1932

The overwintering of Septoria apii (Br. and Cav.) Chester, under conditions prevailing at Amherst, Massachusetts in 1931-1932

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THE OVERWINTERING OF
SEPTORIA APII (BR. & CAV.) CHESTER
UNDER CONDITIONS PREVAILING AT
AMHERST, MASSACHUSETTS IN 1931-1932

VAN VEGHTEN - 1932
THE OVERWINTERING OF
SEPTORIA APII (BR. AND CAV.) CHESTER
UNDER CONDITIONS PREVAILING AT
AMHERST, MASSACHUSETTS
IN
1931 - 1932

By
Grant Bernard Van Veghten

A Thesis Submitted in Partial Fulfilment
of the Requirements
for the Degree of Master of Science,
Massachusetts State College
Amherst, Massachusetts
June, 1932
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The Overwintering of *Septoria apii* (Br. and Cav.) Chester

Under Conditions Prevailing at Amherst, Massachusetts

in 1931 - 1932

By
Grant Bernard Van Veghten

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**INTRODUCTION**

*Septoria apii* is a fungous parasite which causes the late blight of celery. The overwintering of this fungus is of practical importance as well as of scientific interest, since this knowledge should be at hand to combat successfully the disease in celery fields.

**Brief Review of the Literature**

A brief review of the literature shows that several investigators have attempted to solve this overwintering problem and other phases of the life history of this pathogene. Voglino (55:153)* reported that in Italy the organism overwintered as pycnidiospores in pycnidia on infected celery refuse kept outdoors. In 1910 similar results were obtained by Klebahn (20:13) for certain conditions in Germany.

*The first number in parenthesis refers to the article in Literature Cited; the second number, to the page in the article.*
Klebhan also mentioned that pycnidiospores which had overwintered in the fruits of celery remained viable and when germinated, their germ tubes produced infection. Krout (23:65) in 1916 and 1917 concluded that the organism overwintered as sclerotia which were intercellular in the petioles of the host.

From a review of the data presented by Voglino and Klebhan, it was noted that the specific conditions of moisture were not considered. A consideration of moisture seemed necessary since observation by the writer showed that the pycnidiospores readily escape in the presence of free water or excessively humid atmosphere leaving the pycnidia empty. From this it seemed that, following the autumnal rains in the climate of Massachusetts, there would be no spores, formed in the fall, that would persist in the pycnidia during the winter. Krout gave no definite description of the sclerotia which he mentioned as the overwintering form. The writer was unable to find sclerotia in host tissues during the autumn of 1931. His observations and review of the literature indicated that further research was necessary to solve the problem of the overwintering of Septoria spii.

**Statement of the Problem**

The writer has considerable interest in celery culture and desired to investigate some aspect of this very important celery pathogone. The work was undertaken to solve
the following problems:

1. Do pycnidiospores in the pycnidia overwinter, remaining viable and pathogenic?

2. Can the fungus overwinter as free conidia either in soil, in culture, or in fruits?

3. Do sclerotia and pycnosclerotia remain viable through the winter?

4. Can mycelium live overwinter either on artificial medium or in host tissues?

5. Does the fungus overwinter in materials stored outdoors and indoors?

6. Which cultural practices are favorable for the dissemination of the organism and the development of the disease? Which are unfavorable?

MATERIALS

Plant

Leaves from a Local Station.

Leaves of Giant Pascal celery, highly infected with *Septoria apii* were collected from a station in Sunderland, Massachusetts. These leaves were collected in four different lots at four different times during the fall of 1931. Material I*

*Roman numerals refer to collections of leaf materials. Their lack of sequence is due to the fact that they were assigned according to the dates when the materials were collected rather than according to the order in the descriptions.*
was collected on August 29 and consisted of infected leaves from young plants about eight inches in height. Material III was collected September 15 and consisted of more mature leaves from plants about twelve inches in height. Material X was collected on October 12 and consisted of whole leaves, while Material XIII, collected on November 27 from mature plants after they had been banked, consisted of terminal leaflets only. When Material XIII was collected, there was one-half inch of snow on the ground and frosted areas were visible on the leaflets.

Material I was sorted for completely infected leaflets and divided into two equal quantities, which were placed in packets, consisting of folded galvanized wire gauze of one-sixteenth inch mesh, which previously had been dipped in paraffin. These packets were about 5" x 10" in area and 2" thick. The material was stuffed in the packet making the whole, pillow-like. On the following day these packets were buried in soil; one outdoors at a depth of six inches, and one indoors at a depth of two inches, or as deep as the greenhouse bench would allow. An effort was made to retain the moisture of the greenhouse soil the same as that of the soil outdoors.

Material X consisted of portions of leaflets and

*Roman numerals refer to collections of leaf materials. Their lack of sequence is due to the fact that they were assigned according to the dates when the materials were collected rather than according to the order in the descriptions.
petioles so selected that the lesions were not more than five millimeters apart. This material was also placed in wire packets as described for Material I. However, the packets were slightly smaller, 5\" x 3\" x 1\". One of these packets was buried outdoors and one indoors as for Material I.

Unpressed samples of Materials I, III, X, and XIII were placed in paper envelopes and stored in the laboratory for testing the viability of the pycnidiospores in dried host materials. Similar leaves in envelopes from Materials X and XIII were stored in an incubator with an average temperature of 17\(^\circ\)C. to test the effect of a variation of temperature on the viability of the pycnidiospores. Pressed diseased leaflets were likewise kept as herbarium specimens of Materials III, X, and XIII.

A handful of Material X was placed outdoors on the surface of the soil as a check to the material buried. Furthermore, diseased leaflets from this lot were placed on galvanized wire platforms in moist chambers; one was stored outdoors and the other in the incubator at a temperature of 17\(^\circ\)C. Certain areas bearing pycnidia on these leaflets were encircled with India ink so that subsequent development of these pycnidia could be observed.

On September 15, 1931, ten infected celery plants in bloom were transplanted from the field to five-inch clay flower pots. Five of these plants were then placed in a greenhouse bench and the other five were buried up to the rim of the pot.
in soil outdoors. From these plants it was hoped to obtain infected fruits. These plants appeared to suffer no setback and continued to blossom and finally formed fruits.

**Leaves from Other Points in Massachusetts.**

Small qualities of representative leaves or leaflets were obtained from two different sources. Material II was collected in Seconk County on September 10, 1931. It consisted of matured leaves of an unknown variety of tall, yellow plants appearing much like Golden Self-Blanching. Diseased leaflets were pressed as herbarium specimens. Some of this material was used for testing the formation of pycnosclerotia by placing selected portions of infected petioles in holes cut in a filter paper laid on the bottom of a Petri dish. These were covered with a second filter paper and kept moist by the addition of sterile water. The location of pycnidia on these bits of tissue was mapped so that further development or any slight change could be observed through the glass of the Petri dish.

Material IV was collected in a garden at Holyoke on September 15, 1931, and consisted of infected leaflets of a green variety much like Giant Pascal. The spots (lesions) on this material were rather large. A few of these leaflets were stored in paper envelopes in the laboratory, while others were pressed for herbarium specimens.

Material VII was collected at a station near Hathorne
on September 23, 1931. This consisted of a few leaves each of both a green and a yellow variety. The latter bore both rather large and small spots. Leaflets showing each type of infection were pressed and others were stored in paper envelopes in the laboratory. Plate 1, Fig. A shows one of these leaflets.

Leaflets from Other States.

Diseased leaves in quantities of one to two pounds were received from Michigan, New York, Ohio, and New Jersey.

Materials V and VI collected by R. L. Cochran in Michigan arrived at the laboratory on September 15, 1931. The leaves were half grown when gathered and having been packed in wet paper were bleached, and had begun to rot when received at Amherst. Material V from near Kalamazoo was judged by the collector to be of the small spot type. Material VI collected near East Lansing was of the large spot type of infection. Upon receipt at Amherst, the material was kept in a low temperature incubator until the sorting for burial. Material V was divided into two parts, each of which was placed in a wire packet of 3" x 5"; one was buried indoors and the other outdoors, as described previously for Material I. Material VI was placed in a single packet of the same size as for Material V and buried outdoors. These packets were buried on September 19, 1931.

Material VIII was collected by A. L. Harrison near
Williamson, New York and arrived at the laboratory on October 3, 1931. It was of the Wonderful (yellow) variety, and consisted of portions of matured leaves above the petioles. It also was bleached and had begun to rot when it arrived. It was stored in a cool basement room until buried.

Some of the material was washed under the cold water tap, before portions of it were selected to be buried, because of the copper lime dust apparent on the leaves. On October 5, 1931, packets 3" x 4" in size were prepared and on October 10, they were buried; one outdoors and one indoors. A sample of unwashed material was placed to dry in envelopes stored in the laboratory. The remainder of the unwashed material was stored in a cool room until November 9 when a sample was placed to dry in a low temperature incubator.

Material IX, collected by Dr. J. B. Wilson near Wooster, Ohio arrived on October 3. It consisted of mature leaflets with parts of the petioles. This material was of two varieties, Golden Self-Blanching, and Emperor (the latter is a green variety). The Golden Self-Blanching was infected with Cercospora in addition to Septoria. This material was divided and one-half buried indoors and the other outdoors.

Material XI and XII. On November 9 and 12, respectively, this material received from New Brunswick, New Jersey, was similar to Material IX and was similarly treated; that is, samples were dried in envelopes, others pressed, and
a packet of Material XII buried outdoors.

**Infected Fruits : Home Grown.**

Infected fruits were produced on the plants which had been brought to the laboratory on September 15 (Material III). These plants were inoculated several times with a spore suspension applied with an atomizer. The first inoculation was made on September 19, when a tap-water suspension of spores from leaflets of Material II was used as the inoculum. On October 14, a similar inoculation was made on two of the plants, using a distilled water spore suspension from Material X, at 5:30 P.M. and again at 7:30 P.M. Two days later this was repeated. On November 17, three plants were inoculated by placing infected leaflets over the flowers and sprinkling the plants. On November 18, two of the plants were sprayed with a tap water suspension of spores from Material XIII in two applications, at 5:00 P.M. and 10:00 P.M. On the morning of the next day, the operation was repeated. Microscope slides were sprayed with the same suspension and placed in a moist chamber near the inoculated plants. One hundred per cent germination of the spores on the slide resulted.

Fruits were gathered from these plants on January 5 and again on January 30, 1932, when the plants were dead from drying. These fruits were placed in envelopes and kept in a cool room.

**Commercial "seed".**

The first sample was obtained September 1, 1931. It
consisted of a store packet of Ferry's Golden Self-Blanching. Other samples were obtained from the Department of Vegetable Gardening of the College and one packet of Golden Self-Blanching from Vaughan. Because of the unknown age of these samples and the small number of infected fruits in them, none were used except Ferry's, from which plants were grown for inoculation.

Another supply was obtained May 3, 1932 from a near-by grower. This consisted of about one-half ounce samples of Newark Market grown in 1930 and Pascal, grown in 1928. This seed was grown by the farmer from whom it was obtained. Because of the rather high percentage of infection and known age, this seed was preferable to the other commercial seed and consequently was used along with the home grown in the infected seed (fruits) studies of May 8.

Plants from Storage.

A dozen Pascal celery plants with the roots attached were received from Burlington, Massachusetts on January 28, 1932. This material was collected by Dr. E. F. Guba at the Waltham Station. This had been in pit storage since November, 1931. The writer tested the viability of spores from this material and made a drawing of a structure that might be called a sclerotium, found in a petiole (Plate 6, Fig. C). Since this material was so slightly infected, it was of little use for disease specimens so was used in celery decoction.
MEDIA

The media used were liquids and solids. The liquids were water and nutrient decoctions; the solids were agars. The kinds of water were cold tap, distilled, and sterilized distilled (autoclaved). The nutrient decoctions were plain carrot, carrot dextrose, plain celery, and celery dextrose. The agars were plain water, potato dextrose, plain celery, and celery dextrose.

Formulae for making the decoctions follow:

Plain carrot decoction:

1. Fresh carrot roots; washed, peeled, and sliced ----------------------------- 300 gms.
2. Distilled water --------------------- 1000 cc.

The carrots were cooked in water for 45 minutes by steaming in the autoclave at 7 pounds pressure, after which the liquid was poured off, filtered, restored to its original volume, tubed and sterilized.

Carrot dextrose decoction:

1. Plain carrot decoction ------------------- 1000 cc.
2. Dextrose (Bacto) --------------------- 20 gms.

Plain celery decoction:

1. Fresh celery leaves (blades and petioles)
   washed -------------------------- 250 gms.
2. Distilled water --------------------- 750 cc.
Plain celery decoction (cont.)

The leaves were boiled 30 minutes, then the liquid was filtered into flasks which were plugged and then sterilized.

This formula was modified by using 500 grams of fresh celery leaves.

Celery dextrose decoction:

1. Plain celery decoction --------------- 125 cc.
2. Dextrose (Bacto) ------------------- 1.25 gms.

This was modified by using 0.125 grams of dextrose.

Formulae for making the agars follow:

Plain water agar:

1. Agar agar (Bacto) ------------------- 17 gms.
2. Distilled water ---------------------- 1000 cc.

The agar in the water was autoclaved for one hour, filtered through cotton into 250 cc. flasks, plugged and sterilized by autoclaving.

Potato dextrose agar:

A. Potato decoction:

1. Potatoes, washed, peeled, and sliced- 200 gms.
2. Distilled water ---------------------- 500 cc.

The potatoes in the distilled water were autoclaved for one hour, filtered, restored to 500 cc. volume.

B. Plain water agar:

1. Agar agar (Bacto) ------------------- 17 gms.
Potato dextrose agar (cont.)

2. Distilled water --------------------- 500 cc.

The agar agar in the water was autoclaved for one hour, filtered, and restored to 500 cc.

C. Potato dextrose agar:

1. Potato decoction --------------------- 500 cc.
2. Plain water agar --------------------- 500 cc.
3. Dextrose (Bacto) --------------------- 20 gms.

These three ingredients were mixed by rotating while hot, then tubed, plugged and sterilized.

Plain celery agar:

1. Fresh leaves ------------------------ 250 gms.
2. Distilled water ---------------------- 500 cc.
3. Plain water agar --------------------- 500 cc.

The fresh leaves were autoclaved for one hour in the distilled water, mixed with the plain water agar when both were hot, then the mixture was filtered, tubed and sterilized.

Celery dextrose agar:

1. Plain celery agar --------------------- 1000 cc.
2. Dextrose (Bacto) --------------------- 20 gms.

The dextrose was added to the melted agar, then the mixture was treated as the other agars.

APPARATUS

Most of the apparatus employed is described under the
title "Methods", since it is not complicated and needs no special description. However, three sets of apparatus need explanation.

The centrifuge employed in some of the experiments was a two-tube, clinical machine, a product of the Shelton Electric Company, New York and Chicago (manufactured by the General Electric Company). It was driven by a 110 volt—60 cycle electric motor at velocities of 800 to 1600 revolutions per minute.

The low temperature incubator for culture work was ice cooled, with electric thermostatic control and heating, operating on a 110 volt—1 ampere current. The incubator was manufactured by the Chicago Surgical and Electrical Company, Chicago, Illinois.

The cool room in which plant materials and media were stored was a small room in the basement, usually open to the outdoors by means of a window. The temperature was therefore near to that outdoors. The atmosphere within the room was somewhat humid. In the cool room was a refrigerator which was used without ice as the storage closet for media, particularly.

METHODS

Testing Viability of Pycnidiospores

Testing viability of pycnidiospores consisted, primarily, in attempting to germinate them in drops of water or
liquid nutrient, uncovered on a microscope slide, in a moist Petri dish, at a temperature of 17° C. Such a culture is hereafter referred to as an open drop. Slight modification of this method consisted in incubating the spores in small (two centimeter) watch-glasses without a moist chamber.

The spores for these tests were secured by touching a bit of tissue bearing pycnidia directly to a drop of liquid on the slide or by transferring spores by means of a platinum loop from a culture or from a spore suspension. Furthermore, pycnidia in dried material were macerated in the culture liquid on the slide.

The method of calculating the germination of spores was to count about 100 spores from one or more fields of the microscope. If the spores were scattered the low power of the microscope was used, or, if they were thick on the slide, the high power was used. This total number of about 100 was taken as the denominator of a fraction of which the numerator was the number of germinated spores. Germinating spores were counted at intervals of one day over a period of two to seven days.

Securing a Pure Culture of the Fungus

Pure cultures were employed for a study of the morphological and physiological characters of the parasite.

Isolations were attempted by means of three methods: (1) tissue cultures on agar (both plain water agar and with nutrient added); (2) spore suspensions on agar (both plain
water agar and agar with a nutrient) from which single germinated spores were isolated; (3) and a hanging drop culture from a spore suspension. In the case of Material II, celery decoction and water, and celery agar as a smear, were the media used. In other cases the media employed for the hanging drop were carrot-dextrose decoction and potato-dextrose agar.

When attempting tissue cultures from Material II, two methods of disinfecting the host tissue were employed. One was by washing the material in running water; the other, by exposing the material for a short time to alcohol, formalin, or a solution of bichloride of mercury. However, these two methods were sometimes combined.

Portions of stalk tissue were dipped in alcohol and flamed, and sections were cut from these with flamed scissors. These sections were transferred to a plate of "thin" celery agar (approximately 15 per cent) and set in the low temperature incubator.

Leaflets were dipped into formaldehyde (8 parts commercial to 100 of distilled water) and rinsed in cold running water for one minute. This material was designated as II B. Against this there was used as check leaflets washed in cold tap water two minutes, as the only treatment, and designated as Material II A. Bits of tissue of both treatments were placed, eight or nine hours after treatment, on celery agar plates and removed to the cool room at a temperature of 26° C.
Other leaves were dipped into a solution of bi-chloride of mercury (1 part to 1000 parts of water) and then rinsed for one minute in cold tap water, after which portions of infected tissue were cut with flamed scissors and transferred to thin celery agar.

Pure cultures of *Septoria spili* were not obtained from tissue culture by either of the aforementioned methods of disinfection or by employing the different media.

When the spore suspensions were prepared, the diseased host tissues were disinfected by washing in tap water for two to five minutes or by shaking a macerated leaf in a test tube of sterile water. A suspension of spores from Material II A was prepared from a leaf washed for five minutes in hot tap water at a temperature scarcely bearable to the hand. Washing host tissue was insufficient for removing all bacteria but did greatly reduce their number.

Plain and nutrient agar were the media employed for the incubation of spores in suspension. Plain water agar was used for a spore suspension from Material II A, washed in hot tap water. Celery decoction was used as the liquid for another spore suspension from some of the same material. This suspension was poured over plain celery agar. Carrot dextrose decoction was similarly employed for a suspension of spores from Material XIII. This suspension was poured over potato dextrose agar acidified with one drop of 25 per cent lactic acid. Spores from Material X germinated in an open drop of a
suspension were transferred to celery dextrose agar acidified with two drops of 25 per cent lactic acid to each ten cubic centimeters of agar. A similar drop of culture of spores from Material IV was transferred to plain celery agar. Of the media tried, plain water agar proved most satisfactory, since it permitted germination of Septoria spores and allowed only slow development of bacterial colonies.

Single spores germinating on agar were transferred along with a small block of agar by means of a platinum loop to slants of potato dextrose or celery dextrose agar. A culture from spores removed from Material IV was obtained free from fungi other than Septoria api but contaminated by bacteria. However, pure monosporous cultures were obtained by this method from Materials VIII and IX.

Germinating spores in a hanging drop of a spore suspension from Material II were transferred to a potato dextrose agar slant by removing the cover glass bearing the young colony to the surface of the agar. The cover glass was placed with the colony toward the agar. A pure culture of Septoria api was obtained in this way.

**Preparation for the Study of the Morphology of the Fungus**

Preparation for the study of the morphology of the fungus consisted of:

1. Measuring pycnidia and spores from fresh leaves.
2. Making mounts of stained microtome sections of infected
leaf material.

3. Mounting stained germinated spores in celloidin.*

4. Mounting a young colony in glycerin jelly after staining it in erythrosin.

5. Making a mount of a stained free-hand section of a sclerotium-like structure from pure culture.

Pycnidia in fresh leaves of Material I were measured under the low power objection, 16 mm. (A.O.25) 10X, with ocular 10X. The procedure followed was to cut a small bit of tissue bearing pycnidia and to mount it in distilled water without a cover glass. The diameter parallel to the leaf surface was measured for each of forty-one pycnidia. The tissue was then removed from the slide and the drop of water containing the spores was covered.

The spores measured were those coming freely from the pycnidia in contact with water. The measurements were taken by means of an ocular micrometer in a Spencer microscope, using the highpower objection, 4 mm. (H.A.P.) 44X with ocular 10X. Not over twenty-five spores, usually ten to fifteen, were examined from an individual mount. This meant that spores were left in water about one hour. One hundred spores were measured for length and width. The width measured was of the broadest part of the spore, usually found to be at a point somewhere between one end and the middle. The actual length of

*Method was that of Davis, W. H. Staining germinating spores. Phytopath. 12:492-494, 1922.
long spores was considered by making measurements of two or three of the arcs of curvature.

The leaf material for microtome sectioning was fresh material designated as X and XI, also, overwintering materials designated as I and X, Out* and In* (four samples in all) removed when an examination was made on February 4, 1932. The materials were killed in weak Flemming's solution. The fresh material was imbedded in celloidin® and stained by means of Haidenhain's iron alumhaematoxylin plus safranin. The overwintering material was imbedded in paraffin and stained with Flemming's triple stain (alcoholic safranin-gentian violet and orange "G" in clove oil, see Chamberlain 5).

The spores mounted in celloidin were killed in weak Flemming's solution, stained in Haidenhain's iron alumhaematoxylin, after 64 hours incubation in open drops of water. These were from fresh material of XI. A portion of a culture from Material VIII on potato dextrose agar was killed in weak Flemming's solution. Mycelium and pycnidia from this were stained with erythrosin and mounted in glycerin jelly (26).

A free-hand section of a sclerotium formed in a culture from Material VIII, outdoors from January 14 to March 14, was killed and stained in lacto-phenol plus light green (lacto-phenol-green).

*Out and In refer to outdoors, and indoors where the materials were stored, as described on pages 4–6.

Studying the Physiology of the Fungus

The physiology of the fungus in pure culture was studied in relation to temperature and acidity - alkalinity or pH.

Transfers of mycelium and conidia were made from three potato dextrose agar slant cultures to fresh potato dextrose agar in test tubes. Two of these transfer cultures and one original were placed outdoors immediately after the transfers were made. Two other transfers were stored in the cool incubator and after four days, when growth had started, they were also put outdoors. Two other transfers and two originals were stored permanently in the incubator as a check.

The test tubes containing the cultures placed outdoors were kept in soil within a tin can which was sunk into the ground to a depth of five inches. This can was covered by another inverted over it. Nail holes were punched in the side of the inverted can near the top. The cultures kept indoors were held in an upright position in a wire test tube holder.

All cultures were examined from time to time and observations regarding the extent and kinds of growth were

*The term "conidia" is used to distinguish the reproductive structures found in gelatinous masses in culture from those structures similar in appearance, formed in pycnidia in the host tissue. The latter are referred to as "pycnidiospores".*
recorded. Furthermore, the temperature of the soil in the can and of the air five feet above were recorded. Also, the viability of conidia produced in the cultures was tested in open drops. However, the moisture factor was not controlled.

Reaction of the medium (pH) was tested by means of a preliminary experiment with a pure monosporous culture from Material VIII on potato dextrose agar. From this, transfers were made to plates of potato dextrose agar, of which some was acidified with drops of 25 per cent lactic acid, while some of the remainder was made alkaline with drops of approximately normal sodium carbonate (5.3 gms. of the dry crystals to 100 cc. of distilled water). All treatments were duplicated. The number of drops of acid added to each plate (about 30 cubic centimeters of agar) in the acid series was: 3, 6, 12, 15, 18, 21, respectively. The number of drops of alkali added to each plate in the alkaline series was: 2, 4, 6, 8, 10, 12, 14, respectively. For comparison, two similar plates of potato dextrose agar of unaltered natural reaction were also used. (It was found that potato dextrose agar as used in this experiment was acid to phenolphthalein). After inoculation on November 8, 1931, the plates were set in the cool incubator at a temperature of 17° C. Beginning November 11, five observations regarding the extent and nature of growth were made and recorded, until November 23.
Examining Material Overwintered Upon and Beneath the Soil

The examination of material overwintered upon and beneath the soil, consisted of observations of the condition of the host tissue and the genera of fungi found in it. In addition, attempts were made to isolate *Septoria apii* from such material by various treatments and to test its viability on artificial media as well as its pathogenicity on young celery plants.

The first test of the viability of this organism, in whatever form it might be present in the buried material, consisted of pouring a suspension of small particles of the buried material upon potato dextrose agar in plates. The suspension was prepared by shaking some of the material in a test tube of sterile water. Microscopic examination of this material showed that pycnidia and a few spores of the *Septoria* were present, with spores of saprophytes. The rapid growth of these saprophytes prevented observations of viability of *Septoria*.

Because of the inefficacy of this latter, rather gross method, some special means appeared necessary for freeing *Septoria* from its contaminants. The preparation of a suspension in which the spores were more highly concentrated suggested itself.

Centrifuging a spore suspension was the first method tried. The crude suspension used in this experiment was obtained by shaking about half a cubic centimeter of the partially
decomposed buried material in a test tube of sterile water. A preliminary trial was made with a three cubic centimeter volume of such a suspension run at a velocity of 1500 R.P.M. During centrifuging for ten minutes, most of the Septoria spores were thrown down, while most of the bacteria and small fungous spores remained in suspension. However, eight minutes of centrifuging was found more satisfactory. After this, a few drops of the clear liquid above the solid matter was removed by means of a pipette and transferred to potato dextrose agar, some of which acidified with one and some with two drops of 25 per cent lactic acid to prevent the growth of bacteria. Centrifuging failed to produce the desired suspension so this method was discarded.

Since centrifuging failed, a method of sedimentation of a spore suspension was attempted. The spore suspension used was prepared as in the preceding experiment. This suspension was allowed to stand in a test tube and later a few drops of the clear liquid above the solid matter in the test tube were removed from different levels by means of a pipette. These drops were removed in immediate succession at the rate of about one sample per minute. The levels sampled were at the following depths: 0.5 cm. and 1 cm. to 9 cm. at intervals of 1 cm. The periods of sedimentation were: 0.5, 1.5, 4.5, 23 hours after shaking. The period of 23 hours was found to be too long so a period of 13 hours was tried and found to be preferable. However, open drop cultures were made from samples taken after
4.5 and 1 hour of sedimentation. A sample taken, after 4.5 hours of sedimentation, from a depth of 5 cm. was concentrated by evaporation of the water while standing uncovered in a Syracuse watch glass. From the concentrated suspension, a drop was removed and transferred to a microscope slide, then celery dextrose decoction was added and the culture stored in a moist chamber. This method was varied by adding celery dextrose decoction directly to the sample in a watch glass. Contaminations developed in all cultures prepared by sedimentation, so this method, like the other, was discarded.

Simmond's washing apparatus was used to wash Septoria in host tissues free from contaminants. The apparatus was designed for washing small plant parts too delicate to undergo chemical disinfection (45). The operation of this apparatus involves the agitation of the plant parts in sterile water, effected by bubbling filtered air through the water by means of a vacuum pump. By this method, samples of all buried material were washed when examined on February 4, 1932. The washing of each sample was continued for a period of 30 minutes and involved two changes of water. By means of a delivery tube (a third tube on the washing flask, not a part of the original apparatus) some of the suspension containing fine plant debris bearing pycnidia and a few spores was poured over potato-dextrose agar. The remainder of the washed material was kept in test tubes and later used for imbedding and sectioning. The operation of this apparatus failed to remove
the contaminations from the materials and was considered of little or no value.

Because of the failure of all attempts to employ a suspension of material for culturing, hanging drop cultures were finally used for testing the viability of mycelium, particularly that comprising the pycnidia. The nutrients used in the hanging drop cultures were agar and nutrient decoctions. The hanging drop slide chamber was composed of a hard rubber ring temporarily mounted on a microscope slide. A drop of the medium was placed on the cover glass and a pycnidium inserted. The pycnidia used were teased from partly decomposed host tissues, and washed through three changes of water before culturing.

The slides, needles, and cover glasses were dipped in alcohol and flamed to prevent contamination when used. This flaming eliminated contamination except mycelium and bacteria which were in the pycnidia.

A test of the pathogenicity of the organism was employed as a means of detecting Septoria in the buried material. This was accomplished by inoculating celery plants. Single pycnidia, each of which bore mycelium intimately associated with it in culture, and suspensions prepared from the buried material were used as forms of inoculum. The inoculum was applied to both the upper and lower surfaces of leaves on young celery plants.

On March 8 at 11:00 P. M. the suspension was applied
with a wire loop. This suspension was from Material VIII and contained spores and portions of tissue in distilled water. The checks consisted of (1) conidia of a culture from Material VIII in a distilled water suspension, and (2) distilled water only, applied to the plants in the same manner as employed for the test plants. Before inoculation, all plants had been sprinkled with water and following it they were covered with bell jars. On the next day the plants appeared rather dry because they were in the bright sunlight, so the bell jars were wrapped with newspapers for partial shade. The jars were removed at the end of two and one-half days.

On April 6, a tap water suspension of buried material, as inoculum, was poured over all the above ground parts of a young plant. The suspension was prepared, by rubbing the material between the fingers submerged in a cup of water until a murky suspension was formed. The plants were sprinkled before inoculation. The checks consisted of plants inoculated with a tap water suspension of conidia from a culture isolated from Material VIII, and with tap water alone. Following inoculation, the plants were covered with bell jars and stored in a moist chamber for two days.

Testing Fruits and Soil as Sources of Inoculum

The testing of fruits and soil as sources of inoculum consisted of planting specimens of both infected and disinfecte
fruits in both contaminated and clean soil. Some of the fruits were those that had been infected by artificial inoculation in 1931 and others were of Newark Market (grown in 1930) and of Pascal (grown in 1928) received from a grower. These fruits were sown in five-inch tarred paper pots on May 9 and 10, 1932. The soil used was greenhouse compost, not known to be contaminated, and surface soil from a grower's field where the heaviest infection was found during the fall of 1931. Some of the fruits were treated for 24 hours in formalin (one part of 37 per cent to 400 parts of water) and then rinsed twice in distilled water. Part of the soil from the field, about one-half cubic foot, was treated with formalin at the rate of one part of 37 per cent to 50 parts of water per cubic foot. To increase the chances of infection some overwintering infected leaves which had been rubbed through a one-quarter inch mesh sieve were added to another part of the soil. Following the sowing of the fruits, the pots were watered and placed in a damp chamber for twelve days.

THE DISEASE

Common Names

In the United States, several common names have been assigned, by different authors, to the disease caused by Septoria apii,— "The leaf spot of celery", Chester (6); "blight of celery", Humphrey (16); "Celery Blight", Hume (17). However,
"the late blight of celery", Rogers (39), is the common name most generally used in the United States at the present time.

In Europe, the common names that have been applied to this disease are: "Blattfleckenkrankheit", by Klebahn (20) in Germany; the "leaf spot disease", by Chittenden (7) and others, in England (a translation of Klebahn's common name for the disease); "Melanose", a translation of the Russian term used by Dorogin; "Septoriose" and "Melanose", by Campanile (3) in Italy. Of these foreign names, "Septoriose" is the most brief and descriptive.

**Suscepts**

From the literature reviewed, it appears that all the varieties of *Aethion græceolenæ*, i.e., celery and celeriac, are suscepts of this disease. Schenk (44) reported that, in Holland, White Plume and other bleached varieties were more susceptible than green (ordinary) varieties. Of the varieties grown in the United States, Thomas (49) found that Golden Self-Blanching was most susceptible, and celeriac was less so. Furthermore, Pethybridge (34) stated that he found wild celery infected with a species of Septoria which, when inoculated to cultivated celery, produced a more severe infection than the ordinary Septoria of cultivated celery. Thus it is known that both wild and cultivated celery are susceptible to the late blight disease.

Klebahn (21:23) and Thomas (49:10) have shown that
in spite of the similarity among the suscepts to Septorial diseases, within the Umbelliferae, the pathogene on celery cannot produce disease on closely related genera of hosts, including parsley. Laibach (24:191,192) tested the inter-susceptibility of parsley (Petroselinum sativum) and celery (Apium graveolens), attempting reciprocal inoculations within these two host genera. He obtained no evidence of inter-susceptibility of these two hosts. It seems established, then, that the Septorial disease of parsley is biologically different from that of celery.

**History and Distribution**

The history of the disease does not antedate the discovery of the causal organism, as far as is known to the writer. According to Klebahn (20:11) this pathogene and the disease might have been known previously to 1890 inasmuch as Sydow reported a parasite on celery which he named Rhabdospora nebulousa and which answered to the description of a Septoria species. However, Briosi and Cavara's description of the disease and its causal organism is generally accepted as the first authentic report. This report appeared in Funghi Parisitti delle Piante Coltivate od utili, Fasc. VI, No. 144, 1891. This original publication was not available to the writer, but Klebahn's (20:8) description was accepted as authority.

During the following decade, the disease was reported
from several stations in the United States and Europe:

1. In 1891, the disease was reported by Chester (6) in Delaware, who suggested the name 
   *Septoria apii* for the pathogene; Humphrey (18) in Massachusetts; Halsted (16) in New Jersey; and Allescher and Schnabl in Germany. These last named reporters assigned the binomial 
   *Phlyctena magnusiana* to the causal organism (Klebahn, 20).

2. In 1892, the disease was reported by Beach (2) in New York State.

3. In 1893, Rostrup reported it in Denmark (Klebahn, 20).

4. In 1894, Prillieux and Delacroix (36) reported it in France.

5. In 1897, it was found in California (Rogers, 39).

6. In 1913, two forms of the disease were recognized by 
   Dorogin (12) and Laibach (24).

   From these original centers, the disease spread rapidly throughout Germany (20:5), and the United States (Orton, 30,31). Furthermore, in 1902 the disease was already known in Belgium and Norway and it was first observed in England (7:116).

   Some idea of the distribution of this disease can be gathered from the fact of its being reported from South Australia (32); Russia (12:57); Bermuda (26); West Australia (4); Kenya Colony, Africa (27); and Czecho-Slovakia (46). Its widespread occurrence in the United States, alone, is attested by the reports of the United States Department of Agriculture.
given in the Plant Disease Survey Bulletin (50, 51) and Plant Disease Reporter (52, 53, 54). Thus during 1918, twenty-three states distributed throughout the United States, except in the arid southwest, reported the late blight of celery. Eleven more states may be added when other years are considered, making a total of thirty-four states in which the disease was observed from 1915 to 1931. However, the number of states reporting late blight of celery for any one year was never more than twenty-three, 1918. From this evidence, it is safe to say that the late blight of celery may be present in any region where celery is grown.

**Economic Importance**

The nature of the loss from this disease is of two major classes and possibly a third should be added. The one generally referred to in the literature is the reduction in the crop from the stunting of the plants and the severe trimming which is necessary before the infected plants can be marketed. Another very important type of loss is the decay in storage due to the bacteria and fungi which enter through the blight lesions. This disease also occurs on the flowers and their pedicels, and from observation made while attempting to secure matured diseased fruits, the writer thought that infected flowers failed to set fruit.

Estimated percentages of loss from this disease in the United States for some of the years from 1915 to 1931 were
reported by collaborators of the Plant Disease Survey. However, estimates of losses in storage, separately, are less available. The Plant Disease Bulletin (51:146) and the Plant Disease Reporter (52:274; 53:97) together, covering the years 1922, 1924, 1925, show a particularly widespread loss from this disease which was prevalent throughout the celery growing regions of the United States. As a single state, Michigan suffered the severest losses. In 1922, there was a total loss of the last celery crop harvested. In 1915, the loss in Michigan was estimated over $1,000,000 (50:105). These are striking examples of the losses that may result from this disease as it occurs in years when conditions are favorable.

During the same period (1915–1931), Massachusetts did not suffer a general loss of the celery crop on account of this disease. However, in 1918 a general infection resulted and a loss of 10 per cent of the crop was experienced in the eastern part of the state (50:109). In 1928 a loss of 5 per cent was estimated for the crop in the entire state (54:52). The data for Massachusetts show that this disease is of considerable economic importance to most of the celery growing sections of this state.

Symptoms

Morphological Symptoms.

The chief morphological symptoms of this disease are
the necrotic lesions or spots on the leaflets and petioles or leaflets alone. The only signs are the pycnidia borne on leaves and fruits. The lesions vary somewhat in size, shape, color, and the presence of a border. On the basis of certain general differences, Dorogin (1970, 72) has described two classes of morphological symptoms. A translation of Dorogin's Latin description of these symptoms follows:

The first class is characterized by "ochraceous spots, rather obscurely margined or having a continuous yellowish area surrounding, on leaves slightly yellowing, often no distinct spots and the pycnidia 'nesting' in green areas, the pycnidia are scattered on the spots, found on living and dying leaves and also on fruits and their pedicels". Another group of spots within this same class has "spots ochraceous brown or none as in the type, pycnidia with rather an obscure apex, found on living and weakened leaves".

The other class is characterized by, "spots, dirty--ochraceous, indistinct, sometimes a bright pale yellow; 0.5 to 3.0 millimeters in diameter, pycnidia found throughout the entire leaf blade and sparsely on the petioles, aggregated or solitary on the green tissue, lying 'nested' on the spots and around them, numerous, aggregated into 'sori', connected together from upper to lower surface of leaf, erumpent, the affected leaves first turn yellow between the spots." Other spots are "dark ochraceous, rarely white, very irregular, 1-3 millimeters
in diameter; pycnidia in and about the spots and 'nesting' on the petioles, few, amphigenous, erumpent, found more often upon the leaves". Spots of still another group are "sub-orbicular, dark ochraceous or pale, very indistinct, 3-5 millimeters in diameter, pycnidia amphigenous, erumpent, aggregated in the center of the spot, found on living leaves and petioles".

Investigators, other than Dorogin, have observed two types of symptoms, the small spot and the large spot. Among these investigators are Petrak (35), Laibach (24), Foster and Weber, all working independently. Their reports show agreement only in the size of the spots. There is disagreement regarding the color of the spots and distribution of the pycnidia. Dorogin stated that the small spot type was found on both leaflets and petioles and the large spot type on leaflets only, but the other investigators failed to make specific mention of this fact. Still others have subsequently recognized two types of spots. Campanile (3:54) of Italy, and Cochran (3) of the United States, adopted Dorogin's description; while Schenk (44) of Holland, Stirrup and Ewan (48) of England, adopted Laibach's. Dorogin, Campanile, and Cochran agree that the small spot type of the disease is the more prevalent.

A comparison of the specimens collected in Massachusetts in the fall of 1931, (Materials II, III, IV, VII, X), with Dorogin's descriptions of the types of spots showed that
probably all were of one or more of the groups of small spot type, since none of the single spots reached more than 4 mm. in diameter. The spots in Material III and in most of Material X (Pascal variety) compared favorably with those of Dorogin's second, or the small spot, type since the greater number of the spots were one millimeter in diameter and none exceeded 1.5 millimeters. The pycnidia on these spots were for the most part aggregated in the center, thus falling into Dorogin's first group of the small spot type.

Material IV was suspected of having both types of spots, because of the slightly larger size of some of the spots (2.5 mm.) and the arrangement of pycnidia on them. However, Cochran identified leaflets of this material which were sent to him as all of the small spot type.

Some leaflets of a white variety (White Plume) had, besides pycnidia borne on spots, pycnidia in clusters and scattered with no typical spot or only a darker grayish-green area within which the pycnidia were found. These areas had no margin delimiting them. This latter type appears to fall within Dorogin's first type (large or no-spot) under the third group described above.

The occurrence of the spots on leaflets only, was not observed in the Massachusetts material, though the possibility of the occurrence of both forms in the same field and even on the same plant (3:55) may explain how the large or no-spot form might have been overlooked.
It appears then that the second class of symptoms typical of the small-spot form of the disease is generally prevalent in Massachusetts and that the large, or no-spot type, may be in the vicinity of Amherst though definite evidence to support this possibility is wanting.

**Anatomical symptoms.**

The anatomical symptoms which appear during the successive stages of infection have been investigated by Campanile and others. Campanile (3:52) noticed that one of the first evidences of infection after the entrance of the fungus was a separation of the cells and the distortion of the tissues due to the growth of the hyphae. He considered this the internal corollary of the externally visible translucence and convexity of the infected area. Voglino (55:146) noted that the cells of the zone of tissue which marked the outer limit of infection underwent a progressive disorganization of their chloroplasts, which was correlated externally with the yellowish-green border surrounding the spot. This was confirmed by Cunningham (10:731), who stated that this plesioneecrotic area had apparently healthy cells at its outer extremity, where the tips of the hyphae were found, while farther back the contents of the cells broke down and were finally lost. At this same time the walls became brown and collapsed, thus forming the true necrotic lesion which was not bounded by a cicatrice. The necrotic area is thinner than the
healthy tissue and it is upon this area that the pycnidia are borne beneath the epidermis. Campanile (3:51) stated that the pycnidia originate in the intercellular spaces of the palisade layer when they appear on the upper surface of the leaf, but in the spongy mesophyll when on the lower surface. Plate 6, Fig C, drawn from a prepared section, shows this.

Chemical Changes in the Host Induced by the Pathogen.

The chemical changes produced within the host tissue have been reported for two classes of compounds; carbohydrates, and nitrogenous compounds. Concerning the former, Voglino (55:146) found by an iodine test that along with disorganization of the tissue there was a failure in starch formation and instead glucose was formed in that part of the leaf near the hyphae as Fehling's test showed. Regarding the other class of compounds, Coons and Klotz (9:299) found by comparing the nitrogen constituents of healthy celery tissue with that of diseased (the latter infected with *Septoria apii* and *Cercospora apii*), that the diseased tissue had a lower percentage of total nitrogen and nitriles. They believed that these changes were due merely to the decomposition of the host by the parasite in a simple food relation. Voglino (55) considered that specific toxins were involved in the destruction of the host cells.

From the above observations reported in the literature, it is evident that the external symptoms are associated
with equally well marked internal corrolaries in both structural and chemical modifications induced in the host.

STUDY OF THE FUNGUS

Name

The name for this fungus accepted by the writer is the Latin binomial *Septoria apii* (Br. and Cav.) Chester, which, according to Laibach (24:161), is the only correct one that has ever been applied.

The synonymy of the organism with dates of publication and references to Saccardo's description in the *Sylloge Fungorum* are as follows:

- *Septoria petroselini* (Desm.) var. *apii* Br. and Cav. 1891 (41:972).
- *Septoria apii* (Br. and Cav.) Chester 1891.

The above are the original Latin binomials arranged in a chronological order in which they were assigned to this fungus when reported in Italy, Delaware (U.S.), Bavaria, and Denmark, respectively.

History

The history of this fungus begins in 1890 and 1891 with the several original discoveries of the organism, previously mentioned on pages 30 to 32, under the history of the disease.
The first addition to this early history was the report of an extensive investigation by Voglino (55), on the morphology and physiology of the organism as found in its host and in artificial culture. This author used Briosi and Cavara’s name, Septoria petroselini var. apii, but considered that because of the close morphological similarity to the type, S. petroselini, the celery pathogene should not be separated as a variety.

In 1910, Klebahn (20) discussed the Latin names Septoria petroselini, var. apii, S. apii and Phylyctaena magnusiana and pointed out that the latter binomial was inaccurate. In 1912 (21) he proved that the Septoria on celery is not a pathogene of parsley (Petroselinum sativum), but is biologically distinct and should be separated under a different species name as Septoria apii (Br. and Cav.) Rostrup.

In 1913, Dorogin (12) and Laibach (24), working independently, discovered two forms of the fungus. Furthermore, Saccardo (22:1099) described a form characterized by the peculiar pathogenic activity of forming no typical leaf spots. Dorogin compared the two forms that he had found with specimens of other investigators, including those of Saccardo. He noted that the fungus in one of his forms that produced either large spots or none compared favorably with the organisms described by Chester, Briosi and Cavara, Rostrup, and Saccardo. Other specimens of the same general description agreed more favorably with Allescher's Phyctaena magnusiana.
from which Dorogin took the species name to designate his new form. Dorogin did not establish cultural differences between the two organisms that he discovered. However, Laibach showed that the two races he had discovered were different in their reactions to culture media and in their production of peculiar symptoms when pure cultures were used as inoculum (24:187).

The names applied to these forms or varieties and the reference to Saccardo's Sylloge Fungorum are given below:

**Septoria petroselini** Deam. var. apii Br. and Cav.  
forma *emaculata*. Saccardo (42:1099).  
**Septoria apii** Chester var. *magnusiana* (Allesher)  
Dorogin (43:453).  
**Septoria apii** Chester - *graveolentia* Dorogin (43:454).  
**Septoria apii** (Briosi and Cav.) Chester - *punctiformis*.  
**Septoria apii** (Briosi and Cav.) Chester - *maculiformis*.

The last two names were used by Laibach (24:182).

In 1921, Laibach (24) supplemented Klebahn's (21) results concerning the pathogenic relation between *Septoria petroselini* and *S. apii* by attempting reciprocal inoculations within the two host genera, celery and parsley; only negative results were obtained. He gave Chester as the authority for the Latin binomial of this fungus, which was *Septoria apii* (Br. and Cav.) Chester.

It is noticed from the above discussion regarding the proper name for this pathogene that Voglino would agree
with Briosi and Cavara in leaving the established form of Septoria on celery under the species name of Septoria netro-selini, emphasizing the morphology of the fungus alone. However, Klebahn agreed with Rostrup and stressed the biology of the organism as a factor to be considered in naming it. Laibach added evidence in agreement with Klebahn's. On this same basis, if biology be emphasized, Laibach's terms for the races of the pathogene have precedence over Dorogin's, since only Laibach established the difference by culturing the pathogene and performing artificial inoculations.

**Taxonomy**

*Septoria apii* is of the class Fungi Imperfecti, since the perfect stage has not been found (20:13). Because it has a definite, dark colored fruiting body enclosing the sporogenous layer, the genus Septoria has been placed by Saccardo in the family, Sphaerioidaceae of the order, Sphaeropsidales (Allescher 1:5).

**Morphology**

The morphology of this fungus in artificial culture and in association with the host has been fully described in the literature. The formation of the typical structures developed during its life history have been more readily observed in culture and more frequently described. Voglino (55:147) and Klebahn (20:14) have described
and figured the early stages in the germination of a pycnidiospore (Plate 2, Figs. 5-10, from Voglino). The first change in the spore noted was a swelling of the cells except at the septa, thus making the spore appear to be constricted at the septations. At the same time there was an increase in the number of oil droplets within the cells. Usually from one or both terminal cells a short protuberance appeared which, after two days, became a germ tube. Within four days, some, or all, of the remaining cells of the spore sent out from one or both sides tubes which usually elongated to form true septate hyphae. Germ tubes were found to emerge from the parent spore almost perpendicularly. Voglino (55:147) found this was also true for laterals from the main filaments of mycelium. Voglino also noted that under some unknown conditions the germ tube failed to elongate but detached itself in the form of a secondary spore. This failure to elongate was also observed by the writer (Plate 5, Fig B.) in an open drop water culture in which spores from dried Material XIII were thickly sown. Furthermore, the stages in the germination of a pycnidiospore as described by Voglino and Klebahn have been observed by the writer.

Within twelve to fifteen days after spore germination Voglino (55:148) observed that there had formed a dense mat of interwoven mycelium, made up of twisted and anastomosed hyphae of two colors, hyaline and olivaceous brown. From this mat grew bundles of two or more strands that developed short
branches, became olive brown and composed the "nucleus" of pycnidal development (Plate 3, Fig. 48-54, from Voglino). The hyaline filaments developing towards the inside of the young pycnidium formed spores on short lateral branches.

Some of the strands that radiated from the nucleus formed free conidia in clusters of two to six without forming a fruiting structure. Klebahn (20:15) and Campanile (3:47) believed that the formation of such free conidia represented a Hyphomycetoid stage of the fungus.

The role of the hyaline hyphae as stated by both Voglino and Klebahn was the production of conidia, both when found free and within the pycnidia. However, Voglino (55:151) considered the olivaceous hyphae observed in culture, rhizomorphs (55:151) which spread the fungus, but Klebahn (20:15) thought that they represented imperfect pycnidia, especially when found in connection with free conidia. Plate 4, Fig. B., shows a young colony with brown hyphae joining "nuclei" of pycnidia in various stages of development. The writer concludes, from his observations, that the hyaline hyphae are sporogenous and that the brown hyphae on artificial media function as rhizomorphs and as rudimentary pycnidia.

Klebahn (20:16) described the macroscopic appearance of a typical colony. The outstanding fact noted by him and others has been the slow growth of the mycelium which, after a month's incubation, resulted in a colony only a few millimeters in diameter. Around the edge of such a colony, Klebahn
noted fine dark mycelium spreading out in a "brush-like" manner. In the center was dark, irregular mycelium, but upright strands of lighter colored, aerial mycelium were spread over the entire culture. Whitish or rose colored drops composed of conidia appeared on the colony. A microtome section of such a culture showed that the surface of the agar was covered by a dark colored mass of flocculent, thick-walled mycelium, 30-60 microns in depth. Within the agar were delicate mycelial strands bearing conidia in clusters, Klebahn believed the dark mycelial mass represented an imperfect pycnidium.

Observations similar to Klebahn's have been made by the writer. In a culture from Material VIII, incubated outdoors, a lobed, raised, sclerotial-like, hollow colony appeared. The exterior was composed of dark brown mycelium while the interior contained short, grayish-white hyphae. A free-hand section of such a structure is shown in Plate 6, Fig. A. A flat structure was formed in an old culture from Material VIII which, after being hand-sectioned, showed pockets filled with spores. Three of these pockets were found opening into a common large cavity. Such a structure was considered a stroma with incomplete pycnidia formation.

Voglino (55:145) observed that hyphae within the host were hyaline, rather constantly 3-4 microns in diameter and full of oil droplets. Their course through the tissues was very tortuous and hyphal knots were found grouped in the
inter-cellular spaces. Campanile (3:47) reported the formation of stromata in the tissues of the host. However, such structures have not been seen by the writer except in culture, unless the one inter-cellular knot of mycelium represented in Plate 6, Fig. B. can be interpreted as such.

Other types of hyphae have been observed in association with the host. Voglino (55:151,152) described odd, bluntly terminated hyphae found both in culture and on the surface of celery leaves (Plate 3, Fig. 44) and called them appressoria. The brown rhizomorphic hyphae that he found in culture and in the infected leaves, he believed played a saprophytic role and extended the development of the fungus within host tissues after their death.

No true sexual stage of this fungus is known, although anastomosing of hyphae suggesting sexuality has been commonly observed. Voglino (55:141) stated that anastomosing is found at the beginning of pycnidial formation.

Dorogin's (12:72) description of pycnidia and spores of the form *Septoria api* graveolentia as it appeared on the host, is applicable to the organism most frequently found. Dorogin described individual pycnidia as: subglobose, 90-130 microns in diameter, having a dark colored pseudoparenchymatous wall with a darker area around the apical pore; pore sub-orbicular, irregular or elongate with a diameter one-half to two-thirds that of the pycnidium. The conidiophores were 20 microns long. Individual spores were slightly curved or sub-
clavate, obtuse at both ends, hyaline, granulose and septated by three to six cross-walls. The sizes of pycnidiospores (from Material I) as measured by the writer follow: 1.7-3.4 x 27-53 microns (100 spores measured); by Dorogin: 2-3.5 x 45-50 microns. From these measurements it is noted that there is no appreciable variation in width but considerable variation in the limits of length. Many of these pycnidiospores were shorter than those reported by Dorogin.

Voglino (55:151) and Klebahn (20:15) reported dimensions of free conidia formed in culture as similar to those of the pycnidiospores. The writer found the dimensions of conidia from gelatinous drops in culture nearer the upper limits of variation in length, 40-50 microns, than the lower limits of 27-29 microns.

From the previous description of this fungus, it is to be noted that the usual reproductive structures are: stromata, pycnidia, pycnidiospores, and free conidia. The development of these from a germinating spore on artificial medium has been described, as reported in the literature, and as observed by the writer. It has been noted that each of these reproductive structures were found in the host. However, the writer's observations have failed to detect definite stromata formed in the celery tissues which he examined.

Physiology

The physiology of this organism falls naturally into
two parts: first, that concerned with the response of the organism to artificial culture, and second, that concerned with the response of the organism to conditions affecting it while associated with the host, i. e., its epiphytology.

The factors considered in artificial culture are: effect of nutrient media, concentration of spores in culture, temperature, and reaction (pH) of medium. Light, i. e., ordinary illumination, is of little or no importance. Special kinds of light such as ultra-violet are not reported to have been tested on this organism.

The controlling factors under the epiphytology are: condition and age of the host, temperature and moisture.

Artificial Cultures.

The media employed in the present research have already been described on pages 11-13. The beginning of culture of this organism may be successfully accomplished in water. In water, the spores germinate and according to Voglino (55:152) continue to grow and produce mycelium bearing conidia, but never form pycnidia. Slow and incomplete germination in distilled water, sometimes resulting in secondary conidia directly from the parent spore, was found by Campanile (3:49). Furthermore, he found that tap water gave a higher percentage of germination than distilled water though one per cent of glucose added to the distilled water made germination more regular.
Liquid media other than water such as decoctions of celery and carrot, with or without 2 per cent of dextrose, have been used in the present investigation and have aided spore germination. The production of pycnidia from germinated free conidia (removed from an agar slant culture from Material VIII) in plain celery decoction was obtained in a hanging drop culture incubated for seven days.

Several solid media that have been used in culturing this fungus are reported in the literature. Voglino (55:153) germinated spores in manure decoction on soil. Thomas (49:7) found that starch agar favored radial growth of mycelium. Laibach (24:185) noted that germination of conidia was earlier in pure water or on water agar than on prune or carrot agars.

The solid media used by the writer have been enumerated and described on pages 12,13. On plain water agar spores produced small grayish colonies which ceased to enlarge after attaining about one millimeter diameter. On potato dextrose agar germinated conidia were able to form a colony of grayish-white mycelium on which gelatinous drops of conidia were produced. The only noticeable difference between the media potato dextrose agar and the two celery agars was the fact that the celery agar which itself was greenish in color tended to give an olivaceous shade to the grayish mycelium, while potato-dextrose agar generally produced grayish-white mycelium and seemed to favor more profuse production of the
buff-pink (Ridgeway, 38) gelatinous masses of conidia. Of the several agars tested, potato dextrose was generally employed for maintaining permanent cultures.

The effect of concentration of spores in cultures was studied by Stevens and Hall (47:2), who noted that when spores of *S. petroselini* var. *apii* were sown thinly on a celery agar plate they produced dark colonies on germination and later, pycnidia. On the other hand, when spores were sown thickly the resulting colonies were never dark and instead of pycnidia, clusters of free conidia were produced.

In a study of the effect of temperature on pure cultures of this fungus, Thomas (49:7) found that greater radial growth resulted at 13° - 19° C. than at 22° - 27° C. Campanile (3:50) observed that germ-tubes grew more slowly at 25° C. than at 9° - 14° C. No definite statements of the effects of low temperatures were reported in the literature reviewed, although such effects may have been studied by Krout (22:59) and not reported.

No tests of high temperature were made by the writer, but it was observed that a laboratory temperature of 20° - 32° C., during August and September, 1931, was unfavorable for the germination of spores. Therefore, a temperature of 17° C., within the range of favorable temperature reported in the literature, was satisfactorily employed for incubation and storage of the fungus in culture. The effect of low temperature, on pure cultures stored outdoors, was tested during the early
part of 1932. The methods employed have been previously described on page 21 and only a summary of these recorded observations will be given because the growth of the cultures was similar under the same conditions of storage, outdoors and indoors. The lowest temperature for each month that the cultures were outdoors is included in Table 1 (page 54).

Cultures prepared by transferring conidia were placed outdoors on January 14, 1932. An examination of these cultures on February 4 showed short, white hyphae radiating from the inoculum, which was a mere point. On March 14, these colonies had increased to an area of 0.3 x 0.2 cm. Then the colonies were black, lobate, with patches of light gray mycelium on their surfaces. On March 24, the colonies had further increased in area to 0.4 x 0.3 cm., with little if any change in form. On April 14, a further increase in colony area was noted, (total) 0.6 x 0.5 cm., the lobes were more distinct and separated the colonies into dome-like areas. On May 10, a further increase was noted, 1 x 1 cm. Furthermore, they had increased in thickness, 0.9 cm., and were half sunken in the agar. At this stage, many conidial droplets (small, pinkish, gelatinous masses composed of myriads of conidia) were present. The outdoor transfers of mycelium were also examined on the dates above mentioned. In general, the colonies of these cultures were similar in area and type of growth to the
conidial transfers. The cultures placed outdoors after an indoor incubation of four days maintained their advanced growth of nearly three diameters, until April 14 when they were only 1.5 times the diameter of the other transfers. However, they developed conidial droplets much sooner (March 14) than those not incubated four days.

The indoor cultures prepared by transferring conidia showed larger colonies from the first observation which was made on February 9. These had colonies 1.5 x 1.0 cm. as contrasted with a mere point for the outdoor colonies while at the final examination, the colonies were about 4.5 x 1.5 cm., or about 7 times the area of those stored outdoors. The colonies were thicker, 1.4 cm., six-sevenths submerged, and conidial droplets were more abundant, many having appeared as early as March 14. Furthermore, at the final examination, the colony had covered the whole agar surface and was thickly spotted with conidial droplets, many of which were confluent. The cultures prepared by transferring mycelium, and stored indoors, showed larger colonies at the beginning, but on March 14 they were only slightly larger than the conidial transfers; finally, only slightly larger in area. However, they were not so thick as the conidial transfer cultures. The parent cultures stored outdoors and indoors varied as did their respective transfers, so that few if any differences were noted. Germination tests of the conidia from outdoor and indoor cultures showed that a large percentage of the
conidia was viable whenever they were present in culture.

From these observations it is to be noted:

1. Viable conidia were produced in cultures under outdoor conditions during the winter.

2. The mycelium remained viable, grew, and was able to fructify during the winter.

3. The colonies formed in outdoor cultures were more rotund and superficial, showing a modification due to outdoor conditions.
Table 1.* Temperatures of the air and the soil, official and unofficial, as recorded outdoors from September, 1931 to April, 1932.

<table>
<thead>
<tr>
<th>Month</th>
<th>Day</th>
<th>Official (Air) Temperature</th>
<th>Unofficial Temperature</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>Lowest</td>
<td>Mean</td>
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<tr>
<td>September</td>
<td>30th</td>
<td>36°F</td>
<td>64.8°F</td>
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<tr>
<td>October</td>
<td>10th</td>
<td>29°F</td>
<td>53.6°F</td>
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<td>13th</td>
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<tr>
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<td>28th</td>
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<td>8 A.M.</td>
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<tr>
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<td>31.6°F</td>
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<tr>
<td>January</td>
<td>5th</td>
<td>10°F</td>
<td>33.5°F</td>
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<tr>
<td>February</td>
<td>4th</td>
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<td>26.3°F</td>
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<td>0 A.M.</td>
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<tr>
<td></td>
<td>15th</td>
<td>3°F</td>
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<td></td>
<td>16th</td>
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<tr>
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<td></td>
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<tr>
<td></td>
<td>9th</td>
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<tr>
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<tr>
<td></td>
<td>6th</td>
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<td></td>
<td>(6 A.M.)</td>
<td>(29°F)</td>
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<tr>
<td></td>
<td>23rd</td>
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<tr>
<td></td>
<td>(12 P.M.)</td>
<td>(34°F)</td>
<td></td>
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</tbody>
</table>

*Explanation follows on next page.
Explanations of Table 1.

The official temperature readings given in the table were recorded at the Meteorological Observatory of the Massachusetts Agricultural Experiment Station (14,15) located about one-quarter mile from where the outdoor material was kept. Only air temperatures are thus recorded.

Parentheses ( ) indicate the days of the month on which records were also taken by the writer and the lowest temperature and hour at which that temperature was recorded by the Observatory.

Unofficial temperature readings were recorded by the writer. Soil temperatures, taken by the writer at the same time as those of the air were observed by thrusting a thermometer into the soil to a depth of six inches so that the bulb was on a level with the buried material or cultures. An exposure of five minutes in air and soil was allowed before readings were taken.

Two readings for the same day represent temperatures recorded when buried material was dug and again when it was reburied. A.M. or P.M. without any hour being given, under "Day", are readings taken in the forenoon between 9:00 and 12:00 and in the afternoon between 1:30 and 5:00, respectively.
The reaction of the medium was studied by Campanile (3:46,47), who used celery decoction agar, naturally acid; neutral and alkaline by the addition of a saturated solution of sodium carbonate. He found a slow radial growth and a tendency to form dark mycelium on the acid agar, while on the neutral and alkaline agars he noted a dense and extended growth of lighter colored mycelium. Microscopic examination showed that the dark mycelium constituted a stroma bearing abundant pycnidia. On alkaline agar, however, pycnidia were smaller and fewer, while free conidia were much more abundant than on the acid medium. He concluded that alkalinity was more favorable for the growth of the fungus than acidity.

A preliminary experiment made by the writer for testing the development of the organism on media of varied acidity and alkalinity has been described on page 22. Bacterial contaminations developed on all naturally acid (nearly neutral) and alkaline substrata. However, growth of the Septoria occurred only on agar of such reaction. These results lend support to Campanile's observation that alkalinity favored growth of mycelium and production of free conidia, while acidity retarded both.

Epiphytology.

Factors favoring infection of celery by Septoria apii have been investigated by Thomas (49:4-24), who found that
application of sodium nitrate to the soil in pots in which plants were grown favored infection, as did the presence of infected leaves in the soil. Calcium sulphate, and phosphates in a complete nutrient solution; nematode infection of the roots of some of the plants, and etiolation of the leaves decreased the degree of infection. The age of the plant as a whole had little effect, though young leaves were more susceptible. The temperature factor, he found variable and inconclusive. He suggested a possible correlation between the greater susceptibility in young leaves than in old leaves, since a higher acidity was found in old leaves. It appears from Thomas' results that in general, factors favoring rapid vegetative growth of the host favor infection by this pathogene.

The failure of various temperatures in storehouses to prevent the spread of this organism and the disease it produces is accepted by Link (25:507) and Smolak (46:46). Reddick (37:5) tested certain conditions of storage and found that there was no appreciable spread of the disease on celery from October 15 to December 23, 1912 at a constant temperature of 31°F. This result is seen to correspond favorably with the slow growth of the fungus on potato dextrose agar as observed by the writer in pure cultures stored outdoors, January 14 to May 14, 1932. Apparently, a temperature of 31°F is much below the optimum for this organism.

It has been reported by Campanile (3:62) that
humidity is necessary to permit the liberation of spores from pycnidia. Free (liquid) water is generally considered necessary for the germination of spores and for infection of the host by the hyphae.

As a summary of the physiological reactions of the pathogene, it may be said that the organism can be cultured on a variety of artificial media, namely, water decoctions and soil containing manure water, agars of starch, celery, and potato.

The spores of the organism respond to crowding on artificial medium by the production of fewer and smaller pycnidia and more free conidia.

At the rather low temperature of 9° to 19° C., the fungus grows better than at 22° to 27° C.; a temperature a little below 0° C. (31°F.) does not kill it, though such a temperature does retard its growth on both artificial and natural substrata.

Free conidia and mycelium are produced more readily in an alkaline than in an acid medium, and pycnidia more abundantly in the latter.

Regarding the epiphytology of the pathogene, it has been shown that the same factors that favor rapid growth of the host favor the infection by Septoria apií.

It follows, therefore, that any or all of these above mentioned factors may have a definite bearing on the manner in which thus fungus survives the winter.
The Life History in Relation to Overwintering.

The overwintering form of the pathogene is obviously the source of the primary inoculum for the first infection of the host in the spring. The source of such inoculum may be one or all of three possible structures of the fungus, pycnidia and pycnidiospores on the fruits and on the leaf refuse, and sclerotia or stromata in diseased material. The ability of spores from pycnidia on fruits to survive the winter and upon germination to produce infection of celery seedlings has been proved by Klebahn (20:14) in Germany, Chittenden (7:264), and Pethybridge (33:690) in England. Klebahn inoculated seedlings artificially, using a spore suspension. Chittenden made no special inoculations but obtained infection of seedlings with pycnidiospores from pycnidia on the fruits. Results contrary to those obtained by Chittenden were reported in New Jersey (23) and in Ontario (29). However, the experiment in New Jersey was divided between two different localities, and that in Ontario was not reported to have had a check in parallel, so that the conclusion of the two American workers, that infected fruits are not a source of inoculum, was less well-founded than the conclusion of the European, that infected fruits are a source of inoculum.

The alternative source of inoculum considered by Krout and the experimenter in Ontario was some form of the fungus persisting over winter in the soil. Killian (19:6)
has definitely shown that \textit{Septoria lycopersici}, under the conditions tested, can infect tomato seedlings grown on soil containing refuse from an infected crop of the fall before. Voglino and Klebahn did not report a similar fact for \textit{Septoria apiit} in relation to celery seedlings.

The writer's experiment to test the possibility that fruits and refuse in the soil are a source of inoculum has been described on page 27. As additional treatment, the pots were watered daily so that they never became dry. On May 21, five days after the first seedlings had germinated, the plants were removed outdoors to partial shade under a tree because the greenhouse temperature was frequently 35 to 40° C. The pots containing infected seed and contaminated soil were covered with bell jars which were removed in the morning of May 24 when the plants were placed in direct sunlight.

The fruits used in this experiment were found to be infected as follows: Newark Market, 10 per cent; fruits from artificial inoculations, 10 - 25 per cent. Pycnidiospores obtained from these fruits failed to germinate. Moreover, only healthy seedlings developed from these fruits (June 10).

The structures of the fungus found in host refuse, namely, pycnidiospores, pycnidia, and sclerotia (or stromata) when present, have been proved to survive certain winter conditions. Voglino (55:153) found that in the environment of Rome, Italy,
spores from pycnidia on buried leaves in soil in flower pots remained viable throughout the winter of 1899-1900. The atmospheric temperature prevailing was 2° to 4° C. Klebahn (20:13) likewise found that spores from pycnidia borne on infected leaves left outdoors, some in flower pots and some in cloth frames, were capable of producing infection on growing plants the following spring. These tests were made in the fall and winter of 1907-1908, and of 1908-1909, in Germany. Krout (22:596) gave it as his belief that the organism survived the winter (in New Jersey) as sclerotia in leaflets and petioles.

It is noted, by combining these last reports with those preceding, that all three of the structures of this fungus mentioned on page 47 have been reported to be concerned in its overwintering under certain conditions. It is further noted, that temperature is the only environmental factor specifically mentioned. Moreover, this was much above the ordinary outdoor winter temperature found in Massachusetts. Thus, the conclusion drawn from the results obtained elsewhere might have no bearing on the mode of overwintering of Septoria api under Massachusetts conditions in the winter of 1931-1932.

The writer performed experiments to determine what structures of the fungus, if any, overwintered in 1931-1932. The structures of the fungus that may overwinter in host tissues are: pycnidia, pycnidiospores, sclerotia (stromata),
conidia, mycelium. The materials employed and the tests were made according to methods previously described on pages 3 to 9. A summary of the results from the observations recorded follow:

1. The buried material in wire packets, stored outdoors, was examined on November 28, 1931; February 4, March 8, April 6 and 23, May 14, 1932. The indoor material was examined on all these days except November 28, April 23, and May 14. However, it was examined on April 30. The examination of November 28 showed that the infected tissues were intact, while the others were disintegrated. All the tissues were moist. The organisms other than Septoria apii present were: Bacteria, Nematodes, Didymaria, Fusarium, Alternaria. The structures of Septoria apii that were found were: empty pycnidia, individual pycnidiospores, and mycelium, closely associated with the pycnidia. In culture, none of these structures showed definite evidence of viability. Observations made at all subsequent examinations were essentially identical with the observation of November 28 as to the conditions of the host tissues, the organisms present, and the non-viability of the fungus. Furthermore, the tests for pathogenicity of the overwintering structures and of cultures containing contaminants showed that all the structures and cultures of Septoria were non-pathogenic. However, the checks gave positive results.

The indoor materials were examined and observations
recorded. The same decomposition of host tissues, the number and genera of organisms present (with the addition of Cephalotheclium and Gloeosporium) and the same structures of the Septoria were observed as recorded for the outdoor materials.

2. The infected leaves stored on the surface of soil, outdoors, were examined March 24, April 16, and May 9, 1932. Observations, together with tests for viability, were made, similar to those described for the buried materials; but the tests for pathogenicity were omitted. These observations and tests showed that the condition of the host tissue was drier, less decomposed, than the buried. The fungi found in these tissues were species of Fusarium, Didymaria, Cerco- spora, and Septoria. During an examination of March 24, a few pycnidia were observed to contain pycnidiospores, but most of the pycnidia were empty. However, pycnidiospores were not observed in subsequent examinations. The test for viability of pycnidia and pycnidiospores produced negative results.

Discussion of the Results of Experiments

1,2. Only doubtful or entirely negative results were obtained from the tests of viability and pathogenicity of spores, pycnidia, and mycelium found in overwintered buried material. Furthermore, only negative results were obtained from viability tests of similar structures of the fungus found
in material that had overwintered on the surface of the soil. Such results are at variance with those reported by Voglino and Klebahn (previously cited), under conditions in Italy and Germany, respectively. Therefore, the negative results obtained by the writer require interpretation.

Such interpretation is logically sought in the different conditions that underlie the experiments which have shown such opposing results. Of these conditions, only the temperature is known to be different and this can be compared only with that found by Voglino at Rome, Italy, in 1899, since Voglino alone has reported temperature.

It has already been stated (on page 61) that the temperature, reported by Voglino was 2° to 4° C. under which positive results were obtained. Table 1, page 54, shows that the mean air temperature at Amherst for the four months, December, 1931 to March, 1932, ranged from 26.3° F. (−3° C.) (February) to 33.5° F. (1.0° C.). Thus, for half of the eight months during which the material was buried the mean temperature was lower than the lowest (2° C. = 35.6° F.) recorded by Voglino. Moreover, low temperatures of 9°, 10°, 3°, and 9° F. (−13°, −12°, −16°, −13° C.), respectively, are recorded for the same four months. (It is understood that the temperature Voglino recorded was air temperature.) It is obvious then that there is a rather wide difference in the condition of temperature alone, between the experiment of Voglino and that of the writer.

Data are not available to permit a general statement
regarding the soil temperature prevailing where the outdoor material was buried. The temperature of both soil and air were taken at each examination and are entered in Table 1 with those of the official report. It is noticed that the outdoor soil temperature at the level of the buried leaves, i. e., about six inches below the surface showed a lag behind the air temperature at any given time. It is noted that on March 8, 1932 there was the low temperature of 33°F. Moreover, observations not recorded in the table showed that on November 28, 1931 there was a thin crust on the soil over the buried material, and on February 4, 1932 the soil was frozen for a depth of four inches below the surface; however, not reaching to the packets of leaf material. Thus, the soil temperature at Amherst is known to have been frequently below that reported by Voglino at Rome, Italy, for 1899.

Two possible factors not mentioned in the literature suggest themselves. These are moisture and competition of other organisms, both of which have been recorded by the writer. The possible effect of these factors individually, together, and combined with temperature, is not known. However, the presence of *Didymaria* and *Fusarium* in such intimate relationship with *Septoria api* as found in the cultures of single pycnidia, logically suggests that under the conditions of moisture and temperature prevailing, these saprophytic fungi were able to compete unfavorably with this pathogene on celery leaves outdoors, both beneath and on the surface of the
soil. This is further suggested by the failure of any structure of *S. apii* to grow after having been in the soil indoors at a higher temperature (12°-20° C.) with the moisture condition similar to that outdoors. The conclusion is that *S. apii*, in or on the soil, is unable to compete with certain fungous saprophytes from the soil when moisture is sufficient and temperature is not prohibitive.

3. Leaflets in a moist chamber; 4. Portions of infected petioles and leaflets in moist Petri dishes. These two tests were essentially alike in that they were to discover whether sclerotia or stromata could form by a saprophytic development of the fungus. As the tests were arranged, such activity could be easily watched since individual spots were under observation. Incidental to the main test, which consisted of mere observation, were tests of viability of pycnidia and spores. These were like those described for buried material. The results showed that: no true sclerotia or stromata were formed under the conditions of the tests. However, clusters of pycnidia like those frequently found in fresh material appeared in some lesions (Plate 6, fig. B). Furthermore, mycelium and spores from these materials failed to give evidence of viability.

5. The test of spores from dried leaves was merely a partial duplication of Krout’s work (1916:596). The methods of culture and calculation of percentage are explained on
Examinations were made on September 9, 1931, February 2, April 7, and May 11, 1932. No appreciable difference was noted between percentages of germination of spores from material dried in the incubator as opposed to those dried in the laboratory. Perhaps the fact that leaves of Material I were allowed to decay somewhat before they were dried, explains in part the very low percentage of germination that was noted at all the examinations except the first. Sixty to eighty per cent of the spores from each sample remained viable except from Material I. At the last examination, May 11, the spores from Materials I, III, X, and XIII had been dried approximately eight and a half, eight, seven, and five and a half months, respectively. It is evident then that a high percentage of spores on dried celery material indoors remain viable for at least eight months.
CONCLUSIONS

Conclusions drawn from the data assembled by the writer may be stated thus:

1. **Septoria api** (Br. and Cav.) Chester can overwinter as viable pycnidiospores in pycnidia under dry conditions while in host tissue.

2. Free conidia so far as is known, are formed by this fungus in artificial culture only. It is doubtful that individual free conidia survive the winter, though they are continually produced in cultures left outdoors.

3. True sclerotia, pycnosclerotia, or stromata, were not observed except in artificial culture. These can overwinter in culture (on potato dextrose agar) under outdoor conditions.

4. Mycelium in leaf tissue of the host has not been found to overwinter when buried either indoors or outdoors or when left on the surface of the soil outdoors. Mycelium in culture can overwinter.

5. The fungus overwinters as pycnidiospores in pycnidia in dried leaves in the laboratory, but not on leaves or portions of leaves when moisture is present in abundance, whether outdoors or indoors.

6. From what has been discovered from the writer's research, it seems that any practices or handling of infected celery which would permit the leaves to become dry and remain thus overwinter, would be favorable for the dissemination of
CONCLUSIONS (cont.)

the pathogene. On the other hand, plowing-under or otherwise burying infected refuse so that this would be kept moist would be unfavorable to dissemination.

These conclusions are based upon data obtained from tests for the overwintering of *Septoria apii* under conditions prevailing at Amherst, Massachusetts during the winter of 1931-1932.
LITERATURE CITED


37. Reddick, D. Decay of celery in storage. Phytopath. 4:45. 1914.

38. Ridgeway, R. Color standards and color nomenclature. 1912.


PLATE 1

Photographs showing *Septoria apii* in the host and on potato dextrose agar.

Figure A. Photograph showing a pressed leaflet of celery with *Septoria apii*. X2
1. A characteristic lesion with scattered pycnidia.
2. A faint halo around the lesion indicates the yellow-green border.

Figure B. Photograph of colonies which have developed on potato dextrose agar after incubating for four weeks. The pycnidiospores were germinated in celery decoction for twenty-four hours, then the spore suspension was streaked in the shape of an "M" on the surface of the agar. The culture was killed in formaldehyde and photographed. X0.75
PLATE 1. Photographs showing *Septoria api* in the host and on potato dextrose agar
PLATE 2

A photograph of drawings showing *Septoria api* in the host and its mycelium in culture. (From Voglino; Plate I)

Figure 1 and 2. Symptoms of the disease.

3. Types of pycnidiospores.

4. A single pycnidium.


14-27. Modification in the structure of the hyphae from which a pycnidium is formed.
PLATE 2. A photograph of drawings showing *Septoria apii* in the host and its mycelium in culture.

(From Voglino)
PLATE 3

A photograph of drawings showing reproductive structures of *Septoria apii*. (From Voglino; Plate II)

Figure 28. Two types of hyphae, hyaline, and olivaceous-brown.

29-36. Ovoid spores in chains.


40-54. The origin and development of the sporo-genous and wall layers of a pycnidium.
PLATE 3. A photograph of drawings showing reproductive structures of *Septoria apii*. (From Voglino)
Photographs of colonies of *Septoria apiil* showing pycnidia and their formation.

Figure A. A photograph of some of the colonies shown in Plate 1, Figure 1A. X4
1-2. Gelatinous conidial masses.
3-4. Characteristic, rough, globose colonies of *Septoria apiil*.

Figure B. Microphotograph of a hanging-drop culture incubated in celery decoction for eight days. The inoculum was removed from a pure culture which had been isolated from Material VIII, and stored outdoors. The culture was killed, stained and mounted in lacto-phenol-green. X125.

2-6. Successive stages in the formation of a pycnidium.
PLATE 4. Colonies of *Septoria apii* showing pycnidia and their formation.
Free-hand drawings of germinating spores of *Septoria api*, and the beginning of a pycnidium.

**Figure A.** Germinated pycnidiospore; incubated for four days in celery decoction on a microscope slide. The inoculum was removed from pycnidia in celery leaves which had been stored in the laboratory for 205 days and heretofore designated as Material III. The culture was killed and mounted in lacto-phenol-green.  

1. Original pycnidiospore with swollen cells.
4-5. Secondary conidia formed directly on the parent pycnidiospore.

**Figure B.** Germinated pycnidiospores; incubated four days in distilled water on a microscope slide. The inoculum was removed from pycnidia in celery leaves stored in the laboratory for 166 days and heretofore designated as Material XIII. The pycnidiospores were crowded in the culture.  

1-2. Two adjacent pycnidiospores.
3. Anastomosing of cells of the adjacent spores.
4-5. Secondary conidia formed directly on the parent pycnidiospore.

Figure 0. A portion of the margin of a colony which developed from conidia incubated for one week in celery decoction, employing the usual drop-slide method. These conidia were removed from a pure culture of *Septoria api* which had been isolated from leaves as Material VII, from New York State. X510

1-3. Free conidia.

4. Hyphae developing thick, brown cell walls, indicating the beginning of a pycnidium.

5-6. Hyphae with thick, brown cell walls formed; more advanced stages than shown in 4.
PLATE 5. Free-hand drawings of germinating spores of *Septoria apii* and the beginning of a pycnidium.
Free-hand drawings of sclerotium-like structures of *Septoria sp.*

Figure A. A somewhat diagrammatic drawing of a free-hand, razor section of a sclerotium-like structure killed and mounted in lactophenol-green. The structure had formed in a pure culture on potato-dextrose agar, incubated entirely under outdoor conditions from January 4 to March 14, 1932. X52

1. Brown, exterior hyphae.
2. Midsection where the hyphae begin to lose their individuality.
3. Hyphae have almost entirely lost their individuality.

Figure B. Drawing from a microtome section stained in Haidenhain's iron-alum-haematoxylin. This shows a cluster of pycnidia in fresh material heretofore designated as Material X. X292

1. Pycnidiospores escaping from the ostiole.
2. Wall common to two pycnidia.
3. Floccose mycelium in the host tissue.
4. Air space.
5-6. Immature pycnidia.
Figure C. Intercellular mycelial knot in the sub-epidermal tissue of a petiole. This tissue was of celery in storage from November to the following January. Drawn from a water mount of a free-hand section. X150

1. Epidermal cell of the host.
2. The mycelial knot.
3. Mycelial strand leading from the mycelial knot.
PLATE 6. Free-hand drawings of sclerotium-like structures of *Septoria apii*.