient Dextrose Agar: Darkness- Black in substrata, mouse-gray to a faint pinkish gray to almost white aerial growth. Exuding amber colored drops of liquid over white aerial mycelium. Spores +.

Diffused light- As in darkness, but with more numerous amber and colorless moisture droplets. Spores ++.

Electric light- Similar.

10. Potato broth: Growth very similar to that in Nutrient broth. Submerged growths more fluffy.

11. Celery Agar: Darkness- Moderate amount (1/2 of surface). Mouse gray to white aerial mycelium in clumps simulating conidiophore fascicles. A black-green pigmentation. No spores.

Diffused light- Same as in dark.

Electric light- Same as in dark.

12. Prune juice Agar: Darkness- Abundant mineral green to black growth with small mouse-brown cottony clumps, of aerial mycelium. No spores found.

Diffused light- Same. Aerial mycelium green near the point of planting.

Electric light- Same as in diffused light.

13. Corn Meal Agar: Darkness- As in (11) but with fewer, more scattering conidiophore-like clumps. Spores ++.

Diffused light- Same.

Electric light- Same.

14. Nutrient Agar: Darkness- Abundant, deeply submerged (.5 cm.) black growth with mouse-gray to white aerial clumps. No spores found.

Diffused light- Same but with less white aerial mycelium.

Electric light- Same as in diffused light.

15. Nutrient Sucrose Agar:

Darkness- As in nutrient glucose agar, with amber droplets exuding from white aerial mycelium. Spores ++.

Diffused light- Similar, but no moisture droplets. Spores ++.

Electric light- Aerial growth shows a slightly pinkish coloration. Spores ++.

It is evident from the above record that light effects are not very marked. Corn meal agar cultures in

preparation dishes were later similarly exposed to light and darkness with similar results.

To repeat the above observations on the effect of the absence of light on the growth of the fungus in pure culture, five corn meal agar slants were planted with mycelium and placed in a black wooden chalk box. This was enclosed in a pasteboard box and the whole set near a north window of the laboratory. Three similar plantings were placed in a wire basket and set alongside of the boxed tubes. Two weeks later the cultures were examined. There was no apparent difference in the growth of the fungus except that the aerial mycelium of the tubes kept in the darkness was slightly darker gray. Typical spores were produced in both groups of tubes.

Growth on Muck and Celery Refuse.

The record given above shows that several media which supply the food necessary for a good growth of the fungus in pure culture may be employed. Particularly may be mentioned carrots, the nutrient sugar agars, rice, and corn meal agar. Cultures on carrot and rice are rather difficult to transfer rapidly, due to the tough resistant growth. The sugar agars, while being convenient to prepare, are good media for bacterial as well as fungus life. They could not be used to good advantage in isolation work. Because of its convenience in handling and because of its apparent suitability

as a food for the isolation of the organism, corn meal agar was the medium most employed in this work (Plate IV). Good growth of the fungus was secured also on sterile lettuce, celery refuse, beets, orange and lemon peelings, Conns' cheap synthetic media, Richards' synthetic, and muck.

To determine whether or not the organism would grow on sterilized muck and celery refuse, and to note the character of the possible growth as influenced by three different treatments of these natural media, the following was performed:

Five separate portions of quantities of muck, muck and refuse in about equal volumes, and refuse alone were treated respectively as follows: One set of the materials was placed in preparation dishes and distilled water added until it was within .5 cm. of the surface of the medium. This set was called "saturated". Another group was treated with just sufficient water to give the soil a nice friable condition, the material put in preparation dishes and labelled "Optimum water content". A third series was treated as the second, using filtered lime water and marked "Lime water". A fourth was saturated with water containing sulfuric acid in the proportion of 250 of H_2O to 1 of H_2SO_4; the preparation dishes containing the media thus treated were called "acid". The fifth set, used as a check, was left untreated. All were autoclaved at 20 pounds pressure for forty-five

minutes and planted with mycelium from a corn meal agar culture. The results are shown in the following table, page 49.

This experiment simply shows further the saprophytic nature of the organism and suggests the possibility of the fungus' overwintering, if not persisting longer, in the soil and being capable of infecting a celery crop the following summer (Plate V).

Temperature Relations.

The season in which the disease appears in the field together with its reactions to controlled conditions in the greenhouse suggest a rather sharp susceptibility on the part of the parasite to heat. To obtain further knowledge of the temperature relations of the fungus use was made of a model of a Ganong differential thermostat. This consists of two galvanized iron chambers, one for a freezing mixture and the other for boiling water, which are connected by a square trough of the same material. This trough is divided into nine small compartments by means of cardboard partitions and is covered by two pieces of glass, making a gable-like effect. Each division is equipped with a small immersion thermometer. Ice was renewed each day in the one chamber, and the water in the other, kept boiling by means of an electric immersion heater, was automatically supplied from an aspirator bottle.

Table showing Growth on Muck and Celery Refuse.

MEDIUM & TREATMENT.	GROWTH	SPORES
Optimum Water Content 1. MUCK	Medium 3/4 covered with mouse-gray and white growth the white immediately around the point of inoculation and amounting to 1/3 the viable fungus.	None found.
2. REFUSE	Entire medium covered by a brownish-gray plushy growth.	Many.
3. MUCK & REFUSE	Surface 3/4 covered. Character of growth as in (1) except less white area.	Found on refuse.
Water Saturated 4. MUCK	Surface 1/4 covered. Character as in (1).	---
5. REFUSE	As (2) where medium is out of water.	Abundant, some germinating.
6. MUCK & REFUSE	Nearly entire surface covered as in (1).	Few spores found on refuse.
Lime Water. 7. MUCK	As in (1).	Few atypical spores found.
8. REFUSE	As in (2).	As in (5).
9. MUCK & REFUSE	As in (3) with a little more white growth.	As in (3) but fewer.
Acidified 10. MUCK	Character of growth as in (1), 1/3 surface covered.	Few.
11. REFUSE	1/2 surface covered as in (2).	Many.
12. MUCK & REFUSE	As in (9), 1/2 of surface covered.	Few.

Table continued.

MEDIUM & TREATMENT.	GROWTH	SPORES
Untreated. 13. MUCK	Appearance as in (1), 1/2 covered.	Few.
14. REFUSE	As in (2).	Many.
15. MUCK & REFUSE	Character of (3), more growth being on refuse than soil.	Found on refuse.

(Vegetative Growth)--Making use of the apparatus described above the series of temperatures as described above the series of temperatures as recorded in the following tabulation was obtained. Plantings were made on corn meal agar in preparation dishes and four cultures placed in each compartment. The temperatures of the several compartments were recorded and the growth of the cultures observed each day for 16 days. The dishes were then removed and measurements and observations recorded.

No. of compartment.	Temp. range of Compartment, (°C.)	Diameter of growth, mm.		Growth.	Spores.
		Max.	Min.		
1.	41-43	---	---	Death	---
2.	36-38	7.5	4.0	Poorest, heavy, resistant mycelial threads.	---
3.	30-31	28.0	25.0	Next best.	+, few
4.	27-29	34.0	31.	Optimum.	+
5.	24-25	23.	23.	Good.	+ few
6.	22-23	27.	22.	Good.	+, few short spores.
7.	20-21	18.	18.	Declining.	++, many more.
8.	15-17	17.	11.	Not so good as 7	+++ Abundant.
9.	4-8	6.	5.5	Poor.	+ few.

These data indicate that the fungus grows well between 23 and 30°C., temperature above or below this range being distinctly opposed to the best progress of the organism. Most abundant sporulation took place between 15 and 21°C. In several attempted repetitions of this experiment upon carrot disks, Coons' synthetic medium and corn meal agar, the cultures were contaminated by mites in spite of a thorough dusting of the apparatus with Pyrethrum powder and spraying with nicotine solution. Observations of test tube cultures confirm the above results except that the greatest numbers of spores were found at 20-21°C. Work with the living host was impossible because potted celery plants small enough for the compartments did not survive there long.

(Spore Germination)--Spores and conidiophores were removed from diseased celery, placed in tap water on a cover slip and inverted over van Tieghem rings as already described[20]. They were then exposed for 14 hours to the temperatures shown in the table and observations made and recorded.

Temp. (°C.)	New Conidiophore Growth.	Spore Germination.
42-43	---	Death.
35-36	About half the number germinated. Growth 16-100μ in length; one was 130μ.	60% germinated. Tubes 30-85μ long.
27-29	Many (50%) germinated; 170-190μ long.	100%. Practically every cell showed a germ tube.
22-24	(50%) 60-120μ long.	As at 27-29°, but fewer cells germinated.
19	(50%) Growth 30-40μ long.	100% tubes 100-170μ long, fewer cells germinated than at 22-24°.
16	Few (10%) tubes 16-50μ	Many (60%) tubes 38-70μ, some attaining 85μ in length.
11	---	Small germ tubes on many (60%). 14-20μ
8-10	---	---

This indicates that new growth is rapidly made in temperatures ranging from 22 to 29°C. At 27° to 29° the greatest quantity of growth was put out and at this temperature conidia, moisture conditions being optimum, are no doubt most efficient in infecting the host.

(Thermal Death Point)--The thermal death point of mycelium as grown in corn meal agar was determined by means of Novy's capillary tube method[62]. By crushing the agar of a culture with a sterile scalpel the mycelium was broken into relatively small bits. Five cc. of sterile distilled water were then added and the test-tube vigorously shaken making a suspension of the broken hyphae. Sterile capillary tubes, 4 inches in length, were dipped into this suspension which was permitted to rise 4-5 centimeters. The capillary thus charged was sealed at both ends in the flame of a micro-burner. Using heavy iron water baths, the temperatures of which were carefully regulated by the size of the flame below and by having the room as nearly devoid of air currents as possible, the capillaries were exposed as shown and then discharged onto corn meal agar in petri dishes.

Temp. °C.	Time of Exposure.				
	1 min.	2 min.	5 min.	10 min.	20 min.
40	+	+	+	+	+
45	+	+	+	+	+
50	+	+	+	-	-
55	-	Contaminated.	-	-	-
60	-	Contaminated.	-	Contaminated.	-
65	-	-	-	-	-
70	-	-	-	-	-

Using aerial mycelium and spores, scraped from cornmeal agar slant cultures, the above was repeated.

In this instance the temperature intervals were smaller and the capillaries exposed for 10 minutes in all cases.

Capillary No.	TEMPERATURES.							
	42.	43.5	45.	48	50	51	52	54
1.	+	+	+	+	+	+	-	-
2.	+	+	+	+	+	-	-	-
3.	+	+	+	+	-	-	-	-

For an exposure of ten minutes the thermal death point of the fungus mycelium, therefore, dies between 50 and 52°C. Two of the three ten-minute exposures at 51°C. were lethal.

It being found impossible to obtain a sufficient number of typical spores and conidiophores from pure culture to carry out the above work satisfactorily, these fruiting bodies were obtained from diseased leaves. They were removed with small forceps and placed in 2cc. of water in a preparation dish cover. The suspension thus made was taken up with capillary tubes subjected to temperatures as above described and discharged on cover slips and inverted over van Tieghem rings. The hanging drops were examined at intervals for three successive days and germination or its absence noted.

Conidiophores: Tube No.	TEMPERATURES °C. 42	43.5	45.	48.	50	51	52	54
1.	+	+	+	+	+	-	-	-
2.	+	+	+	+	+	-	-	-
Conidia Tube No.								
1.	+	+	+	+	-	-	-	-
2.	+	+	+	+?	-	-	-	-
3.				-	-	-	-	-
4.				-	-	-	-	-

Spores of the two of the four capillaries exposed to 48°C. for 10 minutes failed to germinate; conidiophores put out no new growth after the 51°C. exposure. While this experiment is faulty, a pure culture not being employed, it at least indicates something of the relative reaction to high temperatures of spores and the heavier walled mycelium.

Relation to Reaction of Medium.

Germination or non-germination of spores in several solutions which have a range of Hydrogen Ion concentration values give an indication of the relation of the parasite to the reaction of the medium.

As a preliminary test conidia were placed upon corn meal agar, distilled water and city water and the rapidity of germination noted by measuring the length of the germ tubes from time to time. The results are recorded on Plate X. In the same period of time the germ tube of the spores on corn meal agar attained greater

growth than those in tap water, those in tap water in turn growing faster than the ones in distilled water. The P_H of the agar was 6.7, that of the tap water 7.6, while the distilled water was P_H 5.7. This small preliminary test may give a slight indication of the effect of Hydrogen Ion Concentration and possibly of the food and character of the medium on germination.

Corn meal broth solutions were next adjusted to different hydrogen ion concentrations using hydrochloric acid and sodium hydroxide solutions, spores mounted therein and germination observed. The range as determined by Gillespie* colorometric method was P_H 4. to P_H8. Solutions having a higher H^+ concentration than P_H 5. inhibited germination.

To eliminate, more or less, the food and other factors, spores were placed in various buffer solutions of known hydrogen ion concentration and observed from time to time. The buffers used which were made according to the formulae of Clark** are the following:

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*Gillespie, L. J. The reaction of soil and measurements of hydrogen ion concentration. Jour. Wash. Acad. Sci. 6:7-16, Figs. 2. 1916.

Gillespie, L. J. and Hurst L. A. Hydrogen ion Concentration measurements of soil of two types: Carbon loam and Washburn loam. Soil Sci. 4:313-319. 1917.

**Clark, W. M. The determination of hydrogen ions 1-317, Figs. 1-38. 1920.

1. Primary potassium phosphate - Sodium hydroxide solution. Spores germinated in the entire range of P_H values given by this solution (P_H 5.8-8.0), the germ tubes attaining greatest length at P_H 7.

2. Acetic Acid - Sodium Acetate mixture P_H 3.6-5.6. No germination took place at any value. It was reasoned that the H.I.C. was so high as to be detrimental to germination, the salts themselves toxic, or both factors combined may be instrumental in preventing growth. That the high H.I.C. alone was not responsible in some mounts is shown by the third buffer used.

3. Potassium Acid Phthalate - Sodium hydroxide (P_H 4.-5.6). Conidia did not germinate below P_H 4.6 but did from P_H 4.6-5.4.

The conclusions to be drawn are evident. Many more buffer combinations must be tried to reduce the paradoxes due to the toxicity of the soil itself to a minimum. Quantities of corn meal broth or some other medium that proves satisfactory should be treated with various amounts of appropriate acid and alkali or suitable buffer, and the P_H values determined from time to time by the accurate electrometric method. Having thus obtained solutions of media having a long range of H.I.C. values, the organism should be placed in them and observed as to germination and growth. There are probably so many and complex factors to be taken into account to attain these hypothetical media as to make them impossibilities.

Trust could not be placed in such an unknown quantity as corn meal extract. Its reaction varies in solution with the progressive hydrolysis of its various components. Nevertheless, a procedure very roughly simulating the above was carried out.

To definite portions of sterile corn meal broth (P_H 6.7) were added varying quantities of autoclaved hydrochloric acid of known strength, the hydrogen ion concentration determined colorimetrically, making use of Medalia's* short-cut system. This was repeated adding aseptically to proportional quantities of the broth, corresponding amounts of the acid, giving stock quantities of the medium whose reactions were found to be from P_H 6.7-1.9. To obtain the alkaline range the process was repeated using sterilized N/4.0 NaOH. Five cc. portions of the stock solutions were added aseptically to sterile test tubes containing a small filter paper cone. Two transfers were then made for each P_H value, planting a small piece of fungus mycelium near the apex of each cone. The growth was observed from time to time and was begun February 1, 1921.

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* Medalia, L. S. Color Standards for the Colorimetric Measurement of H-ion Concentration P_H 1.2 to P_H 9.8. Jour. Bacter. 5:441-468. Figs. 1-4. 1920.

P_H values	Growth (1 week) 2/8/21		Growth 2/14/21		Growth 2/24/21		Spores.
1.9	-	-	-	-	-	-	
2.1	-	-	-	-	-	-	
2.3	-	-	-	-	-	-	
2.5	-	-	-	-	-	-	
3.0	-	-	-	-	+ Very slt. gth.	+	No normal spores found; a few atypical.
3.5	-	-	-	-	+ Slt. gth.	+	
4.0	-	-	+ Slt.	+	+ .6cm.	+	
4.5	+ .7cm.	+ .7cm.	+ 1cm.	+	+	+	
5.0	+ 1cm.	+	Sclerotium-like Mycelial Mats. + 1.cm.	+	+	+	
5.5	+	+	+	+	+	+	
6.0	+	+	+	+	+	+	
6.38	+	+	+ 1.3cm.	+	+	+	
6.7	+ 1.2cm	+	+	+	+	+	
6.9	--------Contaminated----------						Spores.
7.2	+ 1.2cm.	+	+ 1.5cm.	+	+	+	
7.6	+	+	+	+	+	+	
8.0	+ 1 cm.	+	Sclerotium-like Mycelial Mats. +	+	+	+	Largest number typical spores.
8.4	+	+	+	+	+	+	
9.0	+	+	+	+	+	+	

The inability of the fungus to grow at P_H 4, 3.5 and P_H 3 at first and the ability later to make appreciable growth at these concentrations of the hydrion show the varying character of the medium. This was due either to the effect of the fungus or the medium being unstable itself, or both. However, titration of the stock quantities at the end of the experiment showed that it had varied greatly. That which tested P_H 1.9 at the initial determination showed P_H 4 at the final, P_H 8.4 media had fallen to P_H 7.6, 2.5 had gained 2, and so on. Only those solutions near neutrality showed any constancy. We may say that the figures only in a general way indicate a tolerance of the organism toward the acidity and alkalinity of the medium in which it was grown. It is obvious that exact descriptive statements and conclusions are here impossible, due primarily to the varying of the very factor under consideration.

To observe something as to the ability of the fungus to change the reaction of the medium there were added to 5 cc. portions of corn meal broth (P_H 6.7) the necessary quantities of the various indicators and the organism planted therein. One week later the colors showed the H+ concentration of the broth to have increased to P_H 6.5, and two weeks after planting to P_H 6.1. Observations one and two weeks from the date of the last record showed P_H 6.2, and five weeks after planting 6.5.

The last record, made 10 weeks after the initial

was P_H 6.6.

Hydrogen ion concentration values for the juice expressed from celery leaves and carrot leaves and for water extracts of the celery powder used in the chemical determinations were determined electrometrically. The writer is indebted to Dr. E. C. Robinson of the Chemical Division of the Experiment Station for these data. These represent values for material from only two sources and are not in any way to be considered comprehensive. The leaves were removed from the plants, washed in distilled water, and then subjected to 250 pounds pressure in a hydraulic press. The dry celery powder was moistened with an equal volume of doubly distilled water and before extraction placed in an ice box at a temperature of 12°C. for 16 hours. Two pressings of the material were made, the extract obtained in the second being labelled "residue" in the following:

Material Expressed Juice from:	P_H	Material Expressed Water extract:	P_H
Carrot leaves	6.36	Healthy powder	5.33
Celery leaves	6.42	Blighted powder	6.08
Carrot residue	5.68	Blighted powder residue	5.00
Celery residue	5.66		

The H.I.C. of this material was considerably higher than that at which the fungus made its best growth in culture. It is believed that the figures for "residue" more nearly represent the correct values for

the true sap of the plant leaves, the liquid from the first being diluted by the wash water. More determinations should be made as more material becomes available, dry leaves from living plants that for several days previous have been washed with distilled water being used. Heart leaves and mature leaves should be determined separately.

Oxygen Relations.

To study the relation of the amount of air to the quantity of growth on corn meal agar slants, test tubes of various lengths and the same diameter were used. The tubes were closed with rubber stoppers and sealed with paraffin. Comparing the longest and shortest tubes a small difference in the amount of growth was noted. There was, however, no appreciable difference between the other tubes and checks all compared.

Plantings were then made on various media and covered with paraffin oil. The fungus was apparently unchecked, it making a white cottony growth, most abundant at the plane of contact of the medium with the oil. In this plane the most mycelium was found on the glass of the enclosing test tube.

Much further investigation was evidently necessary. Making use of the H-tube method* the oxygen of the air

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*Giltner, W. A laboratory manual in General Microbiology i-xiv, 1-422, Figs. 1-74. 1916.

above corn meal agar slants and carrot plugs was absorbed by means of alkaline pyrogallol, the tubes being quickly and carefully closed by rubber stoppers and paraffin. On the corn meal agar and all but one of the carrot plugs absolutely no growth took place. One carrot culture showed a very slight growth at first but this was soon checked. In the control tubes, which were treated the same as the others except that the chemicals were not permitted to come in contact with each other, the cultures made an abundant growth completely covering the medium. The tubes were observed at intervals for several months. The stoppers were then removed and the mycelium plantings broken into pieces and placed upon freshly prepared corn meal agar slants. These were observed for two weeks, no growth taking place. The mycelium was evidently lifeless.

It is known that pyrogallic acid under the conditions of this experiment gives off a trace of carbon monoxide gas, which in sufficient quantity is detrimental and in larger proportions even lethal to many forms of life. To obviate this possible objection test tube cultures were placed in atmospheres made of mixtures of carbon monoxide, carbon dioxide and air as shown in the tabulation. These atmospheres were obtained by displacing water in liter bottles with CO and CO_2 gases prepared by heating together oxalic and sulphuric acids.

Where the CO_2 was not wanted, the mixture of the two gases was bubbled through strong sodium hydroxide solution. Three corn meal agar slant cultures were first placed in the inverted bottle, the bottoms of the test-tubes resting on the flange of the bottle and the cotton plugs pressing tightly against the bottom. The mouth of the bottle was then put into a battery jar of water and the water made to rise to the mouths of the test tubes by removal of the air. This was accomplished by sucking out the air with a U-shaped glass tube, one arm of which extended upward to the bottom of the inverted bottle. In this way practically all the air, except that remaining in the test tubes, was removed and the water ready to be displaced by the gas desired. The desired atmospheres obtained, the bottles were closed under water by means of rubber stoppers. The tubes of each bottle were then shaken off the flange onto the bottom of the stopper, thus permitting free diffusion of the air of the tubes with the surrounding gases.

Atmosphere	6 days' growth			Growth after removal.		
	Tubes			Tubes		
	1.	2.	3.	1.	2.	3.
1/2CO-1/2CO_2	-	-		-	+	
1/3CO-1/3CO_2-1/3Air	Diam. .8cm.	.8cm.	.8cm.	+ Pink color develops.	+	+
1/2CO-1/2 Air	+	+	+	+ Pink color develops.	+	+
1/3CO-2/3 Air	Diam. 2.0cm. Pink.	+ Pink.	+ Pink.	----PINK-----		

All the plantings grew except those in an atmosphere of half CO and half CO_2, (minus, of course, the 30-40 cc. of air contributed by the volume of each of the test tubes). The purpose of the experiment was thus accomplished. Carbon monoxide in relatively high concentrations is not inhibitive to the growth of the fungus.

It was noted in the above that a pink coloration appeared in the agar immediately below the fungus growth. This color extended into the agar about a centimeter. It gradually disappeared as the fungus made further growth although some remained as long as the cultures were kept in atmospheres containing CO. Microscopic examination of sections of the pink agar did not reveal the presence of any fungus hyphae. This pigment had on several previous occasions been noted under young cultures which to all knowledge had not been exposed to any gas. It was thought, however, that possibly some illuminating gas may have entered the tube during flaming. Accordingly an experiment which had the dual purpose of revealing something of the relation of the fungus to oxygen and oxygen diluting gases, and of the production, if possible, of the pink coloration by means of illuminating gas was carried out. The method used was exactly as described under the CO experiment. The gases used and their proportions were as indicated in the following table. The work was begun March 6, 1921 and results recorded on the three dates shown.

ATMOSPHERE	GROWTH		
	3/9/21	3/13/21	3/18/21
All CO_2	Slight. No pink color.	Growth very slight. Concentric darkened rings.	As 3/13/21. Shallow.
3/4 CO_2, 1/4 Air.	1/4 of check. No pink.	As 3/9/21.	do.
1/2 CO_2, 1/2 Air.	do.	do.	do.
1/4 CO_2, 3/4 Air.	1/2 check. No pink.	do.	do.
All N_2 *	Good growth No pink.	Thin spreading growth. No pink.	1/2 check. Many dark brown sclerotium-like mats on surface.
3/4 N_2, 1/4 O_2.	As check. No pink.	Check.	Check exactly.
1/2 N_2, 1/2 O_2.	Do.	Check.	2/3 check. No pink.
1/4 N_2, 3/4 O_2.	Good growth Slightly pink.	Check.	Check size. 1 cm. in med. Intense brown color at inner surface of growth.
All O_2 *	Good No pink.	As check but slightly pink.	3/4 check. As above. No pink.
All Gas* (Lab.Illum.)	No growth. No pink.	No growth.	1/4 check. Very pink.
3/4 Gas, 1/4 Air.	+2/3 check Slt. pink.	2/3 check. Pink.	2/3 check. Pink.
1/2 Gas, 1/2 Air.	1/2 check. Very pink.	1/2 check. Pink.	3/4 check. Pink.
1/4 Gas, 3/4 Air.	3/4 check. Very pink.	As check. Pink.	As check. Pink.
Check- All Air.	.7cm.	1.5 cm.	7/8 surface covered.

All cultures had a few atypical spores.

*Except the air contained in the test tubes.

The presence of the pink in the culture agar remained as long as the tubes were kept in the illuminating gas. The slight pink which was seen at first in other atmospheres disappeared while the tubes still remained in those gases. The fungus may have simply overshadowed it, used it in its metabolism, or excreted something which changed it into a leuco-form. Judging from this and the CO experiment it seems there is a possibility that the CO gas is responsible for the production of the color by the fungus. The CO content of the illumination gas used was 4.4%. The poison gas even a short exposure to it, probably so influences the organism that it excretes the something which is either colored or which reacts with the constituents of the nutrient producing the pigment. Time did not permit of more investigation of this interesting phenomena.

Another experiment was performed to learn more concerning the relation of the fungus to atmospheric oxygen. Four corn meal agar slant cultures were placed into each of several chancel flasks and the air exhausted by means of a May-Nelson vacuum pump. This quickly lowered the pressure to 3 mm. mercury, at which point it was held for 10 minutes. The glass cock was then closed and the pump stopped. Two cc. of water were placed in the bottom of each flask before evacuation to prevent excessive drying of the agar. This gives practically an atmosphere of water vapor. The plantings

were examined from time to time and no growth was observed. The checks showed normal growth, being 1.cm. in diameter 4 days after the planting and two weeks later completely covering the agar surface. Ten days after the beginning of the experiment one of the flasks was tested with a mercury manometer and was found to have maintained its vacuum, the reading being 20 mm. or about the vapor pressure of water at the existing temperature. Air was admitted to this flask and its plantings examined 3 days later. A good growth was evident in all three tubes. For ten days the fungus had either been able to remain alive on the very small quantity of oxygen not removed from the agar and surroundings, or it is able to respire anerobically for some time, or it can remain dormant for this period. The air was again exhausted from the flask and growth stopped. It was reopened two weeks after the second evacuation. At the same time another flask, whose vacuum had been maintained continuously for 26 days, was tested and opened. Growth was not resumed in either flask, showing that two weeks exposure to these conditions is fatal. The last flask was tested after a month's time and although it had leaked until the pressure was only 20 mm. lower than atmospheric pressure, the fungus in no one of the tubes had grown. How long it had maintained a good vacuum it is impossible to say, but it probably leaked very gradually.

Further experiments on the effects of air exhaustion and reduced pressure, more exact and conclusive, were not carried out because of lack of time.

These experiments demonstrate that the fungus, Cercospora apii , while able to maintain apparently normal growth in small quantities of free oxygen, is killed by the almost complete absence of this gas. Tolerance and reaction to the poisonous carbon monoxide gas was also noted.

Resistance to Desiccation.

To determine the effect of drying on the life of the fungus a spore mycelium suspension was made as described under the Thermal Death-point experiment. Using aseptic precautions, small, loose wisps of absorbent cotton were dipped into the suspension and then plac . in sterile petri dishes. Several one-inch cover slips were covered with the suspension and placed in another petri dish. Both petri dishes were placed in a desiccator where they were allowed to remain until just the last trace of visible moisture vanished. The dishes were then removed and each, for protection against contamination, was placed in a cover of a deep culture dish which had been lined with cotton. The covers of the petri dishes were propped up on one side by means of sterile pieces of wire. Desiccation was allowed to proceed at room temperature.

At the time intervals shown in the following table bits of the glass circles and cotton wisps were planted on cornmeal agar slants.

Glass Surface	Time of Drying - Days											
	4	8	16	22	32	38	68					
Set No. 1	+	+	+	+	+	+	+					
2	+	+	+	+	+	+	+					
Cotton Wisps	8	28	30	62								
Set No. 1	+	+	+	+								
2	+	+	+	+								

+ indicates growth.

It is worthy of note that successful plantings were made from cultures which had been permitted to dry for fourteen months. The above results show simply the comparative tolerance of the fungus to desiccation when less protected.

To test the effect of drying on the conidia several blighted leaves were put in a desiccation for 36 hours and then removed to a petri dish where they were permitted to dry at room temperature. One edge of the petri dish cover was raised. At intervals of time shown below spores were removed with forceps and placed in tap water on cover slips and germination observed as in the Thermal Death Point experiment. On the 15th day spores and conidiophores were mounted also from garden material that had been pressed last fall, placed in a pasteboard box and set in the specimen room. Both conidia and conidiophores germinated very readily. The material in the petri dish was consequently

abondoned. Fruiting bodies were then taken from old herbarium material, dating from 1889 to 1912, and germination attempted.

Mount	Time of Drying - Days										Mounts of Material 1889-1912	
	1.5	3	4	6	7	9	13	170				
No. 1 Germination	+	+	+	+	+	+	+	+			--	--
No. 2 Germination	+	+	+	+	+	+	+	+			--	--

These results indicate great tolerance of the conidia to desiccation and the practical application of this to dissemination and infection is obvious. There were no indications whatsoever of living fungus in the mounts from the old herbaruim specimens and, altho allowed to remain several days in the moist chambers no conidiophore or spore produced a germ tube.

Dissemination.

That air currents are to be considered as the main agents in the dissemination of Cercosporae and other fungi of the moniales type is indicated in various writings.[32] With a view toward determining the presence or absence of forcible expulsion of Cercospora spores from conidiophores the following experiment was made. Using paraffin, corks were fastened to the lower surface of the tops of deep culture dishes. To the corks, about 5 mm from the inner glass surface were pinned small pieces of diseased leaves bearing an abundance of conidiophores and spores. Melted cornmeal agar was then poured into the bottoms of the dishes and the tops replaced. Examined with the microscope the dry fruiting structures present the appearance of irregularly twisted, collapsed

rubber tubes.[16] Absorbing the moisture given off by the agar they rapidly become turgid and appear as when mounted in water. On being removed to the dry air again they very readily resume their original shrivelled appearance. Two microscopes were used in the observations. Examinations were made at varying intervals of time for a period of 36 hours. Spores were not observed to leave the conidiophores. The dishes were then set aside and examined a week later to see if any cercospora colonies formed on the agar below. One colony had formed immediately below the leaf in one dish. The others, however, showed no growth. Apparently this fungus is provided with no means for the forcible expulsion of its conidia.

Wind distribution was demonstrated as follows: Pieces of blighted leaves were fastened to the inner top surface of a deep culture dish as described above. The upper surface of the glass immediately over the conidia was cooled by other evaporation. Air was then blown onto the leaf by using a bent glass tube which passed down around the bottom of the microscope stage and up into the dish where it ended in a capillary opening using low power of the microscope and getting the lower surface of the glass in focus, Spores were observed to appear and stick in the condensation moisture which collected on the cool glass.

It should here be repeated that fungus conidia which had overwintered in the garden were found incapable of germination the following spring. They are probably very rarely able to withstand the vicissitudes of winter conditions, being designed for distribution during the

season in which they were produced.

Varietal Resistance.

The literature shows evidence of some little observation on the resistance or susceptibility to the fungus of a few celery varieties. Rolfs (1896) wrote that "self blanching varieties suffer worse than other kinds."(71) Davis (1893) recorded that "White Plume and Golden Self Blanching suffered more than other varieties. Hendersons New Rose has been entirely free from blight.(15)" Taubenhaus (1918) stated that "Boston Market and Gold Heart should be avoided because of their susceptibility to the disease. The white Plume seems to be resistance.(87)" Jones (1910) reported a Milwaukee celeriac grower as losing 90% of his crop in 1909.(61)"

Although observations, both in the field and the green house, were numerous and records were made, they failed to reveal any marked resistance of any variety to the disease. Twelve different varieties of celery were used in these tests and there could be noted in the susceptibility to Early Blight no difference between the green and self blanching kinds or between celery of any one or several varieties.

Control

Being a leaf spot disease it would seem that Early Blight would be amenable to control by a spraying of the plant foliage with some suitable fungicide. The published results of early trials are somewhat confusing. Halsted (1891) obtained good results with ammoniacal copper carbonate.(36)

Scribner (1886) found lime and sulfur of no value. He stated that "liver of sulfur may arrest its progress," but that he hesitated to recommend copper salts for "hygienic reasons".(78) Sturgis found sulfur dust superior to liver of sulfur solutions and the copper salt sprays.(82-85) Atkinson (1893) recommended the use of Ammoniacal copper carbonate.(2) McCarthy (1895) in one of his six rules for combatting the Blight wrote that "liver of sulfur or sulfur flour may be applied in the seed bed after transplanting."(58) Rolfs (1898) stated that the plants being "drenched twice a week with liver of sulfur solution gave complete control," as did the copper fungicides which had the disadvantage of coloring the foliage.(72) Hume (1900) preferred Bordeaux to potassium sulfide and ammoniacal copper carbonate solution.(39) Galloway (1893) wrote Davis of the Michigan Station that celery on upland soil blighted badly in spite of the fact that the leaves were kept covered with Bordeaux, Ammoniacal copper carbonate and other fungicides, but that celery so treated was less attacked than the checks.(15) Lockhead (1900) recommended spraying at two week intervals with ammoniacal copper carbonate.(54)

Later investigators, as indicated in the pathologists reports, have come to the opinion that Bordeaux is the most convenient and effective fungicide to be used in combatting this disease. The reports are varied, "Bordeaux unsatisfactory", "Bordeaux and ammoniacal copper carbonate not very effective," "Bordeaux gives partial control," "Bordeaux good," and "Bordeaux gives excellent results," being some of the statements. The fact that California and Florida celery received here

generally show Early Blight spots in spite of the leaves being coated by Bordeaux, indicates that the spray as applied does not give complete control.

Spray experiments were run in the greenhouse with the object of determining the relative value of several fungicides in preventing Early Blight spotting. Preliminary to these experiments spores were placed in a large variety of chemicals (25 of them) and observed for germination. Copper filings placed in distilled water, 44-50 Bordeaux mixture, ammoniacal copper carbonate (5 oz. $CaCo_3$ and 3 pints 26° Baumé Ammonia to 50 gallons of water mercury cyanide and bichloride (1-1000), a number of other salts whose very poisonous properties or ready solubility made them impossible for use as sprays, and copper sulphate in dilution as high as 1 in 128,000 of water were inhititive to the production of germ tubes. Lime Sulfur (34° Baumé made 1-45) and liver of sulfur (4 oz to 10 gal. water) did not permit germination while the spores were in contact with the fresh solution, but on being allowed to dry and then wet again did not prevent some conidia from germinating. This may partially explain the spraying results obtained with potassium sulfide solution. Sulfur dust did not appear to inhibit germination. It is difficult to understand the good results obtained by Sturgis with sulfur flour.(82-85)

Lime-sulfur (1-40 and 1-45) was found injurious to the celery leaves causing severe burning. It has, moreover only a temporary effect in inhibiting conidial growth.

Of the several small spray experiments run in the

greenhouse the following is an example. Sets of six plants each were thoroughly sprayed, top and bottom of the foliage, with the respective fungicides as shown in the table, allowed to dry, and placed in the rain chamber. They were then sprayed with a spore-mycelium suspension made from 8 cornmeal agar slant cultures and moistened from time to time with the mist. Seventeen days later the results expressed in the number of cercospora spots per plant, were recorded. The identity of about half of the lesions was confirmed by microscopic examination.

Spray	Plant Number						
Bordeaux	1	2	3	4	5	6	6'
No. Spots	1	2	-	3	2	10	12
Am Cu CO_3	7	8	9	10	11	12	
No. Spots	-	18	4	27	12	30	
K_2S	13	14	15	16	17		
No. Spots	33	26	50	20	16		
Lime Sulfur	18	19	20	21	22	23	Bad burns on all
No. Spots	10	12	17	8	3	25	
4 Checks	Showed 40-60 spots each.						

The relative value of the four fungicides, in this small experiment at least, is at once seen. The two copper sprays have a decided advantage over liver of sulfur and lime-sulfur solutions. Because of the growth of the celery, exposing new areas untouched by spray, one should not expect absolute freedom from the disease. However, it is evident that benefits are derived thru a thorough application of an efficient fungicide. In regions where this disease is known

to be bad and during seasons when the climatic conditions, as described are unfavorable for celery, spraying with 4-4-50 Bordeaux mixture is advisable. The applications should begin early in the summer, should be very thorough, covering both leaf surface, and at intervals of a week or not less than ten days. This carries double protection as the Septoria blight also is thus not permitted to obtain a foothold.

The observation of many is that Early Blight is very uncommon on celery grown in rich soils that have plenty of water. Celery, grown for so many years on the cool, moist Kalamazoo muck has rarely been badly attacked by this fungus. Calloway's experiments are very indicative.[30,31,13] The celery grown on a cooperative experimental plot at Mason, which was unusually large and thrifty last year, had been watered by flooding. It showed no evidence of Early Blight. That raised at East Lansing which was watered by the overhead system, produced large edible stalks in spite of the leaves being badly spotted by the disease. It appears that, given an adaptable, fertile soil, water is a very important factor in controlling Early Blight. Where it is possible to irrigate thoroughly or flood the land during the hot, dry parts of summer, spraying may be unnecessary or not of sufficient value to be economically important. However, as the season progresses toward the time of harvest, spraying for the purpose of producing unspotted, more attractive foliage, and more important, as insurance against the destructive late blight, is advisable. To produce a cleaner, more marketable product, ammoniacal copper carbonate (formula 4 oz $CuCO_3$ and 3 pints of 26° Baumé ammonia to 50 gal. of water.) may be substituted for the Bordeaux mixture in the last two sprayings.

After harvest, to remove the big source of infection

to the following crop, the field should be cleaned of all refuse. Infected material would best be burned.

Summary

Celery Early Blight is a disease of celery and celeriac known practically wherever these plants are grown, in Europe and America. It is caused by a specific fungus, Cercospora apii, which was first described by Fresenius in 1863. The disease appears as suborbicular spots on the leaves and also, but not commonly, on the stems. In times of high humidity the lesions are covered with the fruiting bodies which give a velvety appearance.

Given high temperatures supplemented by heavy evening dews, a droughty soil and consequently plants of low vitality, the disease may become of great economic importance. Injury as high as 100% and losses up to 30% of the edible product due to the necessary excessive trimming, may result.

The fungus enters its host thru open stomata by means of conidial germ tubes. It grows intercellularly, absorbing nutriment from the surrounding tissue by means of haustoria. As the food components of a lesion become exhausted, the fungus thrusts thru the stomata in fascicles long fruiting hyphae, each of which bears a long obclavate spore at its end. When mature these spores are easily detached and distributed by air currents. Wind distribution was demonstrated.

Chemical analysis of the nitrogen constituents of the sound and blighted leaves revealed large differences which may indicate something of the metabolism of the fungus and the effect on the invaded host.

The organism grew well on all media employed,

including sterile muck and refuse. It grew in the presence of a hydrogen ion concentration as high as P_H 3.5 and as low as P_H 9. Spores were capable of germination in the range P_H 4.6 to P_H 8. in some buffer solutions.

A study of the temperature relations of the fungus shows that the parasite makes its largest vegetative growth between 25° C and 30° C, that an exposure of 48° C for 10 minutes is fatal to the conidia and 51° C to the conidiophores and mycelium. Spores germinated best at 27-29° C. There was no germination after 12 hours exposure at 42° C.

Fourteen months desiccation was not lethal to mycelium and spores were found capable of germination after six months drying at room temperature.

Though requiring some free oxygen for life, the fungus is very tolerant in this regard.

A study of infection on 12 different varieties of celery showed no varietal resistance to Early Blight .

Control is a matter of keeping the plants vigorously growing; that is, on soil of high fertility and good drainage and having an abundance of moisture. This should be supplemented by spraying with Bordeaux mixture as indicated.

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Description of Plates.

Plate I. Stem bearing naturally infected leaves. Photo by Dr. G. H. Coons.

Plate II. Plants diseased as a result of shaking over-wintered diseased material over them. Photo by Mr. R. Nelson.

Plate III. Naturally infected plant showing broken diseased outer stems. Photo by Dr. G. H. Coons.

Plate IV. Corn meal agar cultures of the Celery Early Blight organism, Cercospora apii Fres. Photo by Mr. J. E. Kotila.

Plate V. Cultures on sterilized muck and refuse. Photo by Mr. R. Nelson.

Plate VI. Camera lucida drawings of-

Fig. 1.-Germ tubes from mycelium entering stoma of celery leaf.
Fig. 2.-Conidiophores of the fungus protruding through a stoma (young stage).
Fig. 3.-Mycelium from corn meal broth culture having H-ion concentration P_H 8.
Fig. 4.-Mycelium from diseased leaf showing knob-like haustoria.
Fig. 5.-Gnarly, tortuous mycelium from culture on Meliotus stems.
Fig. 6.-Sclerotium-like bunches of mycelium formed in corn meal broth P_H .5 and P_H 8.
(Photo by Mr. J. E. Kotila).

Plate VII. Camera lucida tracings of conidiophore fascicles (advanced stage) showing spore (Sp.) germinating in situ, and new conidiophore growths from each of which another spore would have been abscissed.

N.C.G. = New Conidiophore Growth.
G.T. = Germ Tube.

Small letters refer to time showing rate of growth. The mount was made at 9:45 A.M.

Plate VII, cont'd.

a - 11:00 A.M.
b - 12:00 M.
c - 1:00 P.M.
d - 2:00 P.M.
e - 2:30 P.M.
f - 4:15 P.M.
g - 4:30 P.M.
h - 8:00 P.M.
i - 5:00 A.M. (next day).

(Photo by Mr. J. E. Kotila).

Plate VIII. Camera lucida drawings of-

Fig. 1.-Base of conidiophore fascicle (Oil immersion).
Fig. 2.-Tracings of conidiophore tips showing characteristic scars and new growth (Oil immersion).
Figs. 3, 4, 5.-Showing attachment of conidium to conidiophore (Oil immersion).
Figs. 6-12.-Tracings of permanent mounts (paraffin method), showing conidiophores, spores, scars and geniculations.
(Photo by Mr. J. E. Kotila.)

Plate IX. Camera lucida drawings (high power) of-

Figs. 1-5, 8,9,10.-Germinating conidia.
Figs. 6,7.-Germinating conidiophores.
Fig. 11 (a to g, i) Forms of conidia.
Fig. 11h.-An atypical spore from pure culture.
Fig. 11j,k.-Two attached conidia showing tendency toward chain formation.
(Photo by Mr. J. E. Kotila.)

Plate X. Camera lucida drawings (high power) of germinating spores, showing relative rate of growth of germ tubes in tap water, distilled water, and corn meal agar. The experiment was begun at 9:00 P.M. and the temperature was 28-30°C. The small letters refer to time:

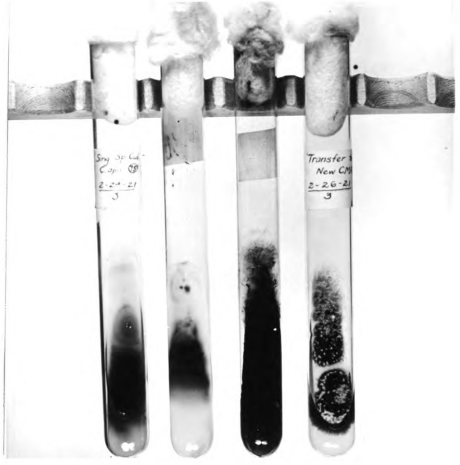
a - 10:30 P.M.
b - 12:00 P.M.
c - 2:00 A.M. (the following day)
d - 5:00 A.M.
e - 7:00 A.M.
f - 7:30 A.M.
g - 9:00 A.M.
h - 9:45 A.M.
i - 11:00 A.M.

PLATE I.

PLATE II.

PLATE III.

PLATE IV.



1915
Isolation

1921
Isolation

PLATE V.

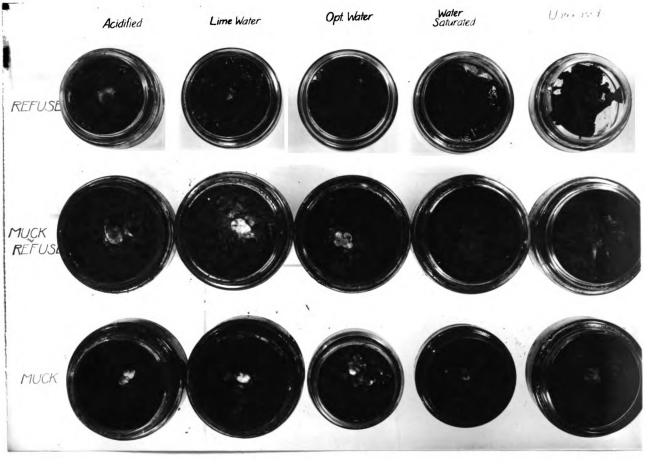
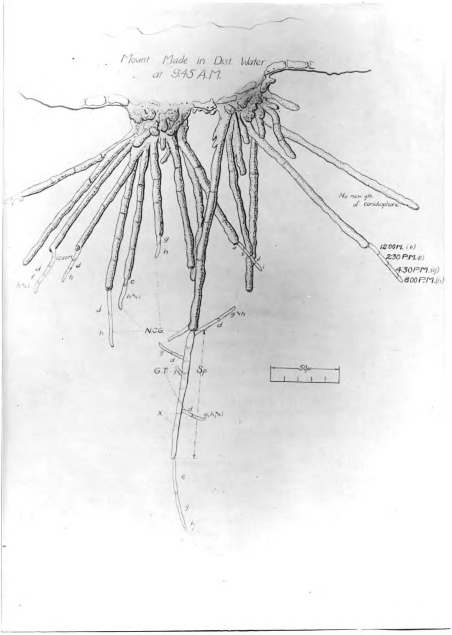

Acidified
Lime Water
Opt. Water
Water Saturated
REFUSE
MUCK
REFUSE
MUCK

PLATE VI.

PLATE VII.



Mount Made in Dist. Water
at 9:45 A.M.
No new gth. of conidiophore.
12:00M. (b)
2:30 P.M. (c)
4:30 P.M. (g)
8:00 P.M. (h)
N.C.G.
G.T.
Sp
50μ
X

PLATE VIII.

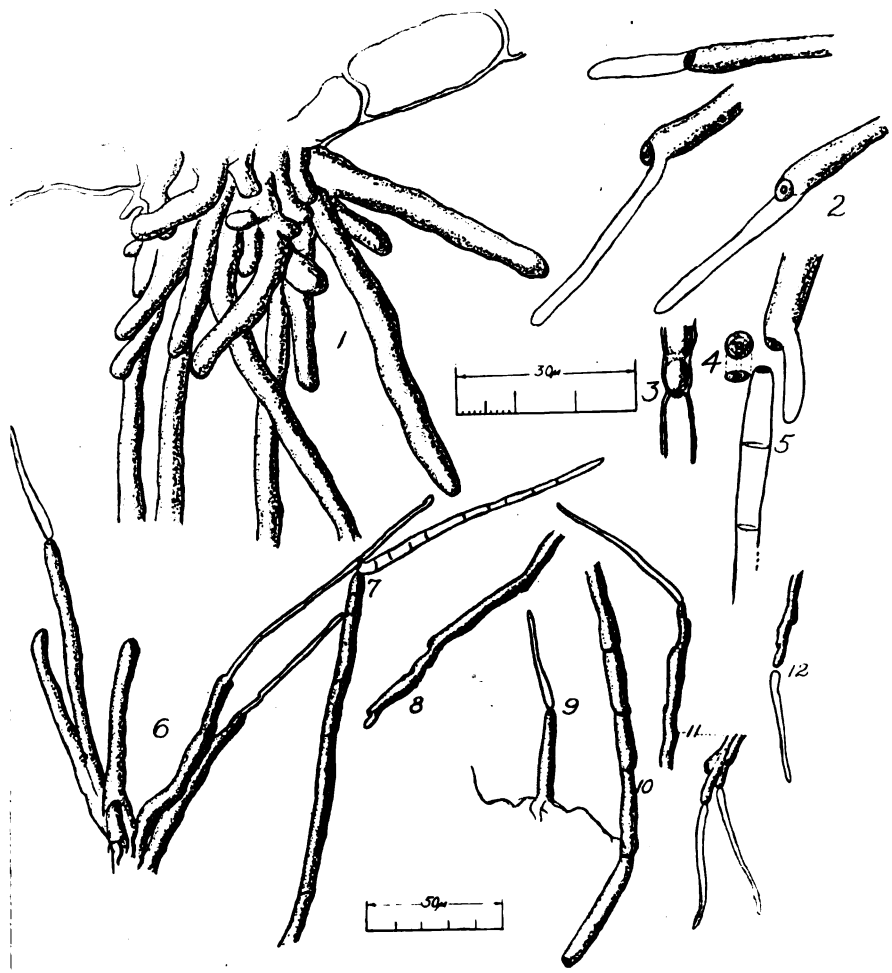


PLATE IX.

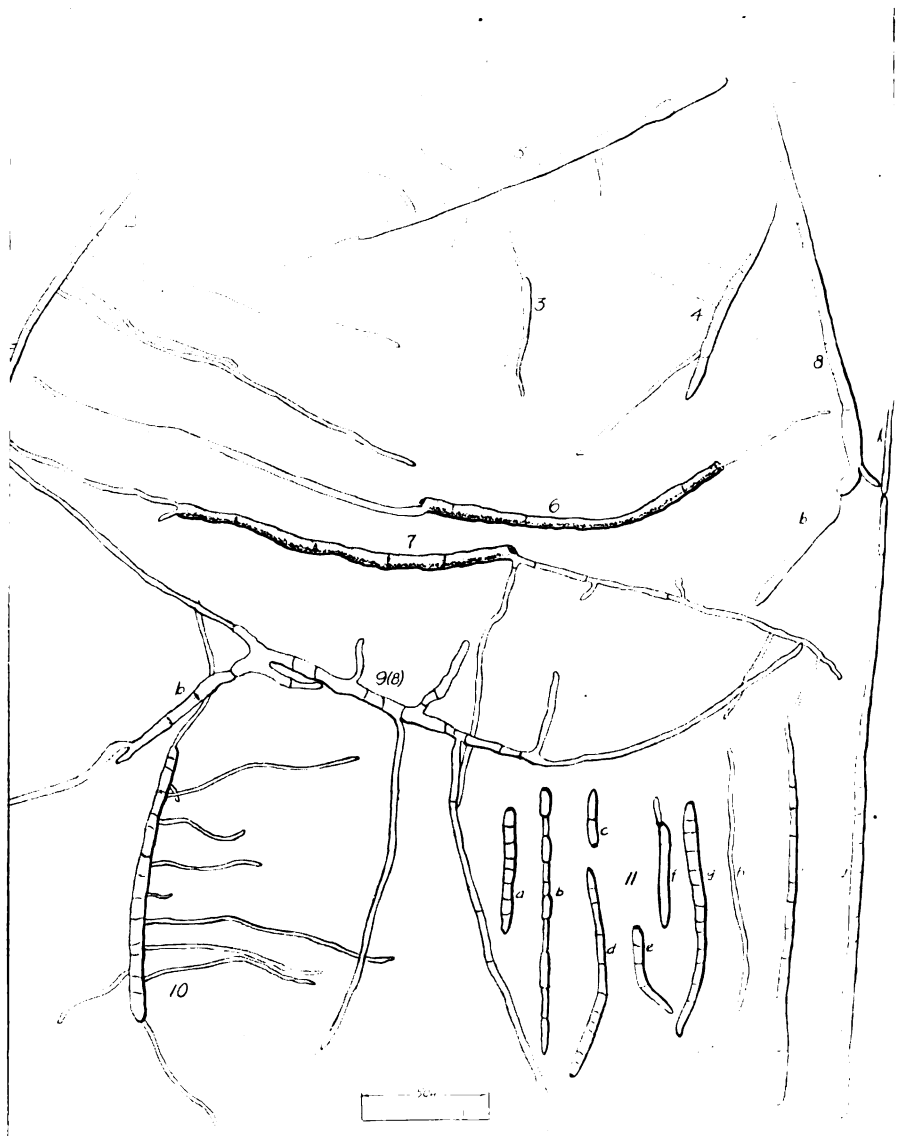


PLATE X.