A STUDY OF TWO SEPTORIA
LEAF SPOTS OF CELERY

THESIS FOR THE DEGREE OF M. S.

L. C. Cochran

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Introduction

Celery is one of the most popular salad crops and is becoming a more common article of diet each year. Formerly it was considered a luxury, but now it is available practically throughout the year. California leads in production with approximately one-fourth of the total acreage. Other states of importance are New York, Michigan, Florida, and New Jersey. Celery growing in these states is mostly limited to regions where the soil type is peat or muck. It is this type of soil which makes celery growing important in Michigan. Ottawa, Kalamazoo, Kent, and Muskegon are the counties in which most of Michigan's celery is grown. Although there are nearly three million acres of land adaptable to celery growing in Michigan, the crop has been grown in comparatively small areas for many years without regular rotation of the crops on the land. This fact along with the use of infected seed has made celery growing in Michigan a battle against disease. The most important celery disease in the United States from the standpoint of spread and actual loss due to the ravage of the parasite is "Late Blight", caused, as will be shown later, by two different species of the genus Septoria.

Observations made by Ray Nelson of the Department of Botany of Michigan State College some years ago in the Kalamazoo celery marshes revealed the presence of two distinct types of spots due to Septoria on celery. Only one Septoria has been listed (2)* as a parasite on celery in the United States. Because of the difference in the size of the spots

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*This number in parenthesis refers to the list of Literature Cited at the close of the paper.

produced on the celery leaves, these will be referred to as the large spot and small spot form. Since the difference between the two types of spots were so marked and since only one Septoria was listed as a parasite of celery, the following studies were undertaken.

Name of the Disease

As will be shown further on, both types of disease have been confused so that the name applied may refer now to one type, now to the other. In the following few paragraphs the two are treated as one disease. The disease has been given many names. Salmon (34 P. 414) lists celery blight, rust, leaf scorch, leaf blight, late blight, and blight as common names given to the disease in England. Chittenden (7) also in England says that the disease has been known as leaf spot, rust or blight. Krout in New Jersey (24 P. 595) calls the disease late or black blight. Other common names applied to the disease by gardeners and scientific workers are Septoria, Septoria spot, black spot, spot disease, yellows, pepper blight, pepper spot, cool weather disease, etc. The most popular and most widely used name among English speaking people is probably late blight.

The cause of the disease is a fungus which belongs to the Genus Septoria. Two distinct species have been isolated and shown to produce characteristic disease. Both of the organisms will be taken up in detail later.

History of the Disease

Chupp (8 P. 89) says that late blight of celery may have been observed as early as 1840, but the first published report as a distinct disease was not made until 1890 when the casual organism was found in

Italy by Briosi and Cavara. The first report of the disease in the United States was by Chester (6 P. 372) in 1891. Chester discovered the malady on diseased specimens sent him for examination and identified it by comparing them with specimens collected by Briosi and Cavara in Italy.

Duggar and Bailey (14) reported the disease as the cause of enormous losses to celery in Central and Western New York in 1897. From this center, according to Rogers (32 P. 89), the disease spread westward until in 1897 it was reported as serious in California. A few specimens of the blight had been found in California celery fields during the two years previous to 1897. Rogers reports the disease from all parts of California in 1907 and 1908 with great losses. California has suffered especially because of the large acreage grown there and because most of the celery is shipped to other parts of the United States. Late blight is not only a serious disease of celery in the field, but also the cause of much loss on celery in storage and process of shipment. This was shown in California in the epochal winter of 1908 when 1950 cars of celery, valued at 550,000 dollars, were lost. Most of the loss was on celery in the field, but a large part of the loss was after the celery had been started to market.

Link and Gardner (26) in a survey of market diseases at several large markets, report late blight as the cause of heavy losses on celery and celeriac. Losses on California and Florida shipments were especially heavy because the temperatures of refrigeration in transit is, although low enough to normally keep the celery in good shape, optimum for the growth of the fungus.

Pethybridge (29) and Chittenden (7) report the disease as serious in all parts of the British Isles in 1910. Late blight was reported from

France in 1894 by Prillieux and Delacroix (31) and from Germany in 1896 by Sorauer (38). Klebahn (21) in 1910 reports celery leaf spot serious in all parts of Germany. According to Klebahn (21) Rostrup in 1893 reported the disease from Denmark.

Coons and Levin (11) and Coons (9) report late blight as the most serious disease of celery in Michigan. The disease was serious in 1915 and the loss was estimated at over 1,000,000 dollars for Michigan alone.

Chupp (8) and Owens (28) in their textbooks state that at the present time late blight is the most cosmopolitan of all celery diseases. It can be found in all regions where celery is grown. Thus in forty years since the first report of the disease on celery in Italy, it has spread to all parts of the world and is the most serious disease of celery.

A new angle on late blight of celery is revealed in the report of Dorogin (13) in 1915 on the occurrence of the disease near Petrograd, Russia. He found that there were two separate organisms causing two distinct forms of the disease. He identified one with the typical form reported from other regions but considered the other a new species and gave it a new name. Both of these forms will be taken up later. Foster and Weber (15) of Florida in 1924 also reported two forms of leaf spots due to Septoria on celery. They regarded these as strains of the organism described by Briosi and Cavara and not separate species.

Klebahn (21) was the first to mention the possibility of carrying the disease on the seed. He discovered the fruiting bodies of the fungus on the pericarp of the celery fruit and showed that they contained viable spores. This method of carrying the disease producing organism makes it an easy matter for initial infection on the young seedlings to occur.

Chittenden (7) and Pethybridge (29) were probably the first to prove that the leaf spot fungus may be carried over from one year to the next as spores in such infected seed. They found that the fungus could be entirely eliminated from seed by the use of a germicide such as weak formaldehyde. They also confirmed the observation of Klebahn that the fungus once established could winter over on celery refuse and recommended the use of Bordeaux mixture in the same manner as used for late blight of potatoes. One other source of infection is the spread from wild celery.

Krout (24) (25) reported the presence in New Jersey of Septoria fructifications on seed in 1916. In experiments with the use of chemicals he reports it is very difficult to kill all the spores without injuring the vitality of the seed. He found that the spores were not viable on celery seed after two years and spores on the peduncles of the celery seed were all dead after three years, while celery seed germinated from fifty to sixty per cent at four years of age. After five years the germination of seed was low and weak.

Voglino (45) found that young seedlings set in pots of soil, on the surface of which was placed a layer of manure to which a viable suspension of Septoria spores had been added, contracted the disease and developed characteristic spots. He attributed this to air currents which he thought carried the spores to the plants.

Newhall (27) in 1926 definitely proved that young celery plants may become infected in the seed bed when there was no viable source of the disease carried on the seed. He planted seed on muck which had just previously grown a crop of celery and on new muck where celery had never been grown. Infection occurred on both lots, but seven times as many spots

were found on the plants grown on the muck which had just previously grown a crop.

The Name of the Causal Organism.

Septoria on celery may date back to the origin of celery according to Chupp (8). Celery, according to Thompson (44), came from the wild celery by selection. This wild celery has been found in Sweden, south to Algeria, Egypt, and east to India. A wild form may be found growing in New Zealand and some in California. The present celery varieties which are of economic importance have been developed by selection and crossing.

Pethybridge (29) while traveling in Ireland found wild celery infected with a Septoria. The Septoria macroscopically was very different from the type found on cultivated celery, but when transfers were made from the wild to the cultivated type, the typical disease was produced on the latter. He did not try transferring the fungus from the cultivated to the wild celery. He suggested that these wild plants possibly may have been infected from the cultivated celery, but that more likely the transfer is the opposite direction.

The actual date when Septoria was first described on celery is somewhat disputed and uncertain, due to the fact that several workers described fungi resembling Septoria at about the same time. According to Dorogin (13) Spegazzini in 1887 described a fungus on Apium australe from Terra del Tuego and Staten Island under the name of Septoria apiicola. Although this is the first record of a Septoria on Apium, the description is so meager that it is impossible to tell whether the fungus is identical with either one on cultivated celery. It is possible that on study of specimens of the fungus on Apium australe, and after cultural and inocu-

lation studies, this may prove to be the same as one of the celery Septorias, but until such studies can be made, it seems advisable to maintain it as a separate species. Briosi and Cavara in 1890 described a fungus on celery in Italy which they called Septoria petroselini Desm. var. Apii. They collected it and distributed it with description as No. 144 in I Funghi Parassiti etc. They retained the specific name because of its likeness to the Septoria on parsley, yet by the varietal name indicated the difference between the celery and parsley organisms. At about the same time Allescher collected specimens of a fungus in Bavaria which he sent to Bresadola. Allescher in his letter called the fungus Septoria magnusiana, but Bresadola in describing the fungus, noted some subhysterioid ostioles and apparently imperfect pycnidia and transferred the fungus from Septoria to Phlyctaena. These specimens collected by Allescher were distributed under Bresadola's name Phlyctaena magnusiana (Allesh.) Bus. as number 188 of Allescher and Schnabl, Fungi Bavarici. This set was dated 1890, but was not distributed until in 1891. Accompanying the specimen no. 188, there was a printed discussion of the new species, so that the publication of the specific name magnusiana dates from early in 1891, when the set was distributed. The species was published in a periodical in 1892 (Allesch 1).

Chester (5) (6) in Delaware in 1891 noted the presence of the fungus on celery and identified it with Septoria petroselini Desm. var. Apii. Br. and Cav. He compared the forms on celery with the true S. petroselini Desm. on parsley and decided that the differences were sufficient to warrant raising the variety Apii to a specific rank. He published the name S. Apii (Br. et Cav.) Chester in the Torrey Bot. Club. Dec. 9, 1891 (6). According to Klebahn, Rostrup in 1893, ignorant of Chester's work, also described the fungus under the name Septoria Apii Rostrup.

The name magnusiana is the first specific name applied to the fungus and according to the rules of nomenclature would be the correct one to use, except for the fact that Allescher subsequently, in 1892, used the combination Septoria magnusiana Allesher for an entirely different species on Spiraea chamaedryfolia. Then the earliest available name for the celery organism is S. apii (Br. and Cav.) Chester.

According to Klebahn (21) and Dorogin (13) who examined the specimens in the sets of exsiccata, Septoria petroselini Desm. var. Apii Br. and Cav. is identical with Phlyctaena magnusiana (Allesch.) Bresadola. Chester examined specimens distributed by Briosi and Cavara and found them identical with his fungus. The present writer has compared Chester's original specimens (sent through the kindness of Dr. Manns) with those of Briosi and Cavara and confirms Chester's findings.

From the descriptions and specimens of Briosi and Cavara, Klebahn and Chester, it is clear that the fungus studied by them was identical with the large spot type, studied by the writer. The specimens of Briosi and Cavara are also identical but the descriptions accompanying the specimens do not exactly fit the accompanying specimen. The pycnidia are described as crowded and fusing. It seems quite possible that both types of Septoria described later on might have been present in Italy and the small spot type could have confused the description, the author not knowing that there were two Septorias attacking celery. The name Septoria apii (Br. and Cav.) Chester then belongs to the large spot type of disease.

In 1916 Dorogin (11) described two distinct Septorias as parasites on celery. One type he described as causing a large spot, reddish brown in color and when mature having minute pycnidia sparsely scattered near the

center of the spot. The pycnidia were nearly round paraplectenchymatous in nature, 70 - 125 microns in dia., the ostiole 1/5 to 1/4 the dia. of the pycnidium and round or irregular in form. The spores were filiform, 30 - 45 x 1.5 - 2 microns about straight without visible granules, and with three inconspicuous septa. No pycnidia were found on the stalks. This form he identified with the Briosi and Cavara and Chester type, i.e. Septoria apii (Br. and Cav.) Chester. The other he describes as causing a small spot with densely crowded, often fused, black pycnidia. The pycnidia were 90 to 135 microns in dia., tissue paraplectenchymatous characterized by very large ostioles often somewhat slit at the top. The pycnidia were evenly distributed over the spot and sunken in the uncollapsed tissue around the spot. The spores were 45 - 50 x 2-3.5 microns, obtuse at the ends, distinctly granular and up to 6 septate. Lesions with crowded pycnidia were abundant on the stalks.

He concluded that this second fungus was a new species and gave it the name Septoria apii-graveolentis.

One specimen mentioned by Dorogin as examined and found identical with his Septoria apii-graveolentis was collected in London, Ontario, Canada, by Dearness. A specimen of this same collection (Fungi Columbiana) in the herbarium of Michigan State College was found to be identical with the small spot type occurring in Michigan. The name of the small spot type then should be Septoria apii-graveolentis Dorogin.

Most other authors, not recognizing the fact that there are two Septorias on celery, have followed either Briosi and Cavara or Chester in naming the fungus on which they were reporting. Some authors consider the difference between the celery and parsley organisms so slight as not to

warrant a distinct name. Upon examining herbarium specimens from different parts of the United States, the writer found that regardless of whether the specimen was of the small or large spot type, they were all named Septoria apii (Br. and Cav.) Chester or Septoria petroselini Desm. Var. Apii Br. and Cav.

Previous Cultural Work

Klebahn (21) was probably the first worker to isolate the Septoria fungus from celery and grow it in culture. He placed leaves in damp chambers and removed some spores from the ooze which protruded from the ostioles. He noted that the spores became modified before germination, certain cells swelled and the constrictions became very marked. Upon germination and growth the spore gave rise to a branching tangled mass of mycelium.of two kinds. The older parts of the mycelium were brown with hard membranes, thick, short celled, irregularly curving, with irregularly placed lateral branches. The younger portions of the mycelium found near the outer limits were small, hyaline, almost straight and sparsely septate.

A second feature mentioned by Klebahn was the productions of free conidia in broomlike tufts at the end of short lateral branches on the older and semi old portions of the mycelium. On the older mycelium he found these to be very numerous and suggested that they could be compared to incomplete pycnidia.

On agar he found the fungus to grow very slowly. The central portion of the colony was dark olive brown to black and tuberculate with smaller lateral hyphae radiating out from this. The whole culture was covered with a sparse white aerial mycelium. The tubercular masses were clump of pycnidia, externally and submerged in the agar.

Companile (10) found very little spore germination in distilled water. In a 1% glucose solution the spores germinated regularly. The spores were found to germinate directly while still in the cirrus. Some spores elongated, becoming swelled with marked constrictions and were notably rich in oil globules. Others became zigzag and the individual cells of the spore came apart.

Previous Work on Host Relationship

Klebahn (21) reported the incubation period to be thirty-four days in one experiment and several weeks in another. Dorogin (13) did not confirm this but on this basis thought that this might help explain the sudden appearance of the disease after 2-3 weeks of weather apparently unfavorable for the spread of the disease. Klebahn and Dorogin agreed on the necessity of water for the spread of the fungus due to the fact that the spores are exuded in a jelly-like mass from the ostiole.

According to Companile (12) the fungus enters the celery leaf by penetrating the wall of an epidermal cell. Once inside the leaf the fungus establishes itself in the intercellular spaces and food is obtained from the host cells through haustoria which are sent into them. Companile also noted that parts of the host tissue turn brown before the fungus has invaded them, thus evidencing a toxic substance which kills in advance.

Thomas and Muller (42) noted that Septoria of celery on solid media grew better in a temperature between 13 and 19° C than between 22 and 27° C. Companile (12) found better growth at 13 - 19° C than at 25°C. Voglino (45) found spore germination best between 12 - 14° C. Owen (28) says the disease caused by Septoria is a cool weather disease, the fungus thriving best from 60° - 70° F.

Thomas and Muller (42) and Thomas (43) associated infection of celery by the septoria fungus with the general health of the host. They found that factors which were more favorable for growth of the host were also more favorable for growth of the organism. They found all ages of tissue susceptible, but younger leaf infection was limited by surface exposed. They failed to get infection on one lot of plants and an examination revealed the presence of a bad infestation of nematodes. Upon rejuvination the plants when restored to health were easily infected. Pritchard and Porte (30) working on Septoria of tomatoes, called attention to the fact that in their experiments they got no increase in infection from fertilizers which were favorable for growth of the tomato. They believe that infection is due to surface exposed and criticized Thomas' work because he did not count infections per sq. in. of leaf surface. Companile (12) concludes that one of the chief reasons why the older leaves are the first infected are that they are nearest the source of infection and also they protect the young leaves from being spattered with spores.

Dorogin (13) in his description of his new species Septoria apii-graveolentis noted that it attacked the leaf stalks very seriously. This fact he never observed with Septoria apii (Br. and Cav.) Chester. The first serious damage to stalks of celery was reported by Duggar (14) in New York in 1897. The disease became serious that year on storage celery and caused much damage in New York. Duggar called the causal organism Septoria petroselini var. Apii, but apparently did not compare it with Chester's specimens as only the one Septoria was known on celery at that time. Likewise several other workers have described the organism as a serious pest of stalks on storage celery. If Dorogin's observations

are correct, these just previously mentioned damages to celery leaf stalks were caused by Dorogin's new species, Septoria apii-graveolentis.

It has been suggested that many described species of Septoria, which are similar in the main points of their description but differ only in their host plants, may actually be identical. Beach (3) in a few cases was able to transfer Septorias from hosts of one genus to hosts of another genus in the same family, but in general found the genus Septoria to be very specialized and the host range limited to very closely related species or varieties. Thomas (43) tried the Septoria of celery on several other Umbellifers, but obtained infection only on celery and celerias. Pethybridge (29) found a Septoria on wild celery which he was able to transfer to cultivated celery and suggested the possibility of identity among other Septorias on Umbellifers.

Distribution of the Fungi

Immediately after it had been definitely proven that there were two separate species of Septoria on celery, the question of distribution arose. Septoria on celery had been reported from all parts of the United States where the crop is grown, but since only one form was known, the same name has been applied to both forms. Since only the small spot form has been found on celery stalks, the damage reported on celery in storage was most likely due to it. Some of the damage to celery has been accredited to Septoria petroselini Desm. It has been proven that the celery forms do not attack parsley but lack of material prohibited testing the parsley Septoria on celery.

From Literature it seems that the large spot type Septoria Apii Br. & Cav.) Chester is the most common in Europe. From literature, specimens, and personal correspondence it was found that the small spot

type, Septoria apii-graveolentis Dorogin is the most common in the United States.

The distribution of the two forms in the United States may be shown by the following data which is the result of a questionnaire letter sent to the states where celery is grown.

State	Source of Information	Occurrence	
		Small Type	Large Type
California	4 specimens	3 specimens	1 specimen
Oregon	4 "	2 "	2 "
Washington	2 "	2 "	2 "
Minnesota	3 "	1 "	2 "
Wisconsin	letter	most prevalent	wide spread
Indiana	observations	most prevalent	
Michigan	observations specimens	most prevalent 2 specimens	common
Ohio	5 specimens	5 "	
New York	8 "	6 "	2 specimens
Delaware	3 "	1 "	2 "
Massachusetts	7 "	7 "	2 "
New Jersey	3 "	1 "	2 "
Florida	Fla. Ag. Exp. Sta. Bull. 173 2 specimens	wide spread	wide spread

Signs of the Disease

Under optimum conditions, a period of from nine to twelve days is necessary for these fungi to enter the host and establish themselves to the extent of the production of visible lesions. Under adverse conditions of temperature, moisture and health of the host, this period may be lengthened but is never less. The first sign of the disease is in the form of a more or less transparent spot surrounded by greener tissue. The green soon disappears and the tissues collapse forming a characteristic spot. Both types in the very early stages are somewhat alike, but soon become differentiated so that they are easily distinguishable.

The large spot type Septoria apii (Br. and Cav.) Chester usually requires an incubation period of a day or two less than the small spot type. The first definite lesion is marked by collapse of the host tissue containing or adjacent to the fungus. It seems that the fungus may at a given state in its development secrete some toxic substance which actually kills the host cells for a certain distance and this is followed by collapse of tissue even when the hypha are not present. This type of lesion in its early stages is very similar to that caused by Cercospora apiicola Fres. with the exception of size, because of the absence of fruiting structures. In general the spots are from 3 - 6 mm. in size. If the atmosphere is very moist, small black scattered pycnidia may become evident in the center of the spot in from two to three days. These pycnidia are never found along the margin of the spot and are scattered singly, never clustered. If the weather is cool and moist this spot may increase to two - three times its original size. The leaf soon shows a general yellowing as a result of the fungus attack. If only one lesion is present on the leaf, it may not show much change for a week, but if there are two or

three spots present, it may turn yellow in two to three days, wither, and die.

The small spot type differs somewhat from the large in that as soon as translucent spots appear on the leaf, fruiting bodies may be found in them. Pycnidia are also found outside the indefinite margin of the spot in apparently healthy tissue. The killing and collapse of the tissue starts at the middle of the spot and progresses outward. The pycnidia which were buried in the leaf parenchyma are now exposed as erumpent black bodies closely crowded together and often fused together in twos and threes. In general the size of the spot is from less than 1/2 mm. to 2 mm. in dia. and never attains the size of the large spot type. The celery leaf is apparently able to endure from two to five of these spots for a long time, but if of the easy blanching type may become yellow, wither, and die. The green celeries seem to be able to stand numerous spots before the yellowing occurs. If the infection is severe in damp weather, the spots fuse giving a brown appearance and the leaf rots with a soft, brown, wet rot.

The striking difference in appearance of the two spots are first in size and second in the time and way the fruiting bodies are produced. The mature or completely developed large type spot is definite in outline, brown to reddish brown in color and surrounded by a darker reddish brown border with scattered pycnidia near the center. The small type spot is indefinite in outline, brownish to black or sometimes gray, with black pycnidia which may be found in the spot or in the green tissue surrounding it. These pycnidia in the spot are erumpent, closely crowded, and some are fused in two threes. The small spot is usually about one-third to one-half the size of the large type.

Etiology

Proof of Pathogenicity

Single spore cultures of the organisms were obtained by the use of the ordinary poured plate method. Both types of the Septoria were collected from several fields in the Kalamazoo marshes. From these specimens spore suspensions were made in sterile water and dilution plates were poured. After the agar was firm the petri dishes were inverted and placed on the stage of the microscope. By the use of the low power objective, spores were located. They were transferred immediately to potato dextrose agar slants and allowed to grow for two weeks.

At the end of two weeks both types of the fungus had grown and produced fruiting bodies. Transfers were made and the original tubes were partly filled with sterile water and agitated. Examination of the water under the microscope revealed a heavy suspension of spores in both cases. Each suspension was poured into sterile atomizer bowls. Six healthy Easy Blanching celery plants which had been grown in the green house where they were kept free from disease were placed in separate Wardian cases. Five of each lot were atomized with a suspension of spores from one of the fungi and then allowed to remain in the Wardian cases for forty-eight hours. The inoculations were made on September 25, and small killed circular areas were noted on the plants atomized with the large spot type on October 5. On October 8 both lots of plants were thickly specked with characteristic lesions on the leaves. The checks remained perfectly healthy. Isolations were made from these lesions by the poured plate method and characteristic growth was obtained on potato dextrose agar. The same type of infection experiment was repeated many times later on in the course of the work.

To avoid individual differences in celery plants a single plant was inoculated with both types of the fungus in the following manner. A large plant was moistened by spray of water from the hose and then divided by placing a large piece of cardboard on edge across the pot so as to leave about half the leaves on each side. The two sides of the plant were atomized respectively with spore suspensions of the large and small spot types of Septoria. The plant was placed in the Wardian case for a period of forty-eight hours and then removed to a bench. After twelve days the characteristic types of spots were apparent on their respective sides. There was a slight bit of mixing due to the impossibility of limiting the spray from the atomizer, but no doubt as to the type of infection on either side. An incidental fact of interest in this experiment was that the side of the plant infected with the large spot type was nearly dead after ten days from the time of the appearance of the disease, although there appeared to be a smaller number of spots per leaf than that infected with the small spot type.

Morphology of the Fungi

Mycelium

The mycelia of these two fungi, as observed in sections of diseased leaves, are very much alike and are composed of twisted and interwoven, irregularly septate, brownish to black, irregularly thickened threads. At the corners of host cells where the mycelia are intercellular, there may be whole fascicles of knot and bulb like formations of irregular outline. The mycelium of the small spot type is 1.5 to 4.5 microns in diameter while that of the large spot type is 1.0 to 5.5 microns in diameter.

In sections through spots the mycelium of the small spot type is much more extensive than the other type. Strands of mycelium may be found as far as 1500 microns beyond the edge of the collapsed tissue. In the large spot type the fungus hyphae never reach the edge of the collapsed tissue. This fact indicates the production of a toxic substance by the large spot type which may help to account for the larger size of the spot and also for its shorter incubation period.

On media of different composition the mycelium differs markedly in color, character and amount of growth according to the food content of the medium. On potato dextrose agar there are two kinds of hyphae, the heavy brown hyphae described above in the leaf and a smaller hyaline type. The smaller type is found near the edge of the colony and changes to the brown type as it grows older. This type is without doubt present in the outer regions of the spot in the leaf but is not easily apparent in sections.

Spores

The spores (plate I.) or conidia are long, slender, filiform hyaline bodies borne in closed nearly sphearical structures, the pycnidia. The size, shape, and appearance of the spores of the two fungi are markedly different.

The spores of the small type Septoria apii-graveolentis Dorogin are slightly flexuose blunt at the ends, granulose with several indistinct septa. The size of the spores varies (plate I.) from 22.5 - 58.5 x 1.5 -3 microns. The mean is 35.0 microns for length and 2.2 microns for diameter. In plotting a curve of the measurements of the lengths of spores, it was found that 92 per cent of the spores would be included in lengths

between 28.5 - 50.2 microns; and 66 per cent in lengths between 31.5 to 42.7 microns. The number of septa varied from 0 to 7. Ten per cent had less than 3 septa, 60 per cent had 3 septa, 18 per cent 4 septa, 10 per cent 5 septa, and 2 per cent with more than 5 septa.

The spores of the large spot type Septoria apii (Br. and Cav.) Chester were straight or slightly curved, minutely granular, with indistinct septa. The size of the spores varies from 13.5 - 34.2 x 1-2½ microns. The mean is 24.6 microns for length and 1.8 microns for dia. In plotting a curve as before it was found that 86 per cent of the spore lengths were between 21 and 28.5 microns. The number of septa varied from 0 - 4. One and one-half per cent had less than 2 septa, 34 per cent had two septa, 64 per cent had 3 septa, and one-half per cent had four septa.

The above measurements were made from a fresh spore suspension taken from spots of several plants. Small sections of diseased spots on leaves from isolated plants which had been infected with a pure culture were placed in a test tube containing sterile water. The tube was agitated so that the mature spores were washed from the cirri on the ostioles of the pycnidia. The suspension was mounted on a slide and the spores were measured under the oil immersion lens.

The septa in both types of spores were very indistinct. Spores from this same suspension were killed, fixed, and stained with gentian violet. The staining made the septa present much more distinct but as already noted some spores showed no septa at all.

Pycnidia

The pycnidia of the two types differ even more than the spores (Plate [illegible]). The pycnidia in the small spot are visible very early and are one of the first evidences that mark a lesion. At first they are

sunken in the tissue but as the disease progresses, the host tissue collapses and leaves them erumpent. At this time other pycnidia may be found around the edge and between the spots. A very distinctive characteristic of this small type is the crowding together and even fusing in pairs and threes of the pycnidia. The pycnidia are subsphaerical occurring on both sides of the leaf and range from 73 to 147 microns in diameter. The ostioles are mostly round with irregularly lobed edges and range from 29 - 73 microns in diameter, i.e. 1/3 to 1/2 the diameter of the pycnidium.

The pycnidium of the large spot type are seldom evident in the spots on the growing plants. If the atmosphere is very moist or if the leaves are removed and placed in a moist chamber, the pycnidia will develop in 24 hours or more. They are erumpent and are found in the center of the spot. The pycnidia are always found singly unless in old spots where the number of pycnidia is allowed to increase for a considerable length of time. In section the pycnidia are spherical and 65 - 95 microns in diameter. The ostioles are round and range from 9 - 20 microns in diameter, i.e. less than 1/4 the diameter of the pycnidium.

The measurements of the pycnidia were made on infected leaves which had been cleared in carbol turpentine. This solution made the leaves more transparent and the exact limits of the pycnidium could be measured.

Size of Spots

Considerable mention has been made as to the size of the spot produced by these two fungi. This can only be in a comparative way because the exact size of the spot produced depends on several factors. Observations have shown that, though one type produces a much larger

spot than the other, yet when infection is once established the diseased area will increase in size for several weeks or even until the host tissue is entirely killed. To compare these two fungi on the size of spot they produce would require identical time, temperature, humidity, and even the same general state of health of the host plant. Only as a comparative statement we may say that the small type ranges from less than 1/2 to $3\frac{1}{2}$ mm. while the large one may attain a diameter of $1\frac{1}{2}$ to 10 mm.

Search for the Perfect Stage of the Fungi

The perfect stage of the Septoria on celery has never been found. Klebahn (21) found a pleospora on overwintered celery leaves, but failed to get infection when the ascospores were sprayed on healthy celery plants. Companile (12) and others report only the asexual conidia borne in pycnidia in culture. Stevens (39) and Gäumann (17) report the perfect stage of several Septorias other than those on celery which have as their perfect stage members of the genera Leptosphaeria and mycosphaerella. Stevens says that many of the so called imperfect fungi have apparently lost their ability to fruit sexually.

Early in the spring of 1929, the writer visited some celery fields near Kalamazoo and Decatur, Michigan, to observe any evidence of Septoria which had wintered over on celery. He examined stalks of celery in hope of finding a sexual stage of the fungus. He found no evidence of any such stage but did find numerous lesions on the old leaves and stalks in which there were both old and new pycnidia. He brought some of these old celery plants into the laboratory and examined them further and found the pycnidia were filled with conidia.

The writer placed some of the stems and leaves in a cigar box and

covered them with damp newspaper. The box was placed on the window sill outside the window for four weeks. At the end of that time the celery stems were covered with large apiculate perithecia, belonging to the genus Leptosphaeria. Single spore cultures of the ascospores on potato dextrose agar produced a red mycelium, on which after 8 weeks was found a species of Hendersonia. A suspension of the ascospores sprayed on healthy celery plants produced no infection. It is clear then that this Leptosphaeria had no connection with the Septorias.

By using ultra violet light, Stevens (39) was successful in inducing a sexual stage in several heterothallic ascomycetes which did not normally have a sexual stage even though both plus and minus strains of the mycelia were present.

Petri dish cultures of both the large and small type of Septorias were exposed to ultra violet light. Two series were exposed, one in which the two types were far enough apart so that they could not grow together and the other in which plantings of the two types were placed side by side. Due to the relative slow growth of both Septorias, it was necessary to incubate the cultures on potato dextrose agar for three weeks before the colonies were large enough to be used. The cultures in series in open petri dishes were exposed at about 50 cm. from the light for 5, 10, 15, 20, and 30 second periods. Another series of cultures were exposed in the same way except that one half of each colony was shaded.

The cultures exposed to ultra violet light for intervals of 5 to 20 seconds showed marked increase in pycnidial formation on the exposed parts. The mycelia of the two types in the plates where they had been placed side by side met and intertwined, but produced no different results. Above 20 seconds the fungus was either killed or inactivated so

that the growth was greatly reduced. On some of the 15 and 20 second exposures the aerial mycelium was greatly reduced in amount. In no case was any sexual stage observed.

One other attempt was made in search for the perfect stage of Septoria. Cultures of both types of the celery septorias on potato dextrose agar, prune agar, hard oat agar, celery concoction agar, steamed celery stems and steamed celery leaves were incubated 12 days at 20 - 23° C. and then stored for a period of 16 weeks at 2 - 3° C. The cultures were examined from time to time and no evidence of sexual fruiting was observed. The only change noted different than in higher temperatures was the formation of sclerotia like mats of mycelium in the bottom of the tubes of both types. The fungi grew scarcely at all during the period of storage, but upon removal, vigorously resumed growth.

Physiological Studies

Cultural Characteristics

In pure culture the fungi were found to grow well on almost all media employed. Plantings from cultures on potato dextrose agar were made on the various media shown in the following record and the growth observed at intervals throughout the period of study. Except for the amount of growth, the macroscopic appearance of the fungi varied but little from time to time. Cultures were grown on both liquid and solid media. In general both fungi produce a purple brown to blackish submerged mycelium and whitish cottony aerial mycelium. Both fungi fruited on most of the media tried. A marked difference was noted between the character and rote of growth of the two fungi.

The following record was made after 15 days growth on the different media. The temperature in the culture room was between 20 and 24° C.

	Growth	Pycnidia
1. Potato dextrose agar (Plate IX)		
a. Small type	good - brownish to black. Agar not colored	Abundant
b. Large type	more rapid and diffuse than small type reddish purple color at the base of the agar slant.	Abundant
2. Coon's Synthetic agar		
a. Small type	medium - mycelium Advanced in coarse pine tree patter, became tufted and knotty near the middle	Abundant
b. Large type	good -- not so coarse and ropey, more profuse	Abundant
3. Hard Oat agar		
a. Small type	luxuriant, very thick at middle, black, slightly cheesy	Very abundant
b. Large type	massive, very black covered the whole slant in 15 days	Very abundant
4. Corn meal agar		
a. Small type	This medium seemed to be a starving medium as the fungus grew scarcely at all	None
b. Large type	Very scant white growth	Very scarce
5. Prune agar		
a. Small type	aerial mycelium in fluffy tufts, mouse gray in color, submerged mycelium black	Not so abundant as in other media few in older slants
b. Large type	like small only more rapid and diffuse growth	"

6. Celery concoction agar (made from celery stalk)		
a. Small type	sparse growth, blackish brown, slight tufted in middle	Fairly abundant
b. Large type	very scant, white and cottony	Practically none--1-2 in each tube
7. Nutrient Dextrose agar (Plate V)		
a. Small type	dense, cheesy tufts	Less than large
b. Large type	dense, flat, cheesy lobed pelicle like, tawny brown, numerous knotty protruberances	very abundant
8. Steamed celery stalks		
a. Small type	dense black tuft with some whitish aerial mycelium great masses of pycnidia	very abundant
b. Large type	dense mycelium, tending to spread down stem. Pycnidia not clumped as much as small. Black sclerotia were formed in the liquid at base of tube	very abundant
9. Carrot plugs		
a. Small type	dense black, covered with mouse gray aerial mycelia, slight tufts	very abundant
b. Large type	tufted, covered with light mouse gray aerial mycelium	very abundant
10. Sterile filter paper		
a. Small type	limited to small tufts	few
b. Large type	sparse mycelium with scattered pycnidia	abundant

11. Steamed parsley leaves		
a. Small type	scant mycelium black	few
b. Large type	fair growth, whitish aerial mycelium	few
12. Steamed water cress stems		
a. Small type	very little growth	scarce
b. Large type	slightly spreading	scarce
13. Horse dung conct. agar		
a. Small type	rich black tufted	abundant
b. Large type	black, diffuse spreading	abundant
14. Steamed muck		
a. Small type	only from small piece of agar which was transferred	none
b. Large type	"	"

These cultures show a very marked difference in the rate of growth of the two fungi. The large spot type on all media with the exception of the celery concoction grew at nearly twice the rate of the small spot type. On celery concoction agar the small spot type outgrew the large spot type and produced abundant fruiting bodies, while the large spot type fruited only very sparsely. The celery concoction base was made by heating 120 grs. of chopped celery stalks in 1 liter of distilled water at 100° F. for one hour. The better growth of the small type on the celery stalk agar then may be closely correlated with the behavior of the fungus on the stalks themselves. In an experiment which will be described later, only the small type grew and produced fruiting bodies on the stalks. These results may be interpreted to show that the

large type is more saprophytic in its habits, since it will grow luxuriantly on celery stalks which have been killed by steam and only very little on living celery stalks. It seems quite strange though that it does not grow on the stalk extract which had been treated.

Temperature Relations

Thermal Death Point of Spores

The thermal death point of the spores was determined by means of the Novy's capillary tube method. Portions of lesions from diseased leaves containing pycnidia were placed in sterile test tubes and 5 cc. of sterile water added to them. The tubes were shaken and the spores were washed from the cirri of the pycnidia into the water. Capillary tubes 10 inches in length were placed in the suspension and the liquid was allowed to use almost to the end of the tube. The tubes thus filled were sealed off in the flame of a microburner. Using a large ten gallon can for a bath, the temperature of which was carefully regulated by the size of the flame below, the capillary tubes were exposed for ten minutes and the contents then plated in potato dextrose agar.

In the table + indicates only a few colonies developing, + + + indicates abundant development of colonies, and - indicates no growth at all.

Temperature	40°C	41°C	42°C	43°C	44°C	45°C	Ck.
Small Type	+ + +	+	-	-	-	-	+ + +
Large Type	+ + +	+ + +	+ + +	+ +	-	-	+ + +

Many of the spores of the small spot type were killed when exposed for 10 minutes at 41° C. and all of the spores were killed when exposed to a temperature of 42° C. Some of the spores of the large type were killed when exposed for 10 minutes to 43° C. All of the spores of the large type were killed at 44° C. Thus the spores of the large type seem more heat resistant.

Vegetative Growth

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 high temperature. To obtain further knowledge of the temperature relations of the fungus, use was made of a modification of a Ganong differential thermostat. This consists of two chambers, one a galvanized iron chamber for a freezing mixture and the other an asbestos lined chamber for a treating element, which are connected by a long trough. This trough is divided into twelve small compartments by means of wooden and cardboard partitions, each compartment being covered by a tight fitting wooden lid. The compartments, although separate, are inter-connected by glass tubing ½ inch in diameter, which comes from the bottom of the next colder chamber into the top of the warmer one. Each division is equipped with a small thermometer. Ice was renewed each day in the freezing chamber, and heat was supplied in the other chamber by means of an electric light bulb which was automatically controlled by a thermostat.

By use of the equipment described above the series of temperatures was obtained in the following tabulation. Plantings of both types of the celery septorias were placed on opposite halves of petri dishes on potato

dextrose agar and three dishes placed in each compartment. The temperatures of the several compartments were recorded and the growth of the cultures observed each third day for 18 days. The dishes were then removed and measurements and observations made.

Compartment number	Temperature range	Large Type		Small Type	
		Av. dia. of growth in mm.	character	Av. dia. of growth in mm.	character
1	9 - 12	7.0	Very slight growth	None	
2	11½ - 15	7.5	"	Very slight	Small white aerial mycelium
3	17 - 19	18.0	Good growth	6.5	Good growth tufted
4	20 - 21	21.0	" "	6.0	"
5	22 - 23	26.0	luxuriant growth	6.5	"
6	23 - 24	26.0	"	5.0	"
7	24 - 25	23.0	very black	slight	small & black
8	26-- 27	13.0	no aerial growth	none	
9	27 - 28	Only few filaments	slight growth	none	
10	28½ - 30	none		none	
11	31 - 34	none		none	
12	38 - 42	none		none	

From this experiment the two fungi are shown to be distinctly different in their temperature range for growth and their optimum temperature. The large spot type grows in a temperature range from 10 to 27° C. with an optimum of 22 - 24° C. The small type grows in a temperature range from 14 to 25° C. with an optimum from 18 - 22° C. This experiment

indicates the possibility of control of the small type which occurs in storage by lowering the temperature of the storage chambers to a point below that at which the fungus will grow. The exact point where growth is inhibited has not been definitely determined.

Spore Germination

A suspension of mature spores was obtained in the usual way and placed in hanging drops on the under sides of glass slides which had been ringed with vaseline. The slides were placed on supports in petri dishes which were lined with moist filter paper and the dishes were placed in the compartments of the Ganong thermostat. The slides were allowed to remain exposed to these temperatures, for a period of 14 hours when they were removed and the germination stopped. The covers were removed from the petri dishes and the lower halves containing the slides were placed over an open dish of formaldehyde under a bell jar. The spores were soon killed and the germination was measured as shown in the table on the following page.

The spore measurements in the record represent the measurements from fifty spores taken at random from several fields on the slide. The average length of the germ tube was computed from only the spores which showed germination.

The results on spore germination agree fairly well in their temperature range with that of vegetative growth. Before conclusive results can be drawn the experiment should be repeated and a larger number of spores from each lot measured. The only fact worthy of note was that a markedly smaller number of spores of the large type germinated and the incubation period seemed to be longer. Companile (12) did not get spore germination in sterile distilled water.

		Small Type				Large Type			
No.	Temp.	No. spores measured	No. spores germinated	Av. length of germ tube	Per cent germination	No. spores measured	No. spores germinated	Av. length of germ tube	Per cent germination
1	9-12	50	41	10.1	82	50	7	3.1	14
2	11½-15	50	45	11.2	90	50	9	2.8	18
3	17-19	50	47	23.7	94	50	13	6.9	26
4	20-21	50	46	41.6	92	50	15	6.9	30
5	22-23	50	47	43.3	94	50	14	11.1	28
6	23-24	50	44	42.3	88	50	20	17.3	40
7	24-25	50	45	45.4	90	50	19	16.2	38
8	26-27	50	30	27.6	60	50	13	14.7	26
9	27-28	50	10	9.1	20	50	12	6.5	24
10	28½-30	50	none			50	6	1.0	12
11	31-34	50	none			50	none		
12	38-42	50	none			50	none		

Relation of the Fungi to the Reaction of the Medium.

Both of the Septorias on celery grew well on a wide variety of media, which also varied widely in Hydrogen ion concentration. One lot of Coon's Synthetic agar on which both types of Septoria grew well was found to be Ph 4.5. To test the reaction of the fungus to different Ph, a series of media was prepared. It was impossible to harden the agar in solutions of high Ph, so a liquid medium was used. The ordinary base in Coon's Synthetic was used and sterile N/10 NaOH or HCl was added to make a series which was determined by the colorimetric method. The series of solutions ranged from Ph 3.0 to 8.8 by .2 intervals. Thirty cubic centimeters of the base solution was placed in 75 cc. Erlenmeyer flasks in which small filter paper cones had been placed. These were sterilized and the sterile acid or base as mentioned before was added to complete the range. The culture flasks were inoculated by placing small pieces of agar containing the mycelia of the fungi on the tip of the cone. Both fungi grew well throughout the series. The large type grew slightly more luxuriantly on the acid side of the scale. The small type did not show much specificity. Companile (12) working with the large spot type reported more spread of the fungus on neutral or alkaline media.

The question of Ph is very important with many fungi and should be thoroughly investigated. It was planned to elaborate the studies with the Septorias but they seemed to respond so slightly to different Ph that it was decided useless.

Growth on Plant Refuse and Field Soil.

It is important to know whether the celery Septorias are able to grow saprophytically on field soil. A series of test tubes were half filled with muck, moistened with distilled water and sterilized in the autoclave

at 75 pounds pressure for 45 minutes. Two drops of a heavy spore suspension from each type of Septoria was added to each of six tubes respectively. In two more sets of six tubes, inoculations were made by transferring a very small piece of agar. No growth either microscopic or macroscopic was observed in the tubes containing the spore suspension. Only a few small filaments of the fungi were found in the tubes in which the agar had been placed. The filaments probably grew from the food they obtained from the agar as the soil alone did not support growth.

During the first week of April, 1930, partly decayed celery leaves from the previous years crop with definite lesions on them were collected near Kalamazoo. The leaves examined in the laboratory revealed the presence of both new and old pycnidia. A spore suspension obtained from the spots was sprayed on healthy celery plants and after fourteen days the plants were badly infected with the large spot type.

Longevity of Spores.

The actual age at which Septoria spores lose their viability is not definitely know. The conditions under which the spores are kept has a very important bearing. Coons and Levin (11) recommend the use of two or three year old celery seed in Michigan. Krout (24) found that conidia on celery leaves and surface of seed were dead in 8 - 11 months and conidia from pycnidia on seeds and pedicels were dead in from 2 - 3 years. Flocks (16) reported the spores in pycnidia on seed viable from 1 to 2 years.

It was impossible for the writer to test the viability of Septoria on different age seed because of the uncertainty of the age of the seed. A lot of seed obtained from Burpee and Co. in January 1930 was tested and the conidia did not germinate but the age of the seed was unknown. Ger-

mination tests were made on spores from old specimens in the Michigan State College herbarium but no viable spores were found. A specimen of the small type collected in California was placed between two ordinary blotters and sent to the writer. When the specimen arrived, it was thoroughly dried and the writer was unable to get the spores to germinate or to get infection on plants. The life of these spores was less than three weeks.

Oxygen Relation to Spore Germination

Four capillary tubes were filled with a suspension of Septoria spores and sealed at each end so that the liquid entirely filled the cavity of the tubes. Two of the capillary tubes were opened at each end. The tubes were placed in the culture room at 20 - 21° C. for four days. When the tubes were examined, there was no germination in the closed tubes, but the opened tubes showed germination near the open ends.

Host Relations

Host Range

Beach (3) found the members of the genus Septoria very limited in host range. Pethybridge (29) found a Septoria on wild celery which appeared very different from the one on cultivated celery and was able on transfer to cultivated celery to get the typical cultivated form. He suggested that many of the Septorias with similar description but occurring on different hosts were probably the same. Thomas (43) tried the celery Septoria on several umbellifers but got negative results.

Both of the celery Septorias were tried by the writer on a number of other Umbellifers. Seeds were obtained from the following places: Missouri Botanical Garden, St. Louis; Royal Botanical Garden, Edinburg; Museum d'Histoire Naturelle Culture, Paris; Berlin Botanical Garden and

Museum, Berlin; D. M. Ferry Seed Co., Detroit; Michigan State College Botanical Garden, East Lansing; and from local seed stores. The seeds were planted in sand and the seedlings transferred to 3 in. pots where they grew until large enough for inoculation. Separate lots of two plants of each species were inoculated with a suspension of spores of each type of Septoria respectively. The spore suspension was made from sections of typical lesions on leaves. Four healthy celery plants were placed in the Wardian case with each lot of Umbellifers as checks. The plants were left in the Wardian case for 52 hours after inoculation. The following is a list of the Umbellifers inoculated:

1.* Aethusa cynapium L.
2.* Ammi majus L.
3.- Anethum graveolens Mammoth.
4* Apium graveolens L. (e specim. spontan.)
5.- Apium graveolens L.
6.- Apium graveolens L. var. Rapaceum.
7.' Apium graveolens L.
8.' Apium graveolens L. var. Rapaceum.
9.' Anethum graveolens. (Imp.)
10.- Anethum graveolens.
11.* Angelica purpurascens (Lall) Drude
12.) Bowlesia tenera Sprg.
13.- Bupleurum longicaule
14.- Bupleurum longifolium
15.- Bupleurum candollei
16.- Bupleurum longifolium var. Aureum
17.* Bupleurum rotundifolium L.
18.* Bupleurum solicifolium (L.) Soland.
19.* Corum carvi L.
20.- Corum carvi
21.- Corum segetium
22.- Carum petroselinum
23.- Carum carvi
24.* Conium maculatum L.
25.- Daucus carota var. Oxheart
26.* Coriandrum saturum
27.* Didiscus pusillus F. v.m.
28.* Eryngium amethystinum L.
29.* Erynguim campestre L.
30.* Eryngium planum L.
31.- Eryngium heldreichii
32.- Eryngium agavifolium
33.* Eryngium wrightii
34.- Ferula communis

35.* Ferula communis L. s. sp. glauca (L.) Rouy. et. Carn.
36.' Foeniculum dulce (Imported)
37.- Foeniculum vulgare
38.* Heteromorpha arborescens (Thumb)
39.' Levisticum officinale
40.- Levisticum officinale
41.* Levisticum paludapifolium (Lam.) Aschs.
42.* Libanotis montana All. var. Sibirica (l.)
43.- Oenanthe silaifolia
44.- Oenanthe crocata
45.* Oenanthe aqnatica (L.) Pair.
46.* Oenanthe peucedanifolia Pall.
47.* Opopanax chironium (L.) Kock.
48.* Orlaya grandiflora (L.) Hoffen.
49.- Pastinaea satira L. var. Dauvers
50.- Petroselinum sativum L. var. Scotch Curled.
51.† Peucedanum officinale L.
52.* Pimpinella saxifiaga L.
53.- Scandix australis
54.- Scandix balansae
55.- Scandix brachycarpa
56.- Scandix pinnatifida
57.- Scandix pecten-veneris
58.* Scandix balnasae Rent.
59.- Selinum tenuifolium var. Allium.
60.- Selinum tenifolium
61.- Seseli elatum
62.* Seseli gummiferum Poll.
63.* Seseli libanotis (L.) Kock.
64.* Seseli tenuifolium Ledb.
65.* Silaus pratensis (Lam.) Bios.
66.- Thaspuim aureum
67.* Torilis nodosa (L.) gartn.
68.- Trinia kitaibelii.

The following symbols after the numbers indicate:

* -- seeds from Germany, - Seeds from Scotland, ' seeds from the Missouri Botanical Garden, - seeds from local stores, and * seeds from D. M. Ferry Seed Co.

Many more seeds were obtained but have not been tested with the Septorias. Due to the small demand for such seeds, many of the seeds kept in Botanical garden collections are several years old and so did not germinate. One collection of seeds from Paris arrived too late to be included in this study.

Successful infection was obtained only on Apium graveolens L. and a variety of it, Apium graveolens L. var. Rapacum. The check celery plants developed typical infection in every case.

Small pieces of mycelium of both types in agar were placed on leaves of Levisticum, Pastinaca, Petroselinum, and Daucus and covered with wet absorbent cotton. The cotton and mycelium were removed from the leaves after three days. A few small lesions were observed on Levisticium leaves on which the large type fungus had been placed but they did not develop fruiting bodies characteristic to Septoria. The leaves were removed and placed in a moist chamber and the lesions became slimy as in bacterial infection.

Mode of Infection

Companile (12) working with S. apii (Br. and Cav.) Chester described the fungus as entering the celery leaf by penetrating the epidermal cell walls. After the fungus was established in the epidermal cells, it grew into the intercellular spaces and then produced disease.

Spores of both types were placed in drops of water on the lower epidermis of celery leaves which had been removed and placed in a moist chamber. The epidermis was stripped off the leaf at different intervals and the progress of the spores observed.

After three days long germ tubes were found on all parts of the spores (Plate). The first noticeable change previous to germination was the appearance of oil droplets in the spores. The spores of the small type show a markedly greater accumulation of oil. Both types of spores showed an increase in the number of septa just previous to germination. It was very hard to determine whether the germ tubes had entered the leaf

tissue. Many cases were observed where the germinating tubes grew directly over the surface of a stomata without entering it. (Plate I). One single case was observed where the small slender germ tube from a spore of the small spot type entered an epidermal cell near its anticlinal wall and branched inside the cell (Plate I). These observations have led the writer to believe that entrance may be through any portion of the surface. There seems to be no stimulus which attracts the germ tubes to the stomata. Entrance through a stoma is possible, but the fungus may enter the guard cells as well as the opening.

Inoculated leaves which had been kept in the moist chamber for a period of five days were examined. Small hyphal systems had been established in several of the epidermal cells which were not bordered by stomata. Although it was impossibility to find where the germ tube had entered the cells, the mycelial clumps were limited to regions entirely away from the stomata.

Leaves inoculated on separate sides showed the same number of spots per leaf. The ratio of stomata as counted under low power and averaged for several fields was for upper to lower surfaces as 3.4 to 8.2. The size of the openings and guard cells were approximately the same on both sides. Stomata on the celery stalks occurred only in the furrows between the ridges.

Fusing of Spores

Another feature noted in culture was the early fusing of the germ tubes of germinating spores (Plate I). The germ tube from some spores seemed to grow in a given direction until it was at a certain distance from another when, as if attracted by some force, it turned directly

and fused with another. Some spores were hooked together directly by germ tubes, and there was no other germ tube present on the spore. If conidia are merely encysted vegetative portions of an organism, it would be only natural to expect that the germinating conidia would fuse as do other hyphae in culture. It would be interesting to try infection experiments with single and with fused conidia.

Effect of Light

Although pycnidia may be found on both sides of the leaf, it was observed that in natural infection they were most plentiful on the upper side of the leaf near the light. To study this factor, healthy celery leaves were placed in a large glass culture dish. In a series of leaves inoculated with a drop of spore suspension on the top side some were placed with the top side up and some with the bottom side up. A similar set up was made with leaves inoculated from the bottom side. In all cases the greatest number of pycnidia were found on the side nearest the light.

Stalk Infection

Sections of celery stalks were washed in sterile water and placed in a sterile moist chamber. Drops of spore suspensions of each type of Septorias were spread along the stalks. After 16 days typical lesions were developed on the stalks inoculated with the small spot type. The stalks inoculated with the large spot type showed very shallow brown spots but no pycnidia were developed. Dorogin (13) recognized typical lesions on celery stalks by his new species of Septoria which is identical with the small spot type of their study. He did not find lesions of his large spot type on celery stalks.

Another experiment in which market celery was inoculated with both types of Septoria was lost by soft rot.

Varietal Resistance

Several commercial varieties of celery were tested for evidence of resistance. A list of the varieties are as follows:

1. Golden Self-Blanching (New Strain)
2. Golden Self Blanching
3. Easy Blanching
4. M. A. C. Yellows Resistant A. A. 25
5. Simons Golden Plume
6. White Plume
7. Burpees' Fordhook
8. Curly Leaf
9. New French Strain
10. Giant Pascal

Two series of six plants each of each variety were inoculated with one each of the two Septorias respectively. The disease appeared on all the plants after 12 days. Counts were made and all the varieties were found to be nearly equally spotted. The easy blanching types soon lost their color and died, the varieties affected with the large spot type succumbing first. The green varieties seemed to be able to endure the attack of both types of Septoria but after ten days began to show yellow along the edges of the leaves. The spots of the small type septoria on the green varieties grew in size but the celery finally outgrew the infection and the disease leaves were lost by old age.

Age of Tissue

Thomas (43) reported the younger leaves of celery to be more susceptible to Septoria than the older ones, but less easily infected due to smaller surface area. Six old plants in two series were inoculated with both types of Septoria from celery and the leaves tagged with notes on their condition at the time of inoculation. When the disease appeared 50 old leaves and 50 young leaves were removed from the plants. The leaves were placed under a glass on which a circle had been made with a wax pencil. The spots over a given area then as counted inside the circle were averaged. The results showed the young leaves to have 7.3 spots and the old leaves to have 7.8 spots for the large type. In leaves infected with the small spot type the younger ones showed 14.9 spots as compared to 15.3 spots on the old leaves. The young leaves used in this experiment were very nearly expanded so that there was very little increase in size after inoculation. This tends to show that infection is more a matter of contact than susceptibility.

Summary

1. Two distinct species of Septoria were isolated from late blight lesions on celery and definitely proved to cause typical forms of the disease.
2. The two fungi produce on celery large and small types lesions respectively and may be distinguished accordingly. The small spot type was found to be Septoria apii-graveolentis Dorogin. The large spot type was found to be Septoria apii (Br. and Cav.) Chester.
3. Although both types are present in the United States the name generally applied in literature and in herbaria is that of the large spot type, the other species not having been identified in this country.

4. The disease has been found to flourish in relatively cool weather, 60^0 - 70^0 F and is the most serious disease of celery. The loss each year amounts to many thousands of dollars. The small spot type is also a serious parasite on celery leaf stalks and is responsible for great losses in storage.
5. The fungi enter the host by direct penetration through the epidermal cells. The large spot type seems to be more saprophytic than the small spot type because it seems to kill the tissues in advance of the mycelium. Fruiting bodies of the small spot type are readily apparent and may be found as early evidence of a lesion. Fruiting bodies of the large spot type are not found until a definite region of tissue has collapsed and even then when the atmospheric humidity is high, they are limited to the center of the spot.
6. The fungi grow well on most ordinary culture media, except corn meal, The small spot type grew on celery stalk concoction agar while the large one did only very sparsely. No growth was obtained on sterile muck. The optimum temperature for the small spot type was between 22 - 24^0 C while the large type grew best from 18 - 22^0 C. The spore of the small spot type were killed at 41° C for ten minutes and that of the large spot type at 43^0 C.
7. Both fungi were inoculated on a large number of Umbellifers but infection resulted only on celery and its variety celeriac.
8. Ten varieties of celery were infected and although the green celeries seemed to endure the disease better, there were the same number of spots per leaf.
9. Lesions on old leaves in the field were used as a source of inoculum on healthy plants and the plants responded with infection of the large spot type.

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Explanation of Plates

Plate I. Camera lucida drawings of (about 1400 times)

Fig. 1. Spores of large spot type of Septoria on celery - S. apii (Br. and Cav.) Chester.

Fig. 2. Spores of small spot type of Septoria on celery - S. apii-graveolentis Dorogin.

Fig. 3. Small spot spore in distilled water after five days.

Fig. 4. Fusion of two germinated spores of the large type.

Fig. 5 & 6. Germinated spores of the large spot type.

Fig. 7. Double fusion between two germinated spores of the small spot type.

Fig. 8. Small spot type spores fused on a leaf. The germ tube passed directly over a stoma and did not enter.

Fig. 9. Small spot type spore showing that there is no attraction to the stoma.

Fig. 10. Two large spot type spores fused on a leaf.

Fig. 11. Spore of the small spot type showing the germ tube inside an epidermal cell.

Plate II. Celery leaves showing typical disease lesions as caused by the two septorias (natural size).

Plate III. Celery leaves showing typical disease lesions enlarged 4 times. The large type on the left has not yet developed fruiting bodies.

Plate IV. Lesion of the large type of Septoria on celery showing a lesion after twelve hours in the moist chamber.

Plate V. Small and large types on nutrient dextrose agar.

Plate VI. Small and large types on potato dextrose agar.

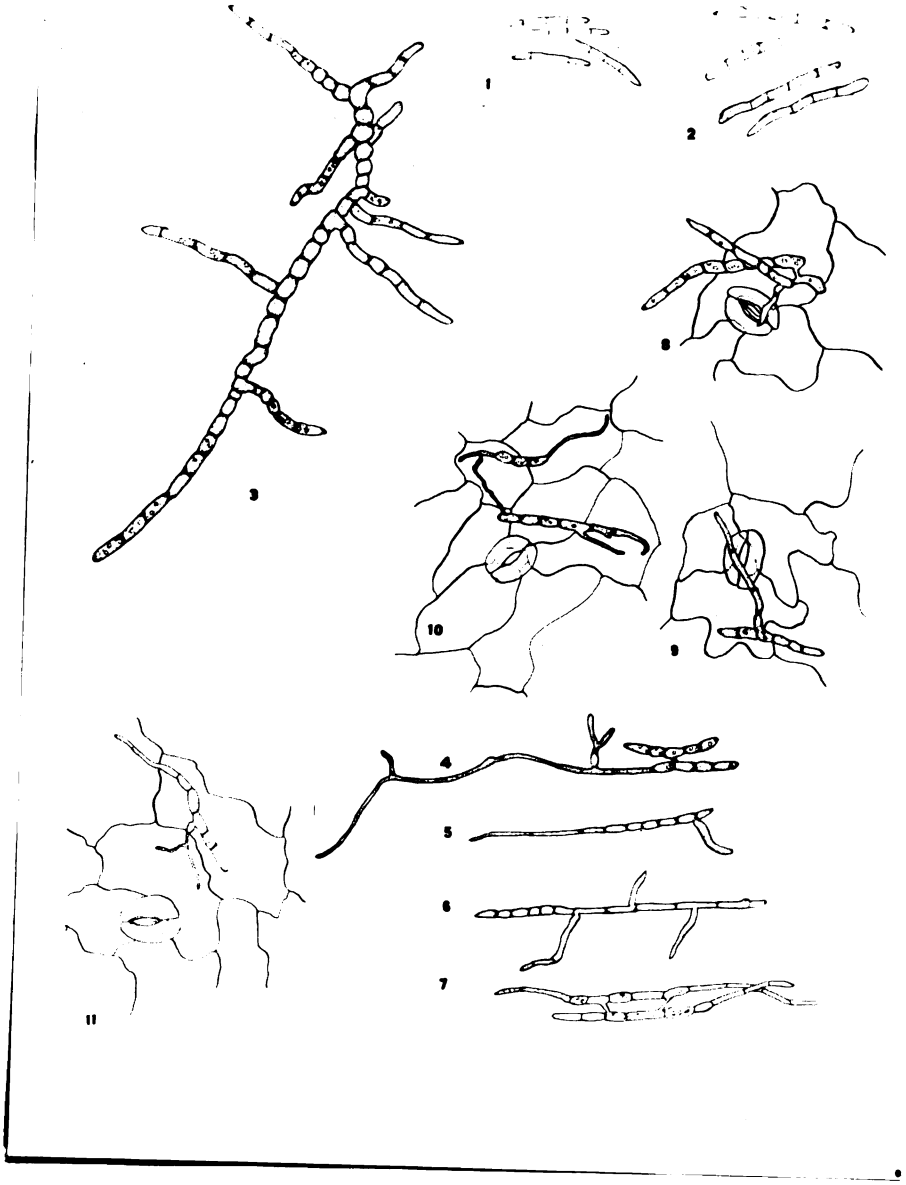


PLATE I.

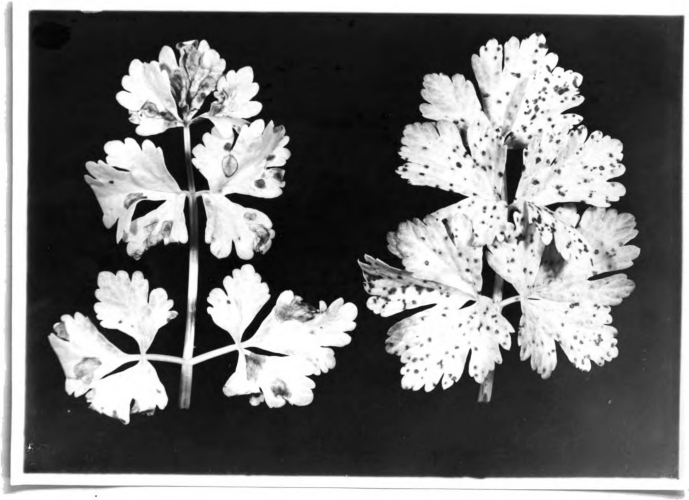
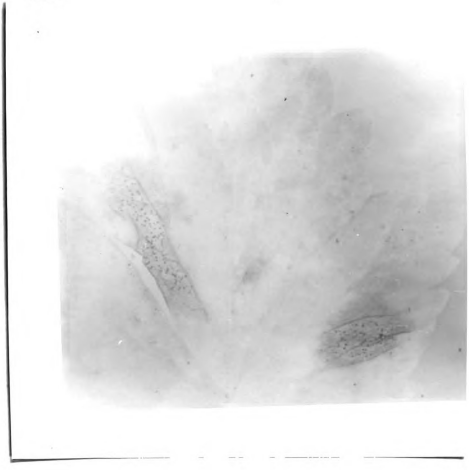


PLATE II.

PLATE III.



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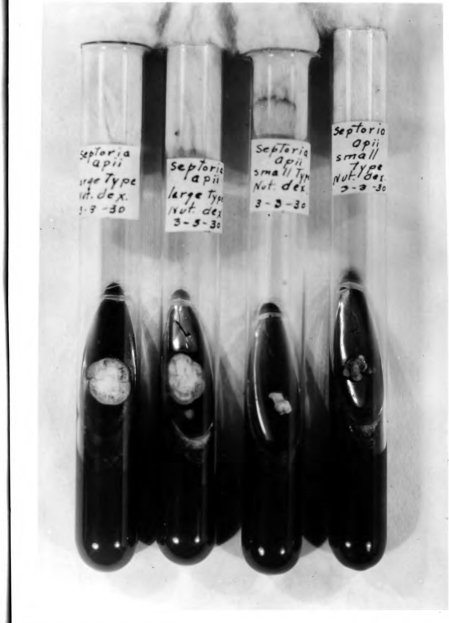
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PLATE V.

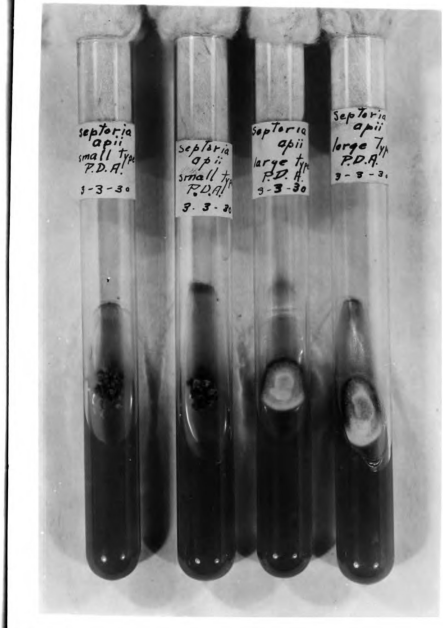
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PLATE VI.