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REPORT

of

COMMITTEE ON THESIS

THE undersigned, acting as a committee of the Graduate School, have read the accompanying thesis submitted by Mr. Raymond C. Rose for the degree of Master of Science. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science.

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May 29 - 1916

A FRUIT SPOT OF THE WEALTHY APPLE.

A thesis submitted to the Faculty of The Graduate School of the University of Minnesota by R.C.Rose in partial fulfillment of the requirements for the degree of Master of Science,
May 19th, 1915.

SUMMARY OF CONTENTS.

INTRODUCTION

During the last few years a fruit spot of the Wealthy apple has been quite serious in Minnesota. In 1911, 1912, and 1913, a large percentage of the Wealthy apples were affected but in 1914 the spot was less prevalent. At the present time there seems to be quite a difference of opinion as to the exact cause of similar spots on other varieties . The purpose of this paper is to report the observations made in an effort to determine the cause of the Wealthy spot.

Description.

The spots vary in size from 2mm. to 1 cm. in diameter and are dark brown or black in color. The epidermis over the spot is slightly sunken and the tissue beneath is dry and brown, but this dry rot seldom extends over 2 mm. deep. The spots enlarge very slowly and unevenly, depending on the temperature and ripeness of the fruit. Generally there is a lenticel in the center of the sunken area, which appears quite often to be the starting point of the spot.

Economic Importance.

The economic importance of this spot lies chiefly in the fact that it mars the appearance of the fruit and makes it less attractive on the market. The spot ordinarily would not make the fruit unsalable but would tend to keep it from being graded as first class and would thus decrease its selling price. From the replies to a circular letter which was sent out to a number of Minnesota fruit growers it seems that the spot is more prevalent after the fruit has been picked and has been in storage, than it is while the fruit is hanging on the tree. One commercial grower estimates that in the latter part of December 33 percent. of his stored Wealthys were spotted with this characteristic spot. Another reports that twenty-five percent. of his crop were affected, while a third states that about ten percent. of his crop were spotted in this manner.

In some orchards however, the spots appear to be very few, and again growers claim that it attacks individual trees in an orchard. It is often found on those trees making a good growth and bearing large fruit.

Besides the Wealthy, several other varieties are susceptible to the spot. In places it has been reported more prevalent on Wolf Rivers than on Wealthys. The following varieties are often affected:- Wealthy, Wolf River, Peter and Peerless. Stakman[10] has also found it on Red Mirror and Hibernal and Patten's Greening. Several Minneapolis cold storage men stated that very few Minnesota apples were stored for any length of time and that they were unfamiliar with this particular spot.

Previous Work

In 1911 Scott[1] found an apple spot on Jonathans which he described as dark brown, circular or angular, one-eighth to one-fourth of an inch in diameter, and superficial. He stated that the cause

of the spot was unknown, but suggested that it might be due to the arsenate of lead used in spraying, altho he himself obtained some cultures of Alternaria and a few of Cylindrosporium from the spots. However he states that the spots developed mostly after the apples were picked, while in temporary or cellar storage or after being removed from cold storage. He found that low temperatures retarded the developement of the spots.

In 1913 Scott and Roberts (2) reported that they had made 400 cultures of diseased spots on apples but they had been able to isolate no organism with any degree of consistency.

Cook and Martin (3) in 1913 described a fruit spot on the Jonathan which they thought was due to an Alternaria. They found that about ninety percent. of their cultures of these spots gave a characteristic growth of Alternaria. They described the spots as being small, circular , black, 1 cm. in diameter, dry and shallow and they concluded from these data

that infection takes place in the orchard and that susceptibility depends on the character of the lenticel. They are of the opinion that three varieties of Alternaria attack apples; one causing a core rot, another causing a surface spot, and a third causing or following a storage rot.

In 1905 Longyear (5) described a species of Alternaria which caused a core rot on several varieties of apples. He describes it as being a slow dry rot.

Norton (6) reported in 1913 that the Jonathan rot of apples can be duplicated by the action of certain gasses, namely : formaldehyde, ammonia, or sulphur dioxide. He suggested that possibly ammonia leaks in cold storage plants may cause the spots.

During the fall of 1911 and again in 1913 , Stakman (10) examined a number of these spots found on Wealthy and Wolf Rivers apples. Alternaria mycelium was found in the spots quite constantly and successful puncture inoculations were made on a number of apples.

INOCULATIONS EXPERIMENTS.

Methods of Sterilizing, etc.

In the following work, all fruit to be inoculated or to be used as checks for the inoculations was first thoroughly washed in either an alcoholic solution of iodine, or a solution of mercuric bichloride (1 to 1000) The fruit was then rinsed in sterile distilled water and then inoculated and placed under sterile belljars which had been washed in a 1 to 1000 mercuric bichloride solution. Inoculations were made either by puncturing the epidermis of the apple and placing some of the spores and mycelium in the puncture, or by smearing the same over the surface of the fruit. The checks were made either by puncturing the apple with a sterilized needle or by placing drops of sterile distilled water on the surface . Generally six or seven inoculations or checks were made on an apple and these marked with a circle of india ink.

Inoculations on Jonathans.

On Feb. 3d,1914, the writer secured from Dr. Stakman several cultures of Alternaria which had been isolated from Wealthy spots two months previously and transfers were made from these to tubes of beerwort agar.

On Feb.7th, two-hundred and sixteen puncture inoculations were made on thirty-six Jonathans and sixty check punctures were made on apples which were subsequently kept under the same conditions. At the same time thirty-five surface inoculations were made and on these were placed drops of sterile distilled water. Another group, on which were placed thirty-five drops of sterile distilled water alone, were used as checks.In about a week there was a heavy growth of greenish grey mycelium over the surface of all of the inoculations but no spots were in evidence at that time. On March 15th, about five weeks after the inoculations were made, there were dark brown sunken spots under the surface growth of mycelium at each

point of inoculation. These varied in size from 2 mm. to 7 mm. in diameter and were circular to oval in shape. The spots increased in size very slowly and the tissue immediately beneath seemed to dry out and shrink, thus leaving the sunken superficial spot. The inoculated spots differed slightly from the natural spots in that the dry rot extended deeper into the tissues. This was due to the fact that the fungus mycelium was forced deeper into the punctures than it goes ordinarily in a natural infection.

Thirty-six puncture inoculations were made on Ben Davis apples on March 3,1914 and twenty check punctures were made on another group. These apples were kept at room temperature in a moist atmosphere beneath belljars. In six days a vigorous growth of mycelium had appeared at the points of inoculation. On April 27th spots had appeared varying in size from 2 mm. to 1 cm. in diameter. The check punctures remained sterile . (See Plate I)

Reisolation of the Alternaria Fungus.

The above inoculated apples were, on April 27th, sterilized over the spots and pieces of the brown tissue beneath were cut out with a sterile scalpel and placed on beerwort agar. In four days all of these except one had developed a luxurient growth of olive grey Alternaria mycelium which was found, by microscopic examination to be the same as that used in making the inoculations.

Inoculations on Green Wealthys.

In order that a comparison of the fungus development on green and ripe fruit might be made, thirty-six half-grown green Wealthys were obtained on July 27th, sterilized with mercuric bichloride (1 to 1000) and placed under sterile belljars. At the same time a hundred and fifty-six check punctures were made on another group of ten apples . This fruit was also kept under belljars at room temperature. A vigorous growth of mycelium was produced over the inoculations in about seven or eight days while there was none on the checks. (Seed Plate IV)

The apples in the above experiment were divided into separate groups which were kept under separate belljars and each group was inoculated with a culture of Alternaria isolated from different spots. The following table gives the results of the test.

Table showing Results of Inoculations on Green Wealthys.

Culture No.	No. of Apples	No. of Inoculations	No. of Inoculations Successful.	Size of Spots Aug.21,1914.
1	3	18	18	1 to 8 mm.
2	7	42	42	1 "
3	3	18	18	1 " 6 "
4	5	30	30	1 " 5 "
5	4	24	24	1 " 3 "
6	4	24	24	1 "
Check	10	60 Punctures, No spots.		

When the apple is first formed, according to Zschokke,[9] there are a number of stomata on the surface, but three or four weeks after the blossoms fall, they begin to become corky and form lenticels. This corky tissue develops as a result of breaks in the epidermis caused by the rapid enlargement of the apple, and the

stomata being the points that yield most readily to the strain develop theis extra tissue quite readily. He further states that the tannin is located largely in the surface cells of the apple and that it decreased in the ripening process. The chemical composition of the cell sap of an apple especially the tannic and malic acid, according to his theory, influences the resistance to decay fungi much more than does any mechanical protection.

The above conclusions indicate that the opportune time for infection of apples would be early in the summer when most of the stomata are still open but that the tannic and malic acid in the fruit at that time would naturally retard the development of the fungus until after the ripening season.

Brooks[11] found that most of the infections of Baldwins by Cylindrosporium pomi takes place in July and the early part of August before the protecting layers of the lenticels are formed.

The similarity of the general characters and

development of the Wealthy spot and the Baldwin spot suggest that the methods of infection may be the same. In order to gain some information along this line, thirty-one green Wealthys were obtained on August 6th for laboratory inoculations. At this time some of the lenticels were perhaps already formed, so naturally a lighter infection would be expected. One-hundred and twenty-six inoculations were made by smearing drops of agar and mycelium over the surface of these apples. For checks sixty drops of sterile agar were spread over ten other green apples which had been sterilized. On September 1st the drops containing the mycelium had produced an abundance of new mycelium over the old, but where the mycelium had been removed there were no evidences of infection. The surface growth of mycelium was removed on October 1st and nineteen small brown spots were noticed about some of the lenticels or stomata. These spots were similar to the small natural occurring spot on Wealthys and were 1 mm. and less in diameter.

Besides these small spots there were a number of darkened lenticels about these smear inoculations.

Forty-two puncture inoculations of Alternaria were made on July 27th, on green Wealthys that were still hanging on the tree. At the same time thirty-six check punctures were made on fruit of the same tree. During the first week of August there appeared to be no noticeable difference between the inoculated apples and the checks. Two weeks after the apples were inoculated the puncture appeared to be healing over with corky tissue, but no spots had developed. Still later on, August 24th, the punctures and inoculations appeared as healed over insect stings but none had produced the characteristic spot. It was the original intention to place these apples in cellar storage to note if the spot would develop later, but the fruit was picked from the tree before ripe.

Inoculations on Ripe Apples.

During the middle of October, one-hundred and seventy-seven puncture inoculations were made on a

number of ripe, well colored Wealthys. The same number of check punctures were made on the same apples opposite the inoculations. Spots developed from all inoculations and in six weeks were from 1 to 5 mm. in diameter.

Bagging of Green Fruit.

While the fruit was still green, in the first part of August, fifty Wealthy apples were bagged on a tree with paraffined bags which were left on the fruit till after ripening. Fifty more were marked and left unbagged. The object was to determine whether infection took place before or after ripening. The apples before being bagged were sterilized with 95% alcohol so that no spores would be left on the surface after the bags were on. A piece of cotton was wrapped about the spur where the bags were tied on so as to prevent injury and at the same time leave no opening. The wrapped and unwrapped fruit were picked on September 15th, and kept in a common store room. At the time of picking it was noticed that the unbagged fruit was better colored than the

bagged fruit but there were practically no spots on either group. There were very few of these spots on any of the Wealthy apples grown in the station orchard but whether this may have been due to the spraying of the orchard is not known as there are very few trees of this variety in the orchard and most of these had been sprayed. Some of the large commercial growers in Minnesota are uncertain as to the effectiveness of the ordinary spraying methods in controlling this spot.

STORAGE EXPERIMENTS

On September 29th, eight bushels of Wealthy apples were secured from a commercial grower whose orchard had shown very little of the spot. The orchard had been thoroughly sprayed during the spring and early summer and the fruit was in very good condition. The purpose of this storage experiment was to note whether the spot would develop in cold storage on fruit that showed none of it when put into storage; also to determine the rate of spread at low temperatures

beneath these spots proved that Alternaria was present in all of the nine large spots but not in the many blackened lenticels and very small spots.

LEAF INOCULATIONS.

As species of Alternaria are quite common in nature and are found causing leaf spots on a number of host plants , it appeared that it might be possible that the Wealthy spot Alternaria might also be able to infect apple leaves.

Scott and Rorer (7) in 1908 made a study of apple leaf spots, and, although they frequently isolated Alternaria from these areas, they concluded that it was non-parasitic and that it occurred only as a saprophyte.

Roberts (8) in 1914 found that under certain conditions Alternaria mali is able to enlarge dead spots of apple leaves and he classed it as a rather strong facultative parasite.

Early in January 1915, ten Wealthy grafts were started in the greenhouse in order to test the parasitism of the Alternaria found in the fruit spot,

and the effect of changing from cold to warm temperatures. Observations were made on the following week, and later at intervals of a week or ten days until February 1st, which is a month later than most Wealthys are kept. During this period very little of the fruit spot was found at any one time, the most being on November 17th, when eleven apples were observed bearing typical Alternaria spots. (See Plate III)

On November 17th a bushel of apples was taken from storage and placed in cellar storage, in which the temperature varied from 35° to 40° F. and in which the air was fairly moist. Another bushel was placed in a dry store room in which the temperature was between 40° and 45° F. The apples were kept under these conditions until the middle of January when Penicillium and natural deterioration made it impossible to keep the fruit longer.

During this time none of the apples in the store room developed spots while six became spotted in the cellar. A microscopic examination of the tissue

on the leaves.

Fifty-two puncture inoculations were made on apple leaves on March 22nd,1915, and a small amount of the Alternaria mycelium with a drop of sterile distilled water was placed over each. Fifty check punctures were made on other leaves and moistened with distilled water. Fourteen days after the inoculations had been made forty-six of them had developed brown spots ranging from 1 mm. to 5 mm. in diameter. The check punctures at that time had made no change.

On the same day that the above inoculations were made Alternaria mycelium and spores were smeared over the surfaces of thirteen leaves. A small amount of beerwort agar was placed on the leaves with the mycelium to prevent it from dying out before it had a chance to start growth. Checks were made by smearing some sterile beerwort agar over ten other leaves. Although there was a vigorous growth of mycelium on the surface of these leaves, none developed spots until after April 14th, when six of the leaves became covered

with a large number of brown spots from 1 to 5 mm. in diameter. The other inoculated leaves had from one to six spots each. During the first part of April the White Flies became very numerous in the greenhouse, and from appearances those leaves that were attacked the hardest showed the most spots. These flies, besides weakening the leaves by sucking out the juices, left many punctures through which the fungus may have found an easy entrance into the leaf. Five spots developed on the checks. This may be explained by the fact that the spores may have been carried by insects and may have entered through insect wounds. From these results it appears that the Wealthy spot Alternaria is a strong facultative parasite on apple leaves and is able to infect them only through injuries.

In the past winter Alternaria has been isolated from the cores of some western Winesaps. It differed from the Wealthy spot Alternaria in several respects:- (1) It did not grow as rapidly on beerwort agar; (2) the inoculations on both leaves and fruit developed much

slower and did not get as large. In other respects this Alternaria resembles the former and produced spots from inoculations similar to the Wealthy spots. Successful infection in thirteen out of fifteen inoculations of leaves and in sixteen out of sixteen inoculations of fruit were made. Core inoculations were made on March 19th from this core Alternaria and in six days it had spread out 4 mm. from the inoculation in the center of the apple.

PHYSIOLOGICAL SPOTS.

A spot has been noticed on western grown Winesaps that is different from the Wealthy spot, but might easily be mistaken for it by anyone not acquainted with it. These spots are more deeply sunken and are lighter in color and the tissue beneath the surface is not affected with dry rot. Several of the spotted Winesaps were obtained on February 8th from some fancy boxed fruit which would indicate that the spots must have appeared after the fruit was boxed. A microscopic examination of the tissue beneath twenty-five of these

spots showed that there was no fungus present. Pieces of tissue from fifty-six different spots were placed in beerwort agar but none produced an Alternaria.

On Feb.11th some western grown spotted Spitzenbergs were obtained . These spots differed from both of the others in that their margins were uneven. They were shallow, light colored and from 4 to 7 mm. in diameter. No dry rot was noticeable beneath the surface of the spots. An examination under a high power microscope of ten of these spots gave no indication of the presence of a fungus. Several of the apples were sterilized in the usual manner and pieces of tissue from forty -four spots were placed in beerwort agar . Alternaria was not produced from any of these nor was any other organism produced consistently .

The results indicate that there are at least three different kinds of superficial fruit spots and that two of these are non-parasitic in origin.

APPLE SPOTS DUE TO GASES

The affect of sulphur dioxide, ammonia, formaldehyde, ether and chloroform was tried on apples. Wealthy apples were placed in 600 c.c. covered jars and a drop of ammonium hydroxide was added to each of the first two, two drops of 40% formaldehyde to the second two, and sulphur dioxide directed into the third two jars from burning sulphur. Two drops each of chloroform and either were added to the other jars. The immediate affect of the ammonia was to cause black spots about some of the lenticels and placed where the fruit surface had been injured. One thing quite noticeable about these spots was that they appeared only on the colored parts of the fruit and when left in the open air these spots would gradually disappear again. This is explained by the action of the ammonia on the anthocyanin which is red in the presence of acids and dark blue in the presence of alkaline substances After a day or more there were slightly sunken areas where the dark spots had been, but these were light colored and did not resemble the

Alternaria spot in the least. When these were inoculated with the Alternaria they became darker in color and enlarged much more rapidly than after ordinary inoculation. In four days after inoculation, the spots appeared quite typical.

The only effect of the formaldehyde on the apples was to cause a softening of the tissue but no definite spots were produced. Sulphur dioxide bleached the color out of small areas about the lenticels and bruises and caused deep sunken yellow spots in these places. These spots ,however, remained yellow instead of turning brown and bore little resemblance to the Alternaria spot. Ether and chloroform had no effect on the fruit other than to soften it.

For the purpose ofdeterminingwhether these gases make an apple more susceptible to Alternaria by sensitizing the areas about the stomata, the following inoculations were made on March 19th, Twenty-four ammonia spots were inoculated by puncture with Alternaria and twenty-four more were left as checks.

Eighteen Alternaria puncture inoculations were made on apples not treated with ammonia gas. The jars containing the fruit were kept in a cool moist atmosphere and in eight days the Alternaria in the ammonia spots had made a good growth and the spots were darker and from .4 to 1 cm. in diameter. The ammonia spots not inoculated were light in color and from 2 to 3 mm. in diameter. The eighteen puncture inoculations on normal apples developed dark spots 2 to 3 mm. in diameter.

THE FUNGUS

The mycelium is branched and varies from 3 to 7 microns in thickness. When growing on artificial media the mycelium is first hyalin but gradually becomes olivaceous as it grows older. The cells vary in length from 3 to 4 microns and contain a number of oil drops of uniform size. From the microtome sections examined the mycelium seems to grow in the intercellular spaces beneath the stomata and seldom goes deeper than the hypodermal parenchyma. In some of the sections examined the

mycelium seemed to pass into the host cell, tho generally they are observed extending between the cells. Conidia are produced in long chains on nutrient media and are from three to seven celled. Under certain conditions chains of thick-walled chlamydospores are produced in the mycelium. These range from 6 to 7 micron in diameter and germinate quite rapidly under favorable conditions by sending out long germ tubes. The conidia vary in size from 15 micron to 17 micron in width, and 24 to 31 micron in length.

CULTURAL CHARACTERS.

On litmus lactose agar- In neutral agar the growth was moderate and in a week or ten days the agar changed from a faint blue to a deep blue color. Only a few spores were produced.

On Oat agar- The growth was very abundant, forming a black stromatic mass over the surface of the slant with abundant greenish grey flocculent aerial hypha becoming darker green near the surface.

On beerwort agar- The fungus made a good growth and resembled that on oat agar.

On potato cylinders in water-The fungus developed quite rapidly producing a black stromatic mass in the potato and hyalin hypha on the surface.

On prune agar- The mycelium growth was fairly good. At first it was hyalin. later becoming olive green with a black stromatic layer under the surfaces. It resembled the growth on oat agar.

On beef agar-

The growth was diffuse, grey in color and aerial hypha were nearly white,and very short.

On cornmeal- Growth was quite rapid. A hyalin mycelium was first produced which later became olive grey in color with a black stroma beneath.

Few spores were produced. The growth resembled that on oat agar.

In water- Small pieces of infected tissue when dropped into sterile distilled water produced a good growth of hyalin mycelium. A few spores were scattered through the mycelial mass.

In beef broth- The development on the surface was very slow and the hypha was olive green in color.

On apple plugs in water- Growth was very rapid. The hyphae were large and of a dark olive color, but few spores were produced.

On sterile apple juice- Growth was slow at first, but later the surface became covered with an olive green mass of mycelium. Few spores were produced.

On apple cellulose agar- The mycelial development was very slight. Only a thin layer of hyalin mycelium was spread over the surface. Conidia were produced abundantly in long chains and later many of the mycelial cells rounded off into chlamydospores.

The above cultural characters agree with those given by Roberts[8] for Alternaria mali except that the fruit spot Alternaria produces a light gray or white diffuse growth on beef agar while Alternaria mali produces on the same medium a diffuse growth that is grayish to nearly black on the surface. Furthermore the spores of Alternaria mali are from 30 to 35 microns long and from 12 to 13 microns in width, while those of the Wealthy spot Alternaria are from 23 to 27 microns long and from 9 to 11 microns wide. A. mali causes a rapid rotting of

ripe fruit while the fruit spot *Alternaria* causes a slow rot.

The apple cellulose agar was prepared by crushing the juice out of apple pulp and then washing the pulp in running water. Then a dilute solution of potassium hydroxide was added to the pulp which was heated for thirty minutes in the Arnold sterilizer, and washed again in running water. The remaining pulp was then used to supply the cellulose in the regular method of preparing cellulose agar.

CONCLUSIONS.

The data reported seem to justify the following conclusions.

1-The Wealthy spot is due to a species of Alternaria.

2-Infection can not take place through the injured epidermis of a ripe apple but green apples may become infected before the stomata become corky and form lenticels.

3-The Wealthy spot Alternaria is capable of infecting apple leaves through injuries only and therefore might be classed as a strong facultative parasite on apple foliage.

4-Non-parasitic spots can be distinguished from the Alternaria spots by color, shape, depth and appearance of the tissue beneath the spot.

5-Certain gases ,namely,ammonia and sulphur dioxide, will cause physiological spots on apples at the lenticels and injuries.

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EXPLANATION OF PLATES.

Plate I- Ben Davis apples inoculated with Alternaria.

Plate II- Green Wealthy apples:

Check punctures on the left and inoculated punctures on the right.

Plate III-Naturally occurring Alternaria spots on a Wealthy apple.

Plate IV- Cross sections of inoculated apples showing the dry rot beneath the spots.

Plate V- Wealthy spot Alternaria growing on beerwort agar.

Plate VI- Spitzenberg apple showing a commonly occurring spot not due to parasitic causes.

Plate I

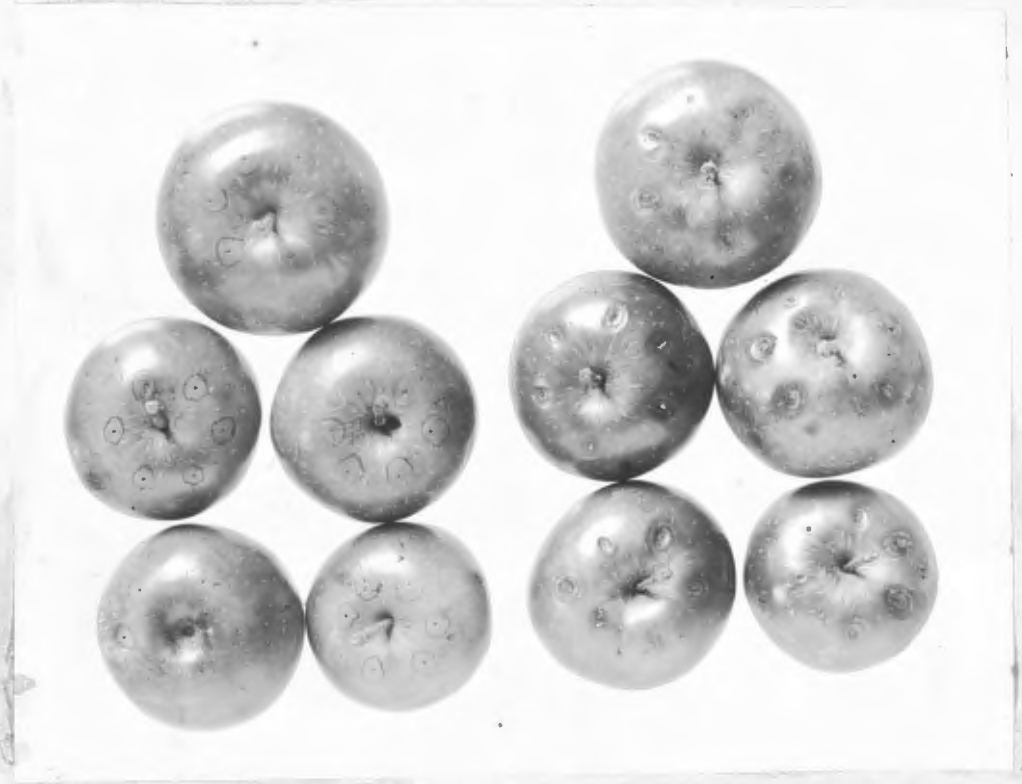

Plate II

Plate III

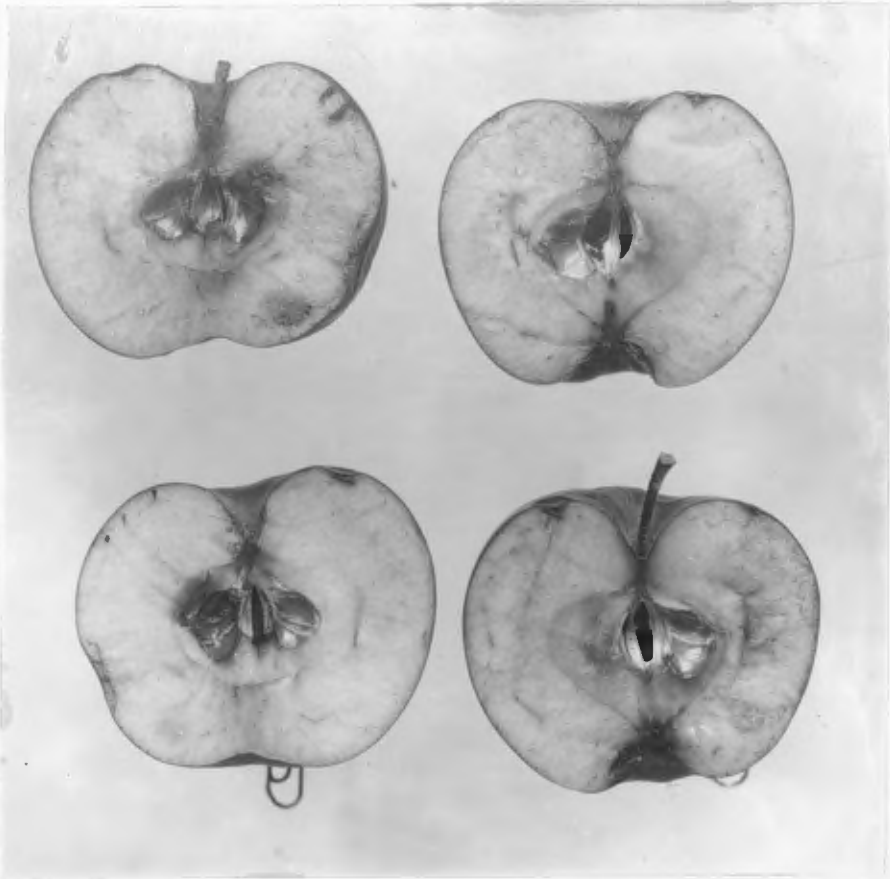

Plate IV

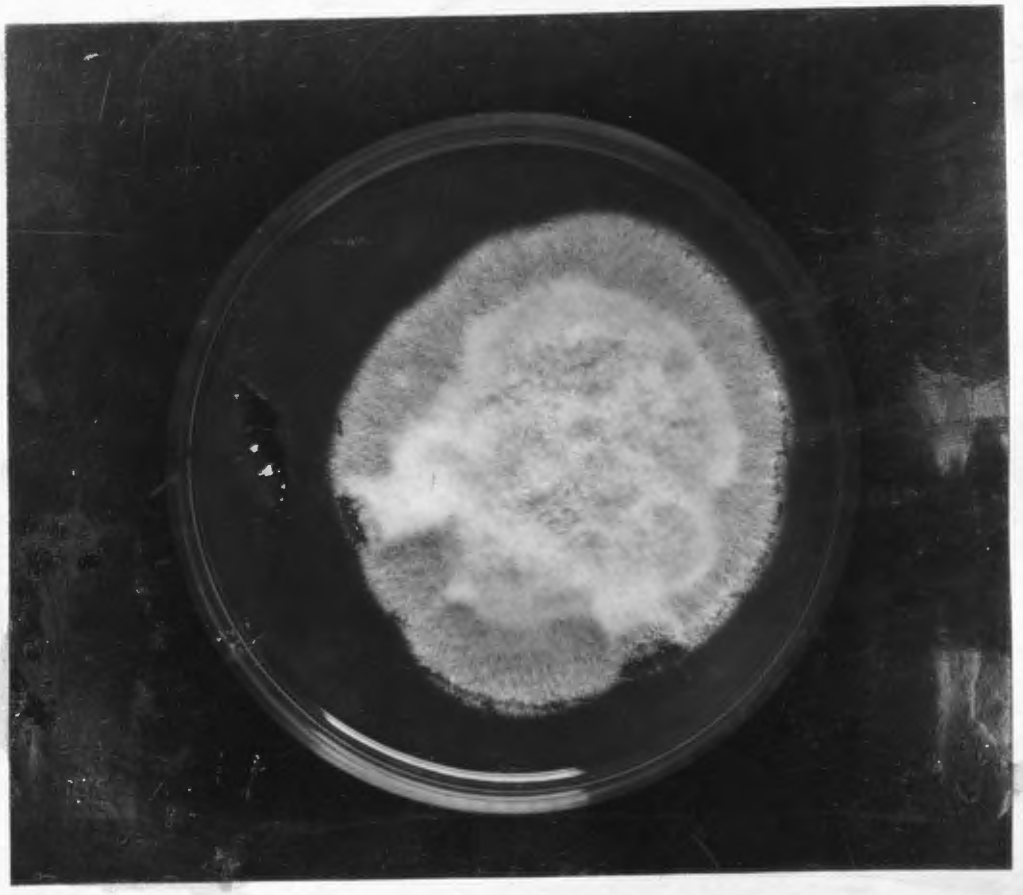

Plate V

Plate VI