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LIFE HISTORIES OF TWO LITTLE KNOWN PARASITES OF THE APPLE AND PEAR.

A Thesis submitted to the Academic Faculty of the University of Missouri in partial fulfilment of the Approved PM Durgan requirements for the degree of Master of Arts.

Columbia, Mo., May 1, 1907.

Contribution No. Botanical Laboratory. University of Missouri.

THE SOOTY BLOTCH.

The Sooty Blotch is a very common fungus upon the fruit of the apple and pear. It was first reported by Schweinitz in 1831 from Pennsylvania. Since then it has been reported from all parts of the United States and Europe. The Sooty Blotch first appears on the apple in the late summer when it has passed the stage of medium growth. (Plate I.)

It is a superficial parasite which does not penetrate the cuticle of the apple, breaking down the tissues; but merely roughens the cuter surface of the cuticle. The fungus is easily recognized by the pale to deep black blotches that appear superficially upon the apple. These blotches are irregular in outline, in many cases coalescing to form large areas.

another whose appearance upon the surface of the apple has suggested for it the mmon name "The Fly-Speck Fungus". Although these two fungi are commonly found together, in many cases only the one may be found. This fly specked and blotched appearance of the apple is prevalent in most orchards, its extent depending upon the variety, the weather conditions, and the situation of the orchard. Such an affection of the pear is not so prevalent, though the extent of the fungus depends upon very much the same conditions.

Historical Account. The fungus causing the Sooty
Blotch was first described by L. Schweinitz (1) in 1831 as

Dothidea pomigena. He found it upon Newton Pippin apples,
in Pennsylvania. The above is practically the only original
technical reference to this parasite in a special botanical
sense; although other references based upon the above may be
found, such as in Saccardo's Sylloge Fungorum. The remaining
references to this parasite are to be found only in the
economic publications of our Agricultural Experiment Stations.

In 1891 Jones (2) in the Fifth Annual Report of the Vermont Experiment Station reported a fruit spot disease of the Baldwin apple which Ellis identified as probably being Dothidea pomigena Schw. In 1899 F. C. Stewart (3), of the Geneva, New York, Experiment Station proved that this fruit spot was not caused by Dothidea pomigena, but was probably the result of an unknown fungus.

In 1898 Sturgis (4), of the Connecticut Experiment
Station, reported it upon the Rhode Island Greening and
Newton Pippin varieties. He also reported that Green and
Selby had noted "its occurrence in Ohio on Peck's Pleasant
and the Rome Beauty as well as on certain varieties of pears,
notably Vicar and Angoulême. Professor Lamson, of New Hampshire, found it common on Greenings, Northern Spies, Baldwins,
and many other varieties, and on pears of the Beurré de Anjou
and Lawrence varieties". Sturgis also conducted some very
successful experiments in spraying for the prevention of this
disease, finding that this operation also improved the general

vigor of the trees and the appearance and keeping qualities of the apples.

In 1897 and 1900 Selby (5), of the Ohio Experiment Station, indicated in a description of these two fungi that the Sooty Blotch was identical with the Fly Speck fungus, but did not produce any evidence to that effect.

Description and Conditions of Growth. The Sooty
Blotch has a world-wide distribution, being found probably to
some extent in every country where the apple is grown. In the
United States it has been reported from New York and New
Hampshire on the north and east, and from Alabama on the south.
Although not having been reported any farther westward than
Nebraska, its range doubtless extends to the coast. It has
been reported from the following States: New York, New Hampshire, Connecticut, Pennsylvania, Delaware, West Virginia,
Alabama, Chio, Indiana, Michigan, Illinois, Missouri, Iowa,
and Nebraska.

Wilcox (6), of the Alabama Experiment Station, reports that it "causes much damage to apples, particularly during wet seasons or where trees are planted in low, damp soils". Kern, of the Indiana Experiment Station, says it is "reported from a number of localities; in some instances one-half of the crop being disfigured". While on a trip in Southern Indiana during November, 1906, the writer observed several orchards in which practically the whole crop was blotched; and in no case was an orchard seen wholly free from the fungus. No data have

been collected as to its distribution through Missouri. Observations of the apples in the local markets at different times show the fungus to have been quite prevalent. In some cases the surfaces of the apples were so completely covered with the blotches that they appeared at first sight to be worthless.

During October, 1906, the writer made a survey of the orchard of the Horticultural Department of the University of Missouri, making a study of the distribution and occurrence of the fungus upon the different varieties. The Janeton, Jonathan, Salome, Kossuth and Mann varieties were found to be the most infested. Upon the Edwards, Kansas Greening, Ferris, Winesap, Ben Davis, Ingram, Rutherford, Andrews' Winter, Wondering and Arkansas Beauty varieties, the fungus had only a medium distribution. While those only slightly infested were the Winkler, Missouri, Gano and Collins varieties. Among the pears the Krull and the Keiffer varieties were found to be the most infested.

It was noticed that the fruit upon different trees of the same variety differently situated in the orchard varied in their susceptibility to the fungus. For instance, one tree of the Improved Janet variety on the extreme north edge of the orchard had its fruit very much disfigured by the fungus; whereas another tree of the same variety situated in the interior of the orchard, had its fruit only slightly infested.

The reasons for this difference in susceptibility involve many ecological factors. No exact ecological data have

been taken, but from observation the following appear to be important factors: (1) moisture, (2) dust, (3) light. in the extreme north to northwest part of the apple orchard was found to be the worst intested. Here the ground is low and moist. Again, along the street to the west of the orchard there are several trees of the Mann. Salome and Kossuth varieties. Here it is also moist but higher ground. All of these varieties were badly infested, the Mann variety being the worst infested of any in the orchard. Adjoining the infested northwest part of the orchard was plowed orchard ground, thus it will be noticed that the two badly infested areas were in close proximity to places where dust was being raised, which would suggest dust as being a factor in the distribution of this fungus. It was also noticed that the fruit attacked to the greatest extent was that located where the limbs were open and not so well protected from dust by the thick leaves.

It has been observed that the fungus is most prevalent during years in which the summers have been cool and damp. Considering the fact that it is very superficial in its growth, as will be shown below, it is easily inferred that moist conditions are most necessary. Longitudinal sections of the infected apple tissue have yet failed to show any penetration of the cuticle by the fungus hyphae. Hence it must be concluded that its food is obtained by breaking down small amounts of the cellulose and the absorption of small quantities of organic matter carried by the air to the apple surface in the form of dust.

There was a noticeable absence of the fungus on the fruit in the south side of the orchard where the trees were The trees here were of the Ben Davis and Jonathan fer apart. varieties, which upon the opposite side of the orchard were The absence must be due to ecomore or less badly infested. logical factors. The ground here is somewhat higher and much less moist than the places previously mentioned. Also, the trees get much more sunlight. the drying effect and higher temperature of the sunlight probably making it difficult for the fungus to infect the fruit. In the interior of the orchard the trees are very close together. The fruit was infected only from a slight to a medium extent. The moisture conditions are possibly good for the spread of the fungus: but the decrease of the dust factor would account for its not being more prevalent.

There is yet another factor which has not been considered; and that is the susceptibility of the apple itself. It is noticed that the greenish varieties of apples are much more often attacked and much more infested than the darker colored varieties. The Mann and Janeton varieties are very susceptible. The large majority of the Janeton apples observed in the local markets were more or less affected by the Sooty Blotch. The Ben Davis and Winesap varieties are also susceptible; but the fungus was never found in nearly such quantities as upon the varieties above mentioned.

Description. The first appearance of the fungus is manifested by a small, slightly colored blotch from 1 to 2 mm. in diameter. It is made up of radiating hyphae, which have

branched and rebranched. forming a network. (Fig. 1.) these blotches increase in size, neighboring ones may combine, forming a blotch very irregular in outline. The hyphae in the youngest stages are olive brown to fuliginous in color. cells are of a more or less irregular barrel shape, the variations giving the hyphae an irregular appearance. (Fig. 2) Branches of the hyphae are formed by buds, which as the young branch extends, are separated from the mother cells by a septum. (Fig. 3). Fusions between neighboring hyphae are common. (Fig. 4.) The next notable stage in the development of the fungus is the aggregation of cells at intervals on the radiating hyphae. The cell walls fuse, forming an early stage in the development of the sclerotium. At this time these cell masses are of a decided olivaceous brown color, having the appearance of a projected, These cell aggregations are at first one convolute surface. layer in thickness. By October they have become several layers in thickness and present the appearance of a more or less irregular mass of cells of a dark color. (Fig. 5.) By November these masses have become of such a thickness that they are black in color, forming a decided sclerotium. (Fig. 6) March the hymenial layer has developed, showing these cell aggregates to be pycnidia. Longitudinal sections at this time show different stages of development; the earliest of which are those showing the radiating character of the hyphee making up the scler-No hymenial layer has yet developed. The next stage otium. seen is that in which the hymenium had developed but no spores

had become differentiated. (Fig. 7.) On April 16 a piece of infested apple tissue was examined under the low powers of the microscope. Each cellular body showed not the usual homogeneous black mass but a large olive brown center with a black border. (Fig. 8.) From teased preparation; it was found that the olive-brown center was an exposed spore mass. (Fig. 9.) The spores were 2-3 x 12-14 and hyaline, slightly wider in the center than at the ends. (Fig. 10.) The pycnidia were 25 - 100 in diameter.

Referring to Fig. 7, it will be noticed that the upper surface of the hymenial layer is covered by a thin layer (a a) of parenchymous tissue. At the time the spores have been differentiated, this layer of tissue is probably broken away, leaving the spore masses exposed as seen in Fig. 9.

Cultural Characters. Numerous attempts have been made by previous writers to secure the Sooty Blotch in culture, but without success. In February, 1907, the writer secured the Sooty Blotch in pure culture by inoculating a piece of sterile filter paper laid upon the sterile surface of a piece of apple with particles of the Sooty Blotch scraped from the surface of an infested apple. The cultures were prepared as follows:

Sterile preparation dishes were used. Into each were placed three glass rings and a small quantity of sterile, distilled water.

Upon these rings was placed a flat piece of apple cut from a healthy apple in such a manner that it was perfectly sterile.

Upon this was placed a small piece of sterilized filter paper

which formed the inoculating surface. The apples from which the Sooty Blotch was obtained for inoculation were washed successively in tap water, distilled water, 95 per cent. alcohol, distilled water, and sterilized water; and then placed immediately into the sterilized preparation dishes.

The growth of the Sooty Blotch was very slow and in many cases contamination, mostly Penicillium, crept in, not allowing the Sooty Blotch to get a starting growth. Cultures were made on February 2, 1907; on February 12 no growth was yet noticeable; on February 25 a small mycelium had developed. Parts of this were transferred to dilution and (Fig. 11.) tube cultures of apple ager. The dilution cultures became so badly contaminated that no growth from the inoculation In the tube cultures spot formations developed, took place. made up of a mass of Dematium-like cells, from which arose a mycelium. This mycelium was composed of fuliginous, irregular, barrel-shaped cells. The hyphae were not branched as much Es those produced on the apples in the field. Hyphal fusions were found to a small extent.

On February 25 a second transfer was made to acid, dilution and tube cultures. Again, the dilution cultures did not grow; but the tube cultures developed the same sort of growth as was noted in the first transfer. It is at this stage that the culture work now stands. The cultures will be carried on to ascertain if the blotch form of the fungus can be obtained by inoculations from these cultures.

Previous to February 2, 1907, a long series of cultures with different media was carried out with negative results. Cultures were tried upon bean, potato and apple tubes; upon dilution and tube cultures of bean, potato, apple fruit, apple bark, bean-apple and maple agars. Apples were halved, placed in preparation dishes so as to rest upon the cut surface, and sterilized. Inoculations were made upon the apple skin. was no resulting growth. Pieces of sterile filter paper soaked in cooked sterile apple juice and fresh sterile apple juice yielded no results. A large number of Van Tieghem cell cultures were prepared, using varying strengths of the following media: Dextrose, dextrose and magnesium ammonium phosphate, dextrose and lactic acid. dextrose and malic acid. dextrose and malic and lactic acids, dextrose and acid potassium phosphate; levulose, levulose and lactic acid. levulose and malic acid. levulose and malic and lactic acids. levulose and acid potassium phosphate, levulose and magnesium ammonium phosphate; magnesium malate; peptone; cane sugar: ammonium molybdate: lactic acid; malic acid; magnesium lactate; magnesium citrate; distilled water. A growth was obtained upon dextrose and magnesium ammonium phosphate solutions, which later turned out to be that of a Chaetomium species.

The difficulty experienced in obtaining a growth would suggest a form of parasitism for this fungus.

Name. The Sooty Blotch was first described, as has been previously mentioned, as <u>Dothidea pom gena</u> by Schweinitz in 1831.

Saccardo in his Sylloge Fungorum has seen fit to change the fun-related gus from the genus Dothidea, placing it under the closely genus

Phyllachora. The pycnidia and spore characters, as already described by the writer, indicate that the fungus does not belong to the genus Phyllachora, but rather should be placed among the Imperfect Fungi, under the genus Leptothyrium, and thus for reasons that will be given later, making it the same as L. pomi (Mont. and Fr.) Sacc.

Phyllachora pomigena (Schw.) Sacc. and Leptothyrium

pomi (Mont. and Fr.) Sacc. being the same, then on account

of the priority of description by Schweinitz, they must

be given the specific name pomigena, the full name becoming

Leptothyrium pomigena (Schw.)

THE FLY SPECK FUNGUS.

The Fly Speck Fungus is another common parasite upon the apple and pear. This name was given it by Chester of the Delaware Experiment Station, on account of its similarity in appearance and size to the ordinary fly speck (Plate I). Other common names for it are "Flies" and "Blackbirds". It appears in late summer as small light colored, orbicular, superficial spots on the surface of the apple. These spots are grouped together, forming circular, sometimes irregular shaped, areas. This fungus is nearly always found closely associated with the Sooty Blotch and as will be shown later in the paper, may be the same organism.

It was first reported and described by Montagne and Fries (1). Since then it has been reported from all parts of Europe and the United States, its range being the same as that of the Sooty Blotch.

Historical Account. The Fly Speck Fungus was first described by Montagne and Fries (1) as Labrella pomi.

Later it was described by Saccardo (2) as Leptothyrium pomi.

Powell (3) was the first to make mention of it in America.

He reports that "all the varieties he examined were affected to a greater or less degree, and some, like the Jackson, were often seriously peppered" with the spots. Selby (4) of the Ohio Experiment Station, made a study of the fungus in 1897. He tried to grow it in culture, but without

success. Clinton (5) gave a short description of it in 1903; and predicted that it wintered over on the apple twigs.

Distribution and Conditions of Growth. The Fly Speck Fungus has practically the same distribution as the Sooty Blotch Fungus, it being reported as being nearly always associated with the Blotch. It is probably to be found wherever the apple is grown. Selby (4) writes that "both sorts of spots may occur upon the same specimen. Indeed it is rare to find an apple affected with one that has not the other." Clinton (5) says "the conditions favorable for the Sooty Blotch also produce this fungus, so the two are often found together. The former trouble, however, proves to be the more serious, as it is more abundant and evident". The writer found it in Southern Indiana: here also closely associated with the Sooty Blotch. Its distribution through Missouri has not been studied. In the local markets during the past winter it was always found more or less prevalent upon the greenish varieties of aprles.

In the fruit survey of the orchard of the Horticultural Department of the University of Missouri, mentioned in the previous paper, it was found to be quite prevalent.

The most infested varieties were found to be the Jonathan,

Cooper's Market, and Arkansas Beauty. Those less infested were Nelson's Sweet, Mihalyfe, Newman, Rutherford, Andrew's Winter, North Western Greening, Beach, Sweet Orange and Winesap. Those only slightly infested were the Janeton, Huntsman.

Edwards, Kansas Greening, Ben Davis, Improved Janet, Missouri, Gano, Devonshire Duke, Dumelow, Ashton, Collins and Wondering. Among the pears, the Krull variety was the only one found infested. The fungus was as widely distributed as the Sooty Blotch but not at all as abundant. In the central part of the orchard the former was much more prevalent.

Apparently the same ecological factors and the same degrees of susceptibility control the Fly Speck Fungus that control the Blotch. Like the Sooty Blotch it is superficial in its growth, never penetrating the cuticle; hence it is very dependent upon the moisture of the air for sustenance.

Description. The first manifestation of the presence of the Fly Speck Fungus on the surface of the apple is a group of small. light colored specks, which separately have the same appearance and size as ordinary fly specks (Plate I). Under the microscope these specks are seen to be a circular layer of olive-brown cells, evidently made up of fused hyphal cells (Fig .1). The main hyphae can often be traced by their increased color. The cells are somewhat varied in shape, being irregular, globoidal to ovoid, resembling very much those seen in the Sooty Blotch. Branching off from these circular layers hyphae can be traced a short distance, where they lose their color and are lost to the eye (Fig. 1).

As the fungus increases in age, these specks increase somewhat in diameter and thickness. Its color becomes deepened until it appears as a smooth, black, shining mass.

At maturity the mass becomes dry and breaks appear just within the margin, allowing the whole center to become free (Fig. 2). This free mass is presumed to be the source of a new infection. Longitudinal sections of these masses on infested tissue, made by the writer, showed a homogeneous mass of cells. Material gathered between October, 1906, and April, 1907, showed no indications of a development of a sporogenous tissue.

Cultural Characters. As has been above indicated, previous attempts at growing this fungus in culture have been In January, 1906, the writer secured the unsuccessful. Fly Speck Fungus in culture upon filter paper soaked in fresh sterile apple juice. The method of preparing the culture has been described under the Cultural Characters of the Sooty Blotch. The culture was made on January 14, 1907, from particles of specks scraped from the surface of a prepared infested apple. On January 21. hyphae made up of fuliginous, irregular, barrel-shaped cells were found growing from points of inoculation (Fig. 3). On February 5 these hyphae had spread radially over a surface from 2-3 cm. in diameter. On February 17 cell aggregations, forming a small layer were noticed. Also many of the hyphae when traced through a distance were seen to have become hyaline at intervals. making them difficult to trace (Fig. 4). this point transfers were made to apple agar tubes, where no growth took place and the culture was lost.

cultures were made on filter paper and the growths transferred to apple, bean-apple and maple agars, with negative results. From this it might be inferred that the fungus was very restricted in its growth, it making a starting growth only upon a cellulose medium. Other media, used with negative results in attempting to grow this fungus, are those outlined under the Gultural Characters of the Sooty Blotch.

Name. This fungus was first described by Montagne and Fries (1) as Labrella pomi. Saccardo (2) placed the fungus under the genus Leptothyrium and it became L. pomi (Mont. and Fr.) Sacc. The writer has found no new characters of this fungus to enable him to determine its exact life history and thus its real position among the fungi.

THE SOOTY BLOTCH FUNGUS IN RELATION TO THE FLY SPECK FUNGUS.

CONCLUSIONS.

In the first paper it is concluded that the Sooty
Blotch is properly referable to the genus Leptothyrium.

In the second paper it will be noticed that the Fly Speck
Fungus has been described as Leptothyrium pomi. The
common generic characters and the more or less constant
association of these two affections suggest a common causal
organism. The facts and observations briefly summarized,
which support the opinion of a common cause are as follows:

- 1. They are superficial in their manner of growth.
- 2. They are controlled by the same ecological factors.
- 3. They are restricted in growth the same by the susceptibility of the host.
- 4. The cells of the hyphae are the same in color and shape.
- 5. There are cell aggregations on the hyphae, though of a different type.
- 6. Their cultural characters are very much the same.
- 7. Lastly, and most important, they are found generally closely associated. By a microscopical examination of a small piece of infested tissue where the two fungi were in close proximity, I have been able to trace connecting hyphae. (Fig. 1.)

I am unable as yet to explain the function of the cell aggregates in the Fly Speck Fungus; but hope by further study of the cultural characters of the two fungi to be able to do so.

In view of the facts above outlined, I therefore conclude that the Sooty Blotch and the Fly Speck are due to the same fungus, namely Leptothyrium pomigena (Schw.)

Synonomy.

Dothidea pomigena Schw.

Phyllachora pomigena (Schw.) Sacc.

Labrella pomi Mont. and Fr.

Leptothyrium pomi (Mont. and Fr.) Sacc.

Leptothyrium pomigena (Schw.)

Leptothyrium pomigena (Schw.) Mycelium superficial, prostrate, olivaceous, sterile throughout growth of host and until early spring; forming speck and blotch-like areas. Pycnidia developing upon the blotch-like areas of the mycelium; demidiate, spheridal, depressed to elliptical, slightly shiny, subradiate in structure and sooty black in color; 25-100 in diameter. Spores 2-3 x 12-14, hyaline, oblong, sometimes obscurely septate.

Habitat. On the surface of the fruit of the apple and pear.

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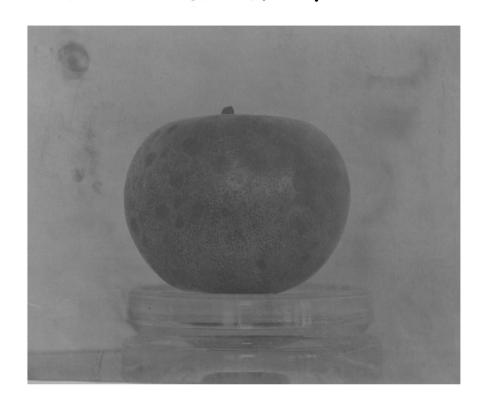
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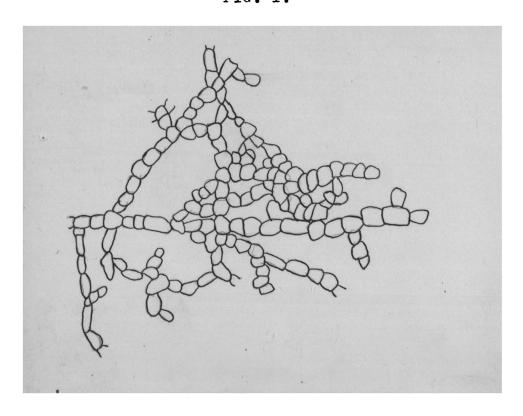
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PLATE I.



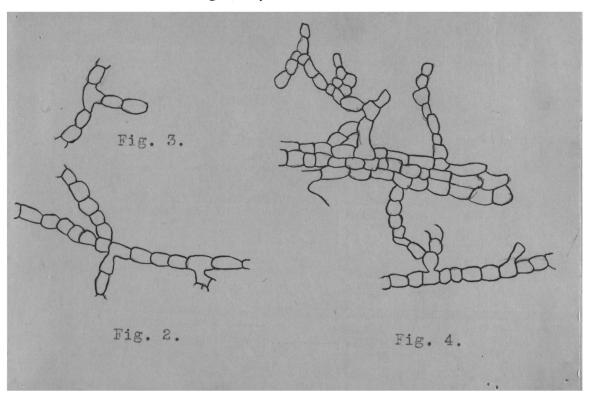
View of the Fly Speck and Sooty Blotch Fungus upon the surface of a Janeton apple.

FIG. 1.

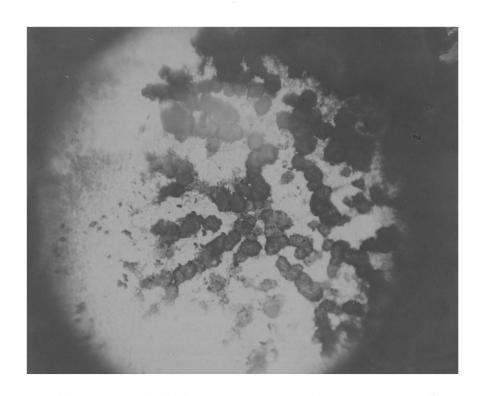


Sooty Blotch. Young stage of hyphae showing branching of the hyphae.

Figs. 2, 3 and 4.

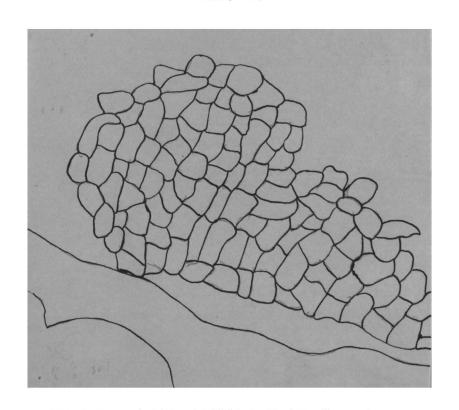


Sooty Blotch. Fig. 2 showing shape of cells in the hyphae; Fig. 3 showing new hyphae arising, cut off from the mother-cell by a septum; Fig. 4 showing the fusing of neighboring hyphae.

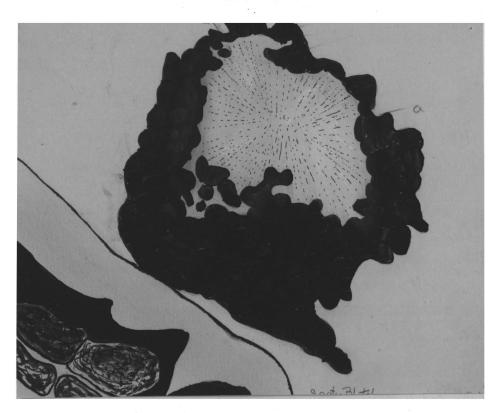


Sooty Blotch. Showing irregular mass of cells of a dark color which go to make up the young sclerotia.

FIG. 6.

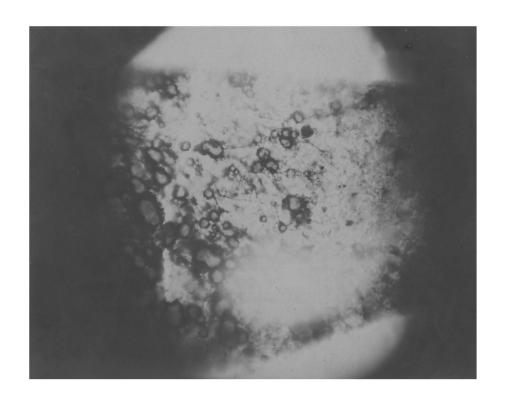


Sooty Blotch. A sclerotium.



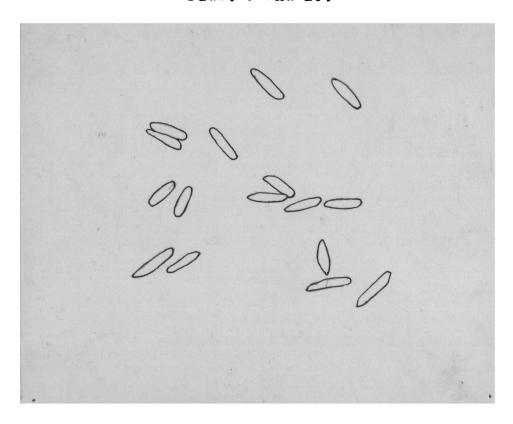
Sooty Blotch. View showing the differentiated hymenial layer. a-a points of breakage of the parenchymous tissue at the time of maturity of the spores.

FIG. 8.

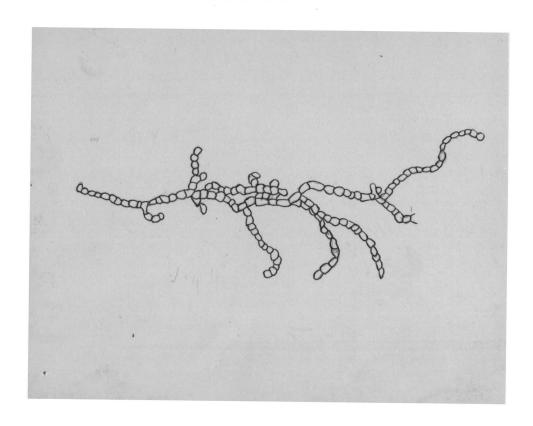


Sooty Blotch. Surface view of exposed spore masses. The mycelium is very evident.

FIGS. 9 and 10.

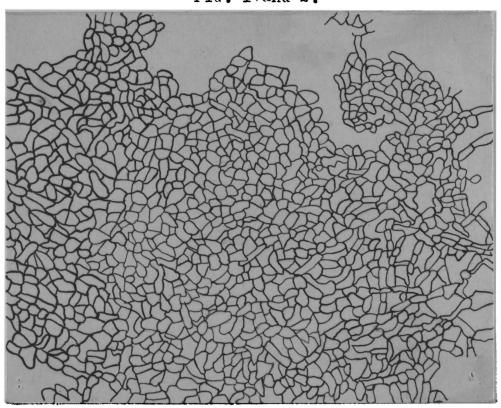


Scoty Blotch. Spores. See Fig. 8.

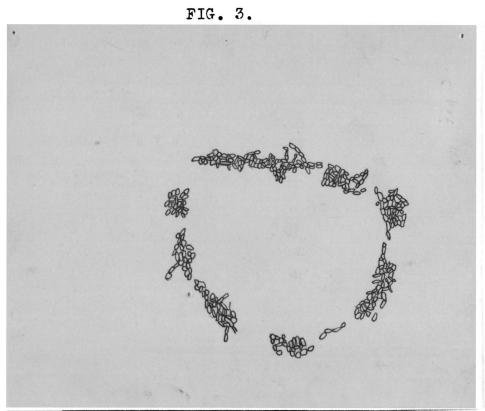


Sooty Blotch. Growth of the mycelium in culture.

FIG. 1 and 2.

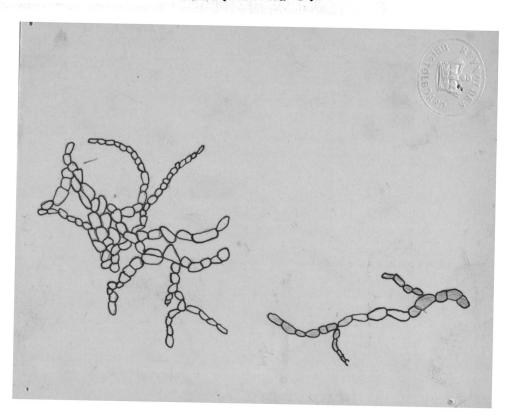


Fly Speck Fungus. Circular layer of cells forming a "speck". Hyphae running outward from the cellular mass become lost.



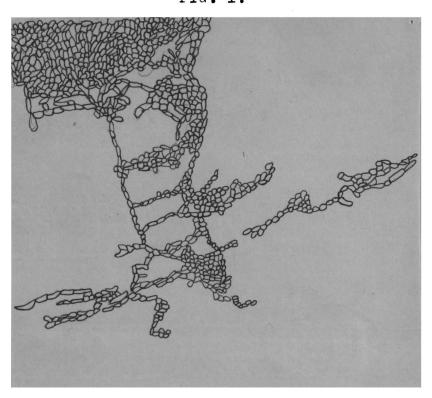
Fly Speck Fungus, showing the center of the mass fallen away.

FIGS. 4 and 5.



Fly Speck Fungus. A cultural growth. A hypha showing hyaline cells.

FIG. 1.



Sooty Blotch and Fly Speck Fungus. Showing connecting hyphae.

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