THE DEVELOPMENT OF STORAGE SCAB OF APPLE

by

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INTRODUCTION

The disease of apples known as "scab" is of major importance in all countries where apples are grown. In England, New Zealand, South Africa, and Australia this disease is called "black spot", while in Germany it is referred to as "scurf" and sometimes as "rust". Apple scab was first reported by Fries (44)* in 1819 from Sweden. Wallroth (110) in 1833 observed the disease on apples in Germany, and de Schweinitz (99) in 1834 reported it as occurring on Newton Pippin apples from New York, and Pennsylvania orchards in the United States. In Maryland apple scab was first reported by Brunk (18) in 1891.

Apple scab is caused by the fungus Venturia inaequalis (Cke.) Wint. In 1819 Fries (44) called the conidial stage of the scab fungus Spilocaea pomi. Again in 1829 Fries (45) writes "Uber nicht immer entwickeln sich Rostflecken, sie sind sogar manchmal warum sie früher nicht beobachet worden sind; aber solche sterile Krusten sind den Mycologen langst bekannt unter dem Namen Spilocaea pomi (Fr.)". Wallroth (110) in 1833 called the same stage by the name of Cladosporium dendriticum. Fuckel (46) in 1869 transferred the conidial stage to the genus Fusicladium and named it Fusicladium dendriticum. The perfect, or perithecial stage was first described by Cooke (23) in 1866; and he named it Sphaerella inaequalis. Winter (119) in 1880 transferred this fungus to the genus Venturia, and called it Venturia inaequalis. Aderhold (3) in 1896 connected the conidial

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* Numbers in parentheses refer to Literature Cited.

stage of the fungus with the perithecial stage and called it Venturia inaequalis.

Apple scab studies have consumed the energies of many ardent research workers in plant pathology, but investigations on the nature of the development of the scab lesions on fruits while in storage have been few and inconclusive. An attempt has been made in this investigation to produce storage scab artificially on harvested fruits by inoculation; to isolate and grow the fungus from different types of storage scab lesions; to study the nature of the host-parasite relationship on stored fruits of several varieties of apple; and to compare in different respects the storage scab with the pre-storage scab lesions.

REVIEW OF LITERATURE ON APPLE STORAGE SCAB

There are numerous references in the world's literature that relate to storage scab. Most of these, however, merely mention the existence of the disease, and but few have made definite scientific contributions to the subject.

Apple scab is a distinct economic problem to the fruit grower; the development of new scab lesions on stored apples adds greatly to the losses of commercial apple growers. Losses are reflected in a slack pack because of excessive evaporation from the affected lesions (10) (88); from unsightly appearance of the fruit, which throws the fruit out of grade, and makes for an unsaleable product; and by providing an avenue of entrance for other saprophytic and parasitic storage organisms, such as Cephalothecium roseum (Cda.), Corticium centrifugum (Lev.) Bres., and Penicillium expansum (Lk.) emend. Thom. (10). Such storage rots cause more rapid deterioration of the stored fruit (94).

Symptoms and History of the Storage Scab Disease

The scab fungus, Venturia inaequalis (Cke.) Wint. produces definite symptoms on the leaves, twigs, calyx, petiole, and the fruit of the apple. This thesis is concerned with the fungus and the symptoms produced by it on the fruit. Most cultivated varieties of apple fruits are susceptible, although degrees of resistance are observed in many varieties. Braun (11) observed in the humid areas of Germany that all varieties become infected with scab, and the fruit may be infested at any stage of development prior to maturity. Young fruit is more readily attacked than older fruit (3) (67) (118). The appearance of scab lesions on the mature fruit which develop while in storage are distinctly different from those that appear on the fruit during the growing period in the spring and early summer. These early season scab lesions have somewhat the following appearance: The young spots are more or less circular in outline, dark olive in color, with a characteristic velvety appearance and the fruit cuticle is often torn away except for a narrow fringe at the edge (17). An older lesion is mostly circular in outline, dark gray to brown in color, with a silvery overlapping border consisting of the raised and frayed margins of the ruptured cuticle.

Storage scab lesions differ so much from those appearing on the fruit at harvest time that they may be mistaken for some form of "functional" spotting as expressed by Staehelin (103), or some other fungal spot like Brooks' spot (12). The symptoms vary with the many varieties of apples on which it is found, and with the climatic conditions so closely associated with scab development in storage. Storage scab lesions appear under the fruit cuticle as small dark brown to black, glistening, smooth spots. Wallace (109) described these lesions as being black and more dense than

the pre-storage spots. Brooks, Cooley, and Fisher (16) described the lesions as being "smooth, black, and sunken, sometimes attaining a diameter of one-quarter of an inch before the fungus breaks thru to the surface, or causing any roughness of the skin". Wormald (120) referred to them as "pin-head, jet-black infections", and described storage scab as having three forms (121), as follows: (1) saucershape, shiny, jet-black depressions with margins well defined; (2) superficial dark brown spots showing lobed growth with irregular margins; and (3) very small dot-like pin-head spots just visible to the naked eye.

Rose et al. (87) concluded that the cuticle is not often broken where there is storage scab development, and that the surface of the lesion is shiny, dark-brown to jet-black, slightly roughened on the surface, and may vary from one-sixteenth to one-quarter of an inch in diameter. Laubert (69) described these lesions as little black or brown specks with or without dark irregular borders, having a diameter of from .5 to 5 millimeters, and an upper surface which is smooth to slightly wrinkled. Wortman (122) related that there are numerous brown spots produced on the surface of the fruit which are separate and distinct and are about 1 to 5 millimeters in diameter.

Cobb (21) in 1892 believed that storage scab was parasitic in origin. He described it as consisting of small brown spots surrounded by green tissue. Faes and Staehelin (33) described storage scab as follows: "On reconnait la travelure tardive a ses premiers stades d'evalution, aux taches noires lisses, a peine saillantes qui sont disseminier a la surface du fruit". Frickhinger (43) found these lesions to be small, brown or black in color, and very apt to mislead scientists not acquainted with this distinct symptom. Osterwalder (85) described a heretofore unknown disease

on unripe fruits, which may be the storage form of scab. He described the lesions as being smooth and glistening, with fimbriate edges and measuring from .5 to 1.5 millimeters in width. The fungus is a weak parasite gradually penetrating the epidermis without rupturing it. This description of the symptoms is much like that of a typical storage scab described by Laubert (70). Wiesmann (113) referred to storage scab as being dark, minute spots with incipient new infection in the epidermis. Fischer (36) noted also that on ripe fruits there is produced on the skin numerous small brown to black dots, which may increase in size in storage.

Many of the recent investigators point to the work of Goethe (48), who in 1889 observed that new lesions appeared on sound fruit while in storage. He stated that, "Das Fusicladium ist im Vergangenen Herbst noch spätin heftiger Weise aufgetreten und hat auf vielen Früchten allerdings nur kleine Flecke hervorgerufen die sich aber im Obsthause unzweifelhaft vergrosserten und neue Infektionen bewirkten anders in wenigstens die entscheidene Zunahmen der Flecken nicht zu erklaren. Es lässt sich genau feststellen, dass mehrere Sorten, die ohne alle Flecken eingebracht werden, jetzt zahlreiche Pilzeflecken aufweisen". Further searching of the literature will reveal, however, that there are possibly earlier references to storage scab. Fries (45) may be referring to storage scab when he stated in 1829, "das nur kugelige, einfache, locken geballte, unter der Oberhaut des Aepfel entstanden und durch aufflosung derselben endlich frei werdende Sporen vorhanden sind". Sorauer (102) in 1879 described a disease of apples which he called the "Stippich werden der Aepfel". He described "stippen" as follows: Brown or blackish-brown spots developed on the surface of the fruit extending into the flesh only .5 to 1.5 millimeters. They may remain isolated without developing for a long time, and later

develop rapidly in storage. He believes it is due to the fungus named by Fries (44), Spilocaea pomi (Fr.). Frank (40) in 1880 thought that Spilocaea pomi (Fr.) was a sterile form of Fusicladium dendriticum (Wallr.) Fckl., thus making the "Stippen der Aepfel" identical with scab. He mentioned storage scab in the following statement: "So lange die Aepfel frisch bleiben, erhalten sich nicht nur die Pilzeflecken, sondern sie leben und vergrossen sich wahren des ganzen Winters". In 1892 Wortman (122) recognized also that Sorauer's "Stippen" disease found on apple fruits may be due to the fungus Spilocaea pomi (Fr.), although he was of the opinion that it is physiological, since the spots are covered by a smooth, unbroken epidermis.

In Australia, McAlpine (75) in 1904 found storage scab appearing in storage on previously clean fruit. Since then the disease has been reported from Norway by Lind (71); from Germany by Braun (11), Laubert (69), and Rothe (88, 89); from Austria by Fischer (36); from Switzerland by Wiesmann (113); and from Russia by Sawsdarg and Yatzinina (95). Numerous reports have come from abroad since 1930 that mention apple storage scab.

In North America Brooks (12, 14), Morse (79), and Morse and Lewis (80) have been constantly referred to as having been the first to report storage scab in the United States during the years 1909-1910. A careful review of the literature will reveal, however, that Detmers (27) in 1891 definitely referred to storage scab when she said, "The spots on stored fruit retain their vitality throughout the winter and produce spores under favorable conditions. It is a well known fact that healthy apples stored with scabby ones may also become scabby". The same year Churchill (19) also referred to this disease. He stated, "The season of greatest activity is during the cool moist weather of spring and fall . . . . the danger is

not past when the fruit is harvested, but that the disease may be transmitted from infected to sound fruit by contact in storage". McCarthy (77) in 1893 related that, "The disease may be communicated from one apple to another in the bin or barrel, therefore in storing the fruit none but unscabbed apples should be packed together". Henderson (55) writing in 1889 stated that, "Germination of the spore . . . (with temperature) so low that apples stored in bins in the East have been infected from fungus bearing apples in the middle of the winter". Finally Jones and Edson (60) reported in 1900-1901 that storage scab developed in barrels immediately after storage in Vermont, except on windfalls picked up on September 12. Since 1900 many contributions have been made on this problem by Brooks (12, 13), Clinton (20), Folsom (37), Folsom and Ayers (38), Gäussow (50), Morse (79), Morse and Lewis (80), Wallace (109), and Bratley (8, 9, 10), the last mentioned being the more valuable contribution of recent investigations.

Factors Associated with the Appearance of Scab in Storage

Infection in Storage by Conidia.

There is much confusion in the literature regarding whether or not storage scab lesions are produced from conidia of the older scab lesions which were present before the storage of the fruit. The older workers were unanimously of the opinion that scab fruit stored along with scab free fruit would ultimately result in infection of the healthy fruit. Waters (111) not only believed this but he also noticed that the disease was able to continue development in storage in spite of the cold temperature. McCarthy (77) also believing this recommended that when the fruit was stored none but unscabbed apples should be packed together.

Morse (79) reporting on his experiments, says he actually observed

that healthy McIntosh fruit placed in a box with scabby fruit became infected from the conidia present on the scabbed fruit only below the diseased fruit, while the remainder of the fruit in the box remained free from storage scab. Morse and Lewis (80) confirm this, although they observed that well-sprayed fruit was more apt to remain free from scab under the same conditions, while Churchill (19) and Detmers (27) firmly believe that healthy fruit stored with scabby fruit will become infected in storage. Cooke (24) recommends that it is best to separate the scabby from the healthy fruit before storage.

Some of the more careful work of recent investigators, however, has shown that it is impossible to spread scab in storage from diseased to healthy fruit. (8, 9, 10), (39), (58), (109), (124).

Artificial Inoculation of Stored Fruit.

Hesler and Whetzel (57) mention that, "If fruits are inoculated just prior to picking the spots appear in storage". This statement is confirmed by Bratley (10), who was able to obtain storage scab infection on fruits by artificial inoculation, provided the fruits were not stored immediately after inoculation and were kept wet for 40 or more hours. Johnstone (58), Folsom (37), and Staehelin (104) failed to obtain scab development on stored fruits by artificial inoculations.

Increase in Size and Number of Scab Lesions in Storage.

Apple fruits that are stored in an apparently healthy condition will appear with numerous small storage scab lesions several months later when taken out of the storage. An increase in the number of new lesions during storage was first reported by Goethe (48). Fischer (36) noticed that the small brown black spots increased in size in storage. Wiesmann (114) also reported that spots not visible to the eye, but observed under the

microscope increased in diameter. However, Bratley (10) concluded that only a small percentage of old scab lesions enlarge on the fruits during storage, the increase being only about 1 to 2 millimeters in diameter. The greatest enlargement of old lesions takes place on fruits which are packed and stored while wet. New lesions are more apt to develop in storage on scabby fruits than on clean fruits and may appear in the storage cellar at any time. Lesions may enlarge more rapidly on overripe fruit (87).

Wiesmann (113) used weak disinfectants, formaldehyde and sulfuric acid, on the scab infected fruits and found that the conidia subsequently germinated. Staehelin (104) and Faes and Staehelin (33) used 70% alcohol, weak disinfecting solutions of formaldehyde and sulfuric acid, but were unable to check the development of scab in storage. They conclude that the fruit had become infested prior to harvest and placement in storage. This opinion is shared by the majority of recent investigators. Eustace (30) has noted that the old scab lesions will enlarge even under a coating of Bordeaux mixture.

Control of Storage Scab by Spraying.

Late applications of sprays are of material benefit in reducing late infection and development of scab lesions in storage. Faes (32), Frickhinger (43), Loewel (72), Sawsdarg and Yatzinina (95), and Wiesmann (113, 114) have all shown that storage scab could be reduced, if not controlled, by late applications of fungicides. Coulson and Godbout (26) noted in Canada that the omission of the final spray resulted in severe storage scab infection especially when there was a late rainy period. Folsom (39) concluded after a five year study on McIntosh apples that he was able to control storage scab with sulfur sprays applied late in the season. Groves

(49) believes that the fruit becomes infected during some rainy period shortly before harvest, and the fungus has not had time to produce a visible lesion on the fruit before it is harvested and packed. He believes this condition could be remedied by proper control of the primary infection in the early part of the growing season. Güssow (50) was able to reduce scab by three late applications of sulfur dust.

Effect of Humidity and Temperature on Storage Scab.

Wallace (109) comes to the conclusion that storage scab may be initiated by weather favorable for the infection of the fruit on the tree just prior to harvest, or by the lodging of the spores on the fruit at harvest and packing time. Morse (79) noted in 1909 that "the entire growing and harvesting season was very wet, and the vegetative development of the fungus continued up to and during the harvest time. The moist apples covered with spores were then placed in rather warm cellars, resulting in the infection of the fruit and the formation of the small scab spots in storage". McAlpine (75, 76) and Wettwer (112) have observed that wet weather and wet storage conditions are responsible for storage scab development on the stored fruit. Braun (11) has found that in humid climates all varieties of apples are susceptible and it is almost impossible to avoid some infection in storage.

Humidity and temperature relations play an important role in the development of scab in storage. Bratley (8, 10) has shown that when the storage temperature is constant, higher humidities promote greater enlargement of the scab lesions. Although small differences in temperature and humidity had little effect on the number of new scab lesions appearing in storage, yet with high temperature and high humidity, the lesions often appeared earlier in the storage season. Brooks (14) points out in

this regard that "Apples but slightly affected with the disease (scab) when allowed to stand in the barrels for considerable time before being placed in cold storage, have been found later to have developed the disease to such an extent that they were scarcely marketable. It is important that apples should be stored as soon after gathering as possible even if entirely free from the disease". He further states, (15) "The scab fungus, like most other plants, is greatly checked in its growth by low temperatures. Its greatest development on stored fruit, therefore, can be expected in barrels from cellar storage, or (on fruits) which are delayed in reaching the cold storage plant". Fischer (36) takes a step further and suggests that apples should be stored in a cool, well ventilated, airy room in order to prevent storage scab.

Germination of Scab Conidia and Infections.

It is questionable whether the scab conidia are able to germinate and penetrate the apple cuticle at the cold storage temperature (33° F.). Adams (1) has found that Venturia inaequalis (Cke.) Wint. grows and produces conidia on the surface of the fruit at 32° F. Groves (49) states that, "Cold storage temperatures are not low enough to prevent the growth of the scab fungus and where such a condition exists, the infection continues to develop slowly and finally becomes conspicuous scab spots". Aderhold (2) believes that the conidia are not viable after three months. In germinating conidia he found the minimum temperature to be 11° C., the spores germinating in 1½ to 18 hours (3). Ewart (31) found that when Fusicladium dendriticum (Wallr.) Fckl. was exposed to a temperature below freezing, the conidia did not germinate as readily as those untreated. Henderson (55) obtained germination of the conidia at temperatures common to apple storage bins (probably 32° F.). Faes and Staehelin (33) found

that conidia of apple scab germinated at 3° C. but it required 18 hours, while the optimum temperature of 17-20° C. required but 4 to 6 hours to bring about germination. Rothe (88) had no trouble in germinating conidia in water drops. Low temperatures, he found, hinder germination but little. Wiesmann (114) found conidia on the surface of stored fruits, but these viable spores would not germinate, although his previous work had shown that even though fruits were disinfected for one-half hour with a weak disinfectant it did not prevent the germination of conidia at 1° C. (113).

Following germination of the conidia there is considerable lapse of time before definite symptoms appear on the fruit. The low temperature of the storage reduces the growth rate of the fungus. Faes (32), Faes and Staehelin (33) believe this period to be as much as 4 to 6 weeks. When very wet weather is encountered prior to harvest, at which time the conidia are readily germinating, Güssow (50) observed that infections, in the form of small scab spots, were easily detectable after 18 days. When inoculations were made the latter part of August, Bratley (8, 10) observed the development of scab in storage six weeks later. These apples remained on the tree three weeks after inoculation. However, when similar inoculations were made on the same variety of apples just removed from the tree, only a slight to no infection was observed until after six months in storage. It appears that the age of the fruit is an important factor in the incubation period of the fungus following either artificial or natural inoculation.

METHODS AND MATERIALS

Isolates of Venturia inaequalis (Cke.) Wint. were obtained from

Arkansas and Wisconsin, and certain preliminary studies were conducted on their growth habits. Since these strains were not common to Maryland, for comparative purposes, certain isolates of storage and pre-storage scab lesions were obtained from several varieties of Maryland-grown apples. Wisconsin strains 17, 22, and D-7 of Palmiter (86) were used because of their ability to produce conidia abundantly, while the strain from Arkansas grew vegetatively on malt agar.

The Maryland isolates from pre-storage scab lesions were obtained by the dilution method. The conidia were scraped from leaves of the McIntosh and Delicious varieties, and from fruits of the McIntosh, Delicious, Stayman, and Williams Early Red varieties. Isolation was made on standard malt extract agar, consisting of 15 grams of agar and 30 grams of Difco malt extract to each liter of distilled water. It was sterilized at 15 pounds pressure for 20 minutes. Ten cubic centimeters of sterile distilled water were used in each tube for making the dilutions. A small drop of water was placed on the scab lesion and a 2 mm. loop needle was used to gently scrape off the conidia into the water drop. A loopful of this spore suspension was transferred to the first tube or water blank. This was well shaken, and a 4 mm. loopful of this spore suspension was transferred to the second tube. This procedure was repeated from tube to tube through five dilutions. A 2 mm. loop was inserted into the last dilution tube and a drop was transferred to an agar slant by the streak method. Agar plates were also streaked in this manner, but because of added chance of contamination from the outside the tube method was found to be more satisfactory. The tubes and plates were watched carefully twice each day for microscopical evidence of spore germination and development of mycelium. As soon as a well-isolated developing colony was observed it was removed

with a 4 mm. special cutter needle and transferred to a tube of malt agar, and grown at room temperature.

Isolations were also made from typical storage scab lesions found on Stayman, Williams Early Red, Black Twig, and Delicious varieties of fruit taken from storage. The fruit was washed in soapy water, then placed in a 1 to 1500 solution of mercuric chloride ($HgCl_2$) for 15 minutes, and rinsed twice in sterile distilled water. With a sharp safety razor blade, sterilized in the flame, one millimeter square pieces of the diseased tissue were obtained by making cross-section cuts through the scab lesion. These were transferred directly to malt agar slants with a special spatula-like needle and incubated at room temperature. Three per cent malt agar, pH. 5.4-5.6, was a satisfactory medium on which to maintain these cultures.

Hanging drop preparations were made by adding a drop of sterile water on a cover glass to which was added a small drop of the spore suspension. It was ringed with vaseline and the slide was inverted over the prepared cover glass. They were incubated at several temperatures, and observed at six hour intervals for forty-eight hours, when the data were obtained on spore germination . Conidia germination studies were made from active scab lesions on early spring scab infections of both leaves and fruits, as well as from fall lesions on fruits and leaves that had been held in cold storage (33° F.) for three months. Hanging drops were also made from 15-day old malt agar cultures of the storage scab isolates.

Healthy and scab infested fruit of the Delicious and Stayman varieties were washed for one minute with certain chemicals used in the removal of lead, arsenic, and other spray residue. The fruit was rinsed through two baths of distilled water, and then transferred to a third water bath which was heavily inoculated with a conidia suspension of the scab organism. The

fruit was removed after three minutes, placed in baskets and stored for five months at 33° F. Checks were similarly rinsed, inoculated and stored. Observations on the development of new scab lesions and the increase in size of pre-storage lesions were made at monthly intervals.

Supplementary information relative to the development of apple scab in the orchard was obtained from a critical examination of primary and secondary scab lesions on the leaves and fruits of Williams Early Red and Delicious varieties on approximately 575 trees of bearing age. These trees had been receiving certain spray treatment for scab control. Following the harvest, data were obtained on the yield and the percentage of scab from each tree, and summated for each of the treatments. The distribution of scab lesions on the stem, middle, and calyx thirds of the fruit was recorded from large samples from each tree, and the data summated for the treatment and for the entire crop. Smaller representative samples of both healthy and scab infested fruit were placed in storage at 33° F. to await further scab development. Each fruit was numbered with black waterproof ink, and the size of the pre-storage scab lesions recorded.

After two months, and again at four months, the fruit was removed from storage and examined for increase in the number of new lesions and increase in size of the pre-storage scab lesions. Samples of fruits from other orchards were also placed in cold storage to observe storage scab development. The home cellar storage was also utilized for some of the studies.

At intervals during the storage period certain fruits showing distinct activity of the scab fungus were removed from storage and photographs were made to record its development. The scab lesions were killed

and fixed with form-acetic alcohol, Flemings solution, or the formal-chrom-acetic mixture. Small blocks of the fruit (not over one centimeter in width and three millimeters thick) containing the scab lesions were removed with a sharp razor blade. Following the regular fixing period the material was washed in water, and following Zirkle's (125) method were transferred through the butyl alcohol series for dehydration of the tissues, and embedded in paraffin.

Sections seven microns thick were cut with a rotary microtome, and fixed on clean slides with egg albumin solution. The paraffin was removed from the sections with xylol and the tissue carried through the alcohols from which it was transferred to the desired stain. Flemming's triple stain was used to study the host-parasite relationship. Heidenhain's iron-alum-haematoxylin was used in studying the fungus. Aceto-carmine was of little value as a vital stain with this organism. Scab infested fruit tissue was cleared and stained by a modification of the Peace method.

Microscopic examinations of the stained sections were made with a 4 mm. high dry objective, and a 1.35 mm. flourite oil immersion objective used with a 15X Spencer compensating ocular. Photomicrographs were made with a Zeiss photomicrographic camera. Artificial light passing through certain green and yellow filters improved the definition of the object. Comparative histological studies were made of the storage scab and pre-storage scab lesions.

EXPERIMENTATION AND RESULTS

Culturing the Fungus, and Germination of the Conidia

Many isolates of the apple scab fungus, Venturia inaequalis (Cke.)

Wint. exhibit different growth characteristics even when grown on the same nutrient media and under other identical cultural conditions. An isolate from Arkansas produced no conidia on malt agar at 20°, 14°, and 8° C. temperature. However, it grew well vegetatively through a range of pH. values from 5.0 to 9.1, with the optimum at pH. 5.6 to 5.9. Wisconsin strain 17 produced conidia abundantly at temperatures of 8-14° C. When it was transferred to a fresh malt agar slant and kept at room temperature for two months it ceased spore production and became vegetative. Wisconsin strain 22 likewise became vegetative at the same temperature, while Wisconsin strain D-7 remained sporiferous at room temperature. Similar variation was observed in the isolates obtained from the Maryland spring infected fruit and leaves. Isolates from storage scab lesions sporulated abundantly at room temperature, as seen in Figure 25, and the production of spores was not altered by repeated transfers to malt agar slants. Figure 23 shows colony characteristics of a malt agar culture of storage scab isolates 1N and 1M. The two tubes to the left are open and granular with abundant spore production. The two tubes to the right show the culture more compact, smooth, and mostly vegetative. Figure 24 also shows the surface characteristics of two storage scab isolates. Both of these isolates produce numerous conidia.

Pea agar, which often alters colonies of fungi, was used with the various scab isolates. It was prepared by the following formula:

Water	1000 cc.
Malt extract	6.0 gms.
Maltose	6.0 "
Peptone	0.6 "
$MgSO_4$	0.6 "
KH_2PO_4	1.25 "
Agar	20.0 "

To this medium was added 400 grams of fresh peas which had been cooked

25 minutes, mashed, and squeezed through cheesecloth. This medium was tubed, sterilized, and slanted. When various isolates of the scab fungus were transferred to this medium, some alteration was observed for many of them in their color, rapidity of growth, appearance, and spore production. Wisconsin strain 17 changed from a rich olive brown to a light gray-green color at room temperature and remained vegetative. Other strains and isolates did not change so greatly on this medium.

Germination of Conidia.

Conidia freshly removed from actively sporulating scab lesions on early summer fruits and leaves will germinate readily in six to twelve hours. Conidia that are found abundant on leaves which have been exposed to the winter temperature, and on fruits that have been in cold storage for several months do not germinate so readily, and are much slower in developing than the spores produced in the early growing season. The percentage germination of scab conidia in hanging drop water cultures from different fruits of the same variety of apple after removing from storage will vary greatly as observed in TABLE I.

In this experiment Delicious apples with typical pre-storage scab lesions, which were producing an abundance of conidia, were placed in storage at 33° F. immediately after harvesting. After three months these fruits were removed and the conidia scraped off lightly by placing a drop of sterile distilled water on the scab lesion and gently brushing the spores off into the water with a fine transfer needle. The hanging drops slides were incubated at room temperature for 57 hours, and then at 10° C. for 15 hours, after which they were examined for spore germination. One scab lesion from each of seven different Delicious apples was examined

and the percentage of germination determined. Only the spores in the margins of the water drop were counted, as those toward the center were not germinating.

TABLE I. GERMINATION, AFTER 72 HOURS, OF SCAB CONIDIA FROM THE SURFACE OF PRE-STORAGE SCAB LESIONS ON DELICIOUS FRUITS STORED FOR 3 MONTHS AT 33° F., IN A HANGING DROP WATER CULTURE.

Fruit No.	Number of Conidia Counted	Number of Conidia Germinated	Per Cent Germ.	Origin of Germ Tube on Conidia: Single Cell Conidia: Base	Single Cell Conidia: Apex	2-Cell Conidia: Top	2-Cell Conidia: Base	2-Cell Conidia: Both
1.	165	61	37.0	58	0	0	1	2
2.	160	15	9.4	14	1	0	0	0
3.	155	7	4.5	5	0	0	0	2
4.	165	22	13.3	15	1	4	2	0
5.	150	54	36.0	45	0	1	0	8
6.	40	1	2.5	1	0	0	0	0
7.	45	3	6.6	2	1	0	0	0
Total	880	163	18.5	140	3	5	3	12

From the above table it will be seen that the spores from some scab lesions germinated to a greater degree than similarly treated spores from other lesions. The germ tube originated most frequently from the basal end of the conidia. Where there were two-celled conidia both cells often germinated. The highest germination was only 37 per cent for these conidia held in cold storage.

After the first examination these slides were incubated at room temperature for another 48 hours when they were again examined and a record made of the germinating spores found in the margin of the water drop. All

germinating cells that could be conveniently found in this area of the water drop were counted and the data, relative to the issuance of the germ tube from the base or apical part of the conidia, were recorded. Where two-celled conidia were observed, the germination of the apical and the basal cell was recorded. The percentage distribution for the origin of the germ tube was determined. It will be observed in TABLE II that where germination took place in the single-celled conidia it was usually at the basal end, and that over two-thirds of all the cells examined showed germination from that region. Likewise, where a two-celled conidium was germinating, the basal cell appeared to be germinating more frequently than the apical cell.

TABLE II. GERMINATION, AFTER 120 HOURS, OF SCAB CONIDIA FROM DELICIOUS FRUITS STORED FOR 3 MONTHS AT 33° F., IN A HANGING DROP WATER CULTURE AT ROOM TEMPERATURE.

Fruit No.	No. Germinated Conidia Examined	Single Cell Conidia		Two Celled Conidia	
		Base	Apex	Basal Cell	Apical Cell
1.	100	70	1	24	5
2.	20	18	1	0	1
3.	55	13	2	32	8
4.	65	44	14	2	5
5.	75	57	4	13	1
6.	2	0	0	0	2
7.	22	20	1	0	1
Total	339	222	23	71	23
% Distribution		65.5	6.8	20.9	6.8

Isolates of storage scab infected fruits grown on malt agar produced conidia abundantly at room temperature. These conidia germinated readily when placed in continuously running water, even though still attached to the growing mycelium as seen in Figure 27. Observations were made on conidia germinating from isolate 1F3. This was obtained from a black submerged storage scab lesion on a Stayman apple. A spore suspension was made from the outer edge of a 20-day old malt agar culture, mounted in water on a hanging drop slide. One group of three slides was placed at room temperature and the other duplicate set at 1-2° C. At the end of 12 hours the conidia at room temperature had begun to germinate. After 24 hours these conidia were germinating freely; those at 1-2° C. showed little evidence of germinating during the first 24 hours.

At the end of 48 hours the hanging drop cultures were observed again and the germination studied. A total of 500 spores were counted from the slides at each temperature. Germination of the conidia at room temperature had progressed so far by this time that the germ tube had branched considerably. Those conidia held at the lower temperature were now beginning to germinate. The latter group of slides were returned to the 1 to 2° C. temperature for a total of 96 hours and a further germination record obtained. The results of this experiment are shown in TABLE III.

TABLE III. GERMINATION OF STORAGE SCAB ISOLATE 1F3 FROM A BLACK STORAGE SCAB LESION FROM A STAYMAN APPLE.

Conidia Counted	Temp. of Germination Chamber	% Germination	Ave. Length of Conidia, Microns	Ave. Length Conidia & Germ Tube Microns
		After 48 Hours		
500	20° C.	60	20.5	421.6
500	1-2° C.	15.4	24.7	48.8
		After 96 Hours		
100	1-2° C.	59	23.0	167.5

The maximum length of any one conidium was 26.3 microns. After 48 hours at room temperature, small spores were beginning to form on the ends of the branched germ tubes. Associated with the germinating conidia were small knob-like structures (appressoria). Germination may proceed from any part of the cell.

It is of special interest to note that the conidia from this isolate were able to germinate at approximately 33° F., the temperature common to apple cold storage chambers. In TABLE III it is observed that the spores which were germinating at 1 to 2° C. after 48 hours, had produced germtubes equal in length to the spore. Those germtubes produced at room temperature were about twenty times the length of the average spore from which they came. The same percentage germination was obtained in one-half the time at room temperature.

Inoculation of Harvested and Stored Fruit.

From the previous germination experiment it would appear that it would be possible to re-infect apples in storage by conidia falling from scab lesions onto healthy fruit. Several experiments were conducted in an attempt to inoculate fruits after they had been harvested and stored, with negative results.

A fruit washing experiment was performed on samples of Stayman and Delicious fruits obtained from commercial orchards where good spray practices were employed. Some of the fruits were badly infested with early summer scab, and others apparently free from such infection. They were washed for 1 minute at several temperatures with standard chemicals recommended for use in the removal of spray residue, rinsed in water, and plunged into water heavily seeded with scab conidia. These chemical materials are tabulated in TABLE V. The washed fruit was placed in cold

TABLE IV. NUMBER AND DISTRIBUTION OF SCAB LESIONS ON APPLE FRUITS BEFORE RECEIVING THE WASHING TREATMENT

Variety	Sample	No. Fruits	Number and Size of Scab Lesions				
			Less Than 1 mm.	1-3 mm.	3-5 mm.	More Than 5 mm.	Total No. Scab Lesions
Stayman	A	25	83	247	28	41	399
"	B	25	79	299	19	30	427
"	C	25	28	343	21	30	422
"	D	25	10	332	26	41	409
"	E	25	41	431	20	26	518
"	F	25	21	356	22	26	425
"	G	25	44	234	13	30	321
"	H	25	0	173	11	4	188
Delicious	I	25	0	106	31	72	209
Stayman	J	25	0	0	0	0	0
"	K	25	4	135	5	6	150
Delicious	L	25	2	70	25	76	173
Stayman	M	25	2	52	3	5	62
"	N	25	0	0	2	0	2
"	O	25	0	0	0	0	0
"	P	17	6	135	5	17	163
Delicious	Q	33	2	98	33	107	240
Total		425	322	3011	264	511	4108
Ave. Lesions Per Fruit			.76	7.08	.62	1.20	9.66

TABLE V. NUMBER AND DISTRIBUTION OF SCAB LESIONS ON APPLE FRUITS AFTER RECEIVING THE WASHING TREATMENT, STORED AT 33° F. FOR 4 MONTHS

Variety	Sample	Washing Treatment 1 Minute	No. Fruits	Number & Size of Scab Spots: Less Than 1 mm.	1-3 mm.	3-5 mm.	More Than 5 mm.	Total No.	Gain No.	Gain %
Stayman	A	.5% HCl 24° C.	25	83	259	28	41	411	12	3.0
"	B	1.0% HCl 24° C.	25	79	312	28	30	449	22	5.2
"	C	1.5% HCl 25° C.	25	29	362	28	31	450	28	6.7
"	D	2.0% HCl 26° C.	25	10	349	50	43	452	43	10.5
"	E	.5% HCl 40° C.	25	47	451	31	30	559	41	7.9
"	F	1.0% HCl 40° C.	25	21	385	42	29	477	52	12.2
"	G	1.5% HCl 40° C.	25	47	253	17	32	349	28	8.8
"	H	50 lbs. SiO_2 to 100 gal. water, 40° C.	25	0	175	24	6	205	17	9.0
Delicious	I	Same as H 25° C.	25	4	112	35	81	232	23	11.0
Stayman	J	75 lbs. SiO_2 to 100 gal. water, 40° C.	25	4	7	0	0	11	11	11.0
"	K	Same as J 25° C.	25	5	140	6	6	157	7	4.6
Delicious	L	Vatsol 3.1% 38° C.	25	4	78	30	77	189	16	9.2
Stayman	M	Same as L + 1.5% HCl	25	10	64	6	5	85	23	37.1
"	N	Same as M + 1% NaCl	25	0	0	2	0	2	0	0
"	O	Vatsol 6.2% + 1.5% HCl 38° C.	25	0	0	0	0	0	0	0
"	P	Check	17	6	142	10	18	176	13	8.0
Delicious	Q	Check	33	15	114	47	115	291	51	21.2
Total			425	364	3203	384	544	4495	387	9.4
Ave. Lesions Per Fruit				.85	7.50	.90	1.27	9.50	.91	

storage (33° F.) while wet and observed monthly for development of scab lesions. An increase in size of old scab lesions was evident after two months in storage. New scab lesions were few at this time. At the end of four months the fruit was examined and the data recorded, when many new scab lesions were observed. New lesions developed more abundantly on the fruit which had old scab lesions at the time of storing. Checks that were rinsed in distilled water containing scab spores developed as many as, if not more, new lesions than any of the fruits treated. Old scab lesions continued to increase in size in storage regardless of the treatment received. The number of scab lesions appearing on the fruit before washing and storage is presented in TABLE IV. TABLE V summarizes the findings regarding increased number and size of scab lesions on the washed fruit at the end of four months' storage.

Pre-Storage and Storage Scab Development

The development of storage scab is related to the amount of primary and secondary scab infection appearing in the orchard prior to the harvest of the fruit. In order to determine the effect of scab in the orchard on the development of scab in storage, the College orchard of Williams Early Red and Delicious apple trees was selected on the Beltsville Farm with approximately 575 trees of bearing age, an equal number of each variety. This orchard had been receiving certain spray treatments for the prevention of scab. It was of interest to observe the course of infection under each of the various treatments. The general plan of the orchard is found in TABLE VI.

Many thousands of leaves were examined on May 10 and again on June 7 to determine relative distribution of scab lesions on the upper and lower

TABLE VI. PLAN OF ORCHARD FROM WHICH DATA WAS TAKEN ON BELTSVILLE FARM - 1938

Washington - Baltimore Highway

Row Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
24	0	0	0	0	2	1	0	0	2	2	0	0	0	0	0	0	0																
23	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X							
22	1	0	0	0	0		0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	
21	2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	1	X	1	1	1	X	X	X		X	X	X	X	X	X	X	X	X
20	0	0	0	0	0	0	0	0	0		0	0	0	0	2	3	2		0	0	0	0	2	0	0	0	0	1	0	0	0	0	0
19	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X		X	3	X	X	X	X	X	1	2
18	1	0	0	0	1	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	3	0	2	1	0		0	0	2
17	X	X	X	X	X	2		1	2	1	X	X	X		X	1	X	X	X	X	X		3	X	X	X	X	X	2	X	X	X	X
16	0	0	0	0	1		0		2	1	2	1	0	0	0		0		0	0	2	2	0		0	0	2	0	1	0	0	0	0
15	3	2	X	X	2		X	X	X	X	X	X	X		3		X	X			X	X	X	X	X	X	X	X	X	X	X	X	X
14	0	0	0	0	2		0	3	0	0	0	0	0	0	2		0		3	0	0	0	2		0	0	0		0	0	0	0	0
13	X	2	X	1	3	1	X	X	X	X	3	X	X	X	3		X	1	2		X	X	X	X	3	X	X	X	X	X	X	X	X
12	0	0	0	0	3		0	0	0	0	0	0	0	0	0		0	1	3	0	0	0	0	1	3	1	0	1	0	0	3	3	0
11	X	X	X	X	X	X	X	X	3	X	X	X	X	X	X	X	X		3	X	X	1	X	X	X	X	X	1	X	X	X	X	X
10	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0			0	0	0	0	0	0	0	3	1	0	0	0	0	0
9	1	X	X	X	1	X	X	X	X	X	X	X	X	X		X	X	X	3	X	X	X	X	X	X	X	X	X	X	X	X	X	X
8		0	0	0	1	0	0	0	0	0	3		0	0	3	0	0	0	0	2	3		2	2	0	0	3	0	0	0	0	0	2
7	1	X	X	3	X		X	X	X	X	X	X	3	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X
6	3	0	0				0	1	0	3	3	0	3		3	0	0	0	0	0	0	0		0	3	1	0	0	0	0	0	0	0
5	2	X	X	X			X	X	X	2	2	X	X	X	X	X	X	3	X	X	X		X	X	X	X	X	X	X	X	3	X	X
4	2	0	0	0		0	2			0	3		0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	1	0		0
3	X	X	X	X	X	X	3	X	2	X	X	X	X		X	3	X	3	X	X	X	X	3	X	X	X	X	X	X	X	X	X	X
2	0	0	0	2	1	0	2			3	0		2		2		3	2	0	0	0	0		0	0	0	0	0	0	0	0	0	2
1	3	X	2		3	X	2		X	X	1	X	X	X	X	2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	1
Tree Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33

Road Through Farm

W

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X -- Delicious trees
0 -- Williams Early Red trees
1,2,3 -- Young trees not in data
Blank spaces -- Trees missing

surfaces of the apple leaves. At the time of the first observation it was found that the greater number of scab lesions appeared on the lower surface of both varieties. Observations one month later on the Delicious variety showed there was a tendency toward equalization of the number of scab lesions on the two leaf surfaces. This later condition is a result of secondary infection from summer conidia, caused by droplets of water falling from scab infected leaves above onto the upper surface of the leaves below, causing new infections to take place.

The fruits were examined at the same time for the presence and distribution of scab. Scab was becoming generally distributed by June 10. The greater number of scab lesions appearing on these young fruits on the tree were found at, or toward the calyx end. This may be a result of secondary infection from adjoining scab infested leaves.

Large samples of the fruit were collected at harvest time and the distribution of scab lesions on the stem, middle, and calyx thirds of the fruits was noted before storing. Excessively high wind storms which blew off much of the fruit just previous to the picking dates of each variety made it necessary to examine and make record on both the windfall and the picked fruit. The Delicious variety had 80 per cent of early summer scab lesions on the calyx third of the fruit, and the Williams Early Red variety, 73 per cent. The smaller percentage of this pre-storage scab was found on the stem third, as will be observed in TABLE VII.

Following this examination the fruit was placed in storage at 33° F. and re-examined at monthly intervals until the end of three months when the final data, as to the increase in number and in size of scab lesions, were collected. Each scab lesion was noted, and its size in millimeters was recorded before and after storage.

TABLE VII. DISTRIBUTION OF PRE-STORAGE SCAB LESIONS ON APPLE FRUIT AT HARVEST.

Fruit Source	Fruits Examined: No.	Fruits Examined: No. Scabby	Fruits Examined: % Scab	Scab Lesions: No.	Scab Lesions: Ave. Per Scab Apple	Distribution on Thirds of Fruit: Stem No.	Stem %	Middle No.	Middle %	Calyx No.	Calyx %
DELICIOUS VARIETY											
Fallen	10207	1411	13.8	4682	3.3	308	6.6	659	14.1	3715	79.3
Picked	1135	145	12.8	346	2.4	25	7.2	38	11.0	283	81.8
Total	11342	1556	13.7	5028	3.2	333	6.6	697	13.8	3998	79.5
WILLIAMS EARLY RED VARIETY											
Fallen	11210	623	5.6	2701	4.3	131	4.9	619	22.9	1951	72.2
Picked	14647	842	5.8	3566	4.2	265	7.4	683	19.2	2620	73.5
Total	25857	1465	5.6	6267	4.3	396	6.3	1302	20.8	4571	72.9

In TABLE VIII it will be observed that with Delicious apples held in cold storage for three months there was about2.5% increase in the number of new lesions and about 12% increase in size for the pre-storage scab lesions.

TABLE VIII. INCREASE IN DIAMETER AND NUMBER OF SCAB LESIONS ON DELICIOUS FRUIT AFTER THREE MONTHS' STORAGE AT 33° F.

Date of Observation	Fruits Examined: No.	Fruits Scabby	Scab Lesions	Distribution of Scab Lesions on Thirds of Apple: Stem No.	Stem Diam. mm.	Middle No.	Middle Diam. mm.	Calyx No.	Calyx Diam. mm.	Total Diam. mm.
Oct. 22	2796	481	1443	98	540	180	976	1155	3929	5445
Jan. 22	2796	487	1479	108	618	188	1118	1183	4369	6105
Increase		6	36	10	78	8	142	28	440	660

A lot of 160 apparently healthy fruits of the Williams Early Red variety was placed in storage. After the end of four months they were examined and only one storage scab lesion appeared on the entire lot of fruit. Fruits that are well sprayed and clean when stored will remain free from scab.

A basket of Williams Early Red fruit containing all scabby apples was examined before storing at 33° F. and the number and size of the scab lesions recorded. A similar lot of fruit of the same variety was stored, which contained mostly scab free fruit with a few scab infested fruits placed among the healthy ones. After three months' storage these were examined and it was found that new scab lesions appeared in both lots, yet the greater number of new scab lesions was found on the fruit in the basket containing all scabby fruits at the beginning of the experiment.

Greater increase in size of the pre-storage scab lesions was also noted from this same basket, as will be seen in TABLE IX.

TABLE IX. INCREASE IN NUMBER AND IN SIZE OF SCAB LESIONS OF WILLIAMS EARLY RED APPLES STORED AT 33° C. FOR TWO MONTHS.

Treatment	Examination			Increase in Size		Increase in Numbers	
	Date	No. Fruit	No. Scab Lesions	Total Diameters mm.	%	Total No.	%
Healthy & Diseased Fruit in Same Baskets	Aug. 1	662	2905	6651		2905	
	Sept. 30	662	3225	8537	28.3	3225	11.1
All Scab Fruit in Same Basket	July 16	92	352	883		352	
	Sept. 19	92	582	1470	66.5	582	65.4

It is observed from the above results that the more scabby the fruit, the greater the increase in size and number of lesions.

Samples of fruits were obtained from trees in other orchards, which were sprayed regularly during the season. These were placed in cold storage at 33° F. except for one lot which was placed in a home storage cellar at approximately 50-55° F. At the end of two months the apples stored in the home storage cellar began to show numerous black and brownish smooth lesions under the cuticle. The cuticle was not ruptured above these lesions. Data were taken on the distribution of these storage scab lesions on the surface of the fruit. Some of these lesions were embedded in paraffin for future histological observations. Some of the fruit was placed in the open on a laboratory table at room temperature for two months, and the cuticle over the storage scab lesions failed to become ruptured (Fig. 20). Isolations of the scab organism were made from some of these lesions.

Another lot of fruit was so badly infested with storage scab at the end of four months (Fig. 22) that it was removed from storage. The number and distribution of the new lesions were recorded. After one week's exposure to room temperature the fruits were badly rotted and were discarded. Isolations from these fruits produced the scab organism, but with a mixture of many contaminating rot producing fungi.

In TABLE X it will be seen that two lots of Stayman apples, wrapped in wax paper, obtained from the same grower were packed in two different containers before storing. The fruit packed in slatted wooden boxes when examined after 7 months in storage had about two-thirds less new storage scab lesions than similar fruits packed in pasteboard boxes.

In all of the above fruits examined it was found that the new storage scab lesions developed most abundantly on the stem third of the fruit, while the smallest number was found on the calyx end. These findings are directly opposite to the distribution of the pre-storage scab lesions on the fruit prior to storage.

During these observations on storage scab development three distinct types of storage scab lesions were observed. The increase in size of the early summer scab lesion already existing prior to storage shows a distinct marginal development of this lesion which varies with the variety (Fig. 1). This margin may be of two general types: coal black in color and regular (Fig. 5), or it may be brownish to black in color and spreading irregularly (Fig. 3, 4). On the Stayman and Delicious varieties the cuticle was not ruptured above the spreading lesion, and no conidia were observed.

The second type of storage scab lesions appears in the form of small brown or black shiny lesions under the cuticle (Fig. 11, 13). These grow

TABLE X. DISTRIBUTION OF STORAGE SCAB LESIONS DEVELOPED IN STORAGE ON FRUIT APPARENTLY FREE FROM SCAB WHEN STORED.

Variety	Storage			No. Fruits Examined	Scab Lesions on Parts of Fruit						Ave. No. per Fruit
	Container	Temp.	Period		Stem		Middle		Calyx		
					No.	%	No.	%	No.	%	
Stayman	Pasteboard Box	33° F.	7 Mo.	94	1138	51.6	835	37.9	232	10.5	23.5
Stayman	Slatted Wood Box	33° F.	7 Mo.	100	440	49.6	363	40.9	85	9.5	8.9
Winesap	Bushel Basket	33° F.	4 Mo.	50	3237	45.4	2900	40.6	992	13.9	142.6
Stayman	Bushel Basket	Home Storage Cellar	2 Mo.	17	555	48.5	369	32.2	220	19.3	67.3
Average				65.2	1342	47.1	1117	39.2	387	13.7	43.6

out under the cuticle, but do not rupture it, nor produce conidia.

The third type of storage scab lesion is much larger. It has a dull gray-black color. The mycelial mat pushes up the cuticle at irregular intervals, thus making the surface of the lesion slightly rough to the touch. Conidia are produced from this type of storage scab lesion (Fig. 14, 15).

Histological Studies

Pre-storage Scab Lesions.

Examination of a typical pre-storage scab lesion will reveal a rough, callus-like brown center with thin cracks extending across its surface in many directions (Fig. 1, 2). Beyond this appears a gray zone with tufts of conidia protruding through the erupted, frayed margins of the cuticle (Fig. 3). Outside of this appears a black zone representing the activity of the fungus during the storage period (Plate I; Plate II, Fig. 3, 5). In this black, storage zone are many small ridges, which give the spot a rough, pimply appearance. This black border consists of several light and dark bands, which may indicate that the organism grows under the cuticle in an interrupted fashion (Fig. 3).

Histological examination of stained sections through a typical pre-storage scab lesion shows distinct differences in the three zones outlined above. In the central brown callus zone there may be either a thin or thick layer of cork cells, which later may slough off (Fig. 8). There may only be remnants of cushions of the scab mycelium with here and there a cluster of conidia-producing hyphae (Fig. 9). In younger lesions this zone may be entirely filled across with conidiophores bearing conidia situated on a mat or cushion of well defined parenchymatous

scab mycelial cells (Fig. 7). In the mature scab lesions the cuticle is missing in this zone, although other cuticle-like material may be deposited on the outer layer of the apple fruit. Below the thin mat of fungus mycelium there are found three to four rows of very heavily stained cork-like cells which have lost their form and structure (Fig. 6), and are devoid of their contents. They appear to be badly crushed and disintegrated.

Immediately below this cork area there appears 5 to 8 rows of slightly crushed, although fairly well formed, small, very thin-walled cells filled with protoplasmic materials (Fig. 6). Their form is more like that of the meristematic cells which lie just below in a plate of 2 to 3 cells. The cell contents stain readily with orange G. These cells appear to be phellogenic and are forming new cells by division in two or more planes, which are being pushed to the outside of the phellogen layer. To the inside of the phellogen cells appear the normal thick-walled cells that make up the outer portion of the cortical tissue of the apple fruit. These merge gradually into the large cells that are common to the cortex.

Sometimes there is observed directly below the phellogen a row of large thick-walled cells, resembling the endoderm dipping down under the scab lesion from its outer margin.

The influence of the fungus may be observed some distance from the location of the fungus itself in stimulating the fruit to produce cork-like tissue (Fig. 8).

In the gray zone to one side of this cork area the cuticle becomes evident (Fig. 7). It is broken loose from the epidermis of the fruit by the pressure of the thick mat of fungus mycelium below. Tufts of conidia may be seen here and there breaking through the nearby cuticle. The

epidermal cells are distorted and badly crushed. The suberized cells are still evident, but they decrease in number, and are losing their ability to take up the red stain, and adsorb more of the green dye. The phellogenic cells still appear directly below, and now there are only 1 to 3 rows of these cells. Toward the inside of the phellogen are the normal, thicker-walled, outer cortical cells which merge into the normal, large-celled cortical tissue of the fruit.

The black zone is further out toward the margin of the lesion, and is that black tissue developed during storage. Masses of fungus tissue may fill up the entire portion of the cuticle layer. The epidermal cells are distinct and but slightly crushed, and may be completely surrounded by the fungus tissue (Fig. 32). There is only slight evidence of suberization of the cells immediately below. These are the outer cells of the cortex, and merge below into the normal large cortical cells. There is no active phellogenic layer in this region. The cells making up the mycelium of the fungus are well defined. Some of the outermost cells take the red (safranin) dye slightly and may be chitinized. The other fungal cells appear to have cellulose cell walls. In the zones of the scab lesion, thus far described, no fungus mycelium has been observed in the fruit tissue beyond the cuticle and epidermal cell layer.

At the very outer margin of the black storage scab zone of the pre-storage scab lesion cells are found which take only the cellulose (fast green) stain. The cuticle is distinct and the epidermal layer of cells is unaltered. The normal cortical tissue appears below, stained with the cellulose differentiating dye. The scab fungus is indistinct, and appears only slightly in the cuticle. Most of the fungus is growing in the cortical region at a distance of one to two cells below the epidermis (Fig. 34).

It appears to be growing between the walls of two adjacent cortical cells. The cells of the fungus are indistinct, and have very thin walls.

Storage Scab Lesions.

There is a second type of storage scab development which is so characteristic macroscopically. This lesion appears dark brown to shiny black in color. Its surface is fairly smooth and the cuticle is not broken (Fig. 19). There are no tufts of conidia associated with this form of storage scab. The lesion may be very small and difficult to identify as a scab lesion (Fig. 16).

The histological picture of the relationship of the fungus to the host is quite different from that described above. A 7 micron section through a typical lesion of this type reveals the absence of suberized cork cells, and no indication of active phellogenic tissue. The cortical tissue appears like normal fruits. The cuticle is intact and somewhat swollen when the fungus is abundant (Plate 17).

The fungus tissue is found abundant in the cuticle, ramifying throughout this layer, but does not break through it. The mycelium grows below and around the epidermal cells (Fig. 38). Some of the epidermal cells become crushed and lose their shape, but they remain evident even in heavily infested portions. Some of the fungal mycelium grows horizontal to the outer surface of the cuticle for some distance without breaking through it (Fig. 42). No conidia were found in this type of storage scab lesion.

The mycelium mat of cells is distinct and some of the older distinct ones take up the red dye indicating the presence of some chitin (Fig. 43). For the most part the cell walls give a cellulose staining reaction. The outer margin of the lesion is made up of mycelium with the cells less

differentiated and with thinner walls.

The mycelium grows to a large extent in the cortical region of the host. The first two layers of cells below the epidermis are regularly inhabited by the strands of hyphae (Fig. 38). The fungus is able to penetrate deeply into the cortex under this lesion. Some sections show that the fungus has penetrated regularly to a depth of eight to ten cells into the cortex, and it has been seen to penetrate even deeper than this.

The host cells in the immediate vicinity of the scab mycelium appear to be normal in outline, except for the slight change of shape due to the pressure of the fungus. If this type of storage scab is present at a location on the fruit which has been bruised in handling, the host cells will be found crushed, while the fungus may or may not penetrate into the crushed cortical tissue. The mycelium usually grows from cell to cell through the middle lamella, or the region where two cell walls are in contact with each other. The scab fungus was observed to be growing in the cortex even when there was no mycelial mat in the cuticle and epidermal tissue directly above it. In a young storage scab lesion, quite often the mycelium is found in the first few layers of the cortical cells rather than in the cuticle.

The presence of any large amount of the mycelium in the cuticle and epidermis usually results in a distinct crushing and necrosis of the tissue below the mass of fungus hypha (Fig. 40, 41). Much of this distortion of the cells may be due to the pressure exerted by the fungus mycelium or the giving off of some toxic material during their metabolic processes. This necrotic condition was shown to exist when a Delicious fruit was inoculated with a culture of the scab organism growing on malt agar. A small piece of the agar was transferred to the fruit on which the culture

was growing. The fungus grew well for one month on this medium and produced a distinct lesion on the fruit underneath the inoculum. This lesion with the inoculum attached was prepared for sectioning by the usual histological technic, stained, and examined under the microscope. It was found that the fungus did not grow through the agar into the fruit, but obtained its nutrition from the agar surface. The portion of the fruit immediately under the inoculum was, however, necrotized and considerably sunken. There was no evidence that the fungus penetrated into the affected fruit.

The last type of storage scab lesions studied from a histological point of view was that which possessed a rough peaked surface, with occasionally a small tuft of conidia protruding through the cuticle at the many peaks on the lesion. These lesions were black to dark brown in appearance, and their presence resulted in a more rapid deterioration of the fruit when removed from storage. These lesions are generally larger than the smooth type of storage scab. This type of scab lesion produces some suberization of the host cells, and the fungus breaks through the cuticle instead of entering the cortical tissue like the previously described type of storage spot. Here, for the most part, the fungus is confined to the cuticle and epidermal cells. Penetration of the fungus to a depth of two layers of cortical cells is observed, especially at the outer edge of this type of lesion. The cortical cells below the lesion are crushed and distorted.

It has been observed above that the fungus grows intercellularly into the cortex. In several instances it is revealed that the mycelium may also be intracellular, and the host cells are packed with the mycelium (Fig. 40). These cases are rare when compared with the intercellular nature

of the fungus.

It is difficult to determine how the fungus penetrates the cell wall of the cortical cells and enters into the area of the middle lamella. In an attempt to locate some avenue of entrance in the cell wall through which the fungus could migrate to the middle lamella region, some sections seven microns thick were prepared from thin pieces of the epidermis and some of the outer portion of the cortex of a storage scab infested Stayman fruit. The material was prepared before sectioning in the following manner: The storage scab infested material was placed in a saturated aqueous solution of chloral hydrate for 3 days, washed thoroughly in water, and then placed for 3 days in 5 per cent potassium hydroxide solution. It was now bleached and cleared of its contents. After washing thoroughly in water it was transferred to 50 per cent alcohol and dehydrated through the alcohol series to paraffin, from which the sections were cut. Various stains as gentian violet, methylene blue, and fast green were used to stain the cell walls. The cell walls were greatly swollen by this chloral hydrate and KOH treatment, and in them certain distinct pits were observed. Some walls had numerous small pits, while other walls had but few although somewhat larger ones. The cells nearer the epidermis contained the greater number, while in the cells further removed from the epidermis they were fewer in number, and by this method of treatment were lacking entirely in some.

In some storage scab lesions the fungus was seen to be crossing from one cell to another through the middle lamella at the ends of adjacent cells. The fungus appeared under the oil immersion objective to be constricted to a fine point as it grew between the cell walls, and thus acted as a wedge in prying the walls apart (Plate XIV). The fungus may enter distinct pores in the cell wall in a similar manner. The fungus was also observed to be

entering what appeared to be intercellular spaces of the cortical cells, from where it was able to grow down between the cells (Plate XV; Plate XVI, Fig. 37).

DISCUSSION OF RESULTS

Some of the cultural characteristics observed with the different cultures, and isolates from storage scab lesions may be explained on the basis of different strains. Some workers have shown that nearly every isolate of this fungus is a different strain. Schmidt (97) obtained 448 distinct strains from 473 isolates from adjacent apple trees. Wiesmann (115) obtained from five nearby apple trees isolates that were different. In Wisconsin, Palmiter (86) found that in 36 isolates no two of them were alike in morphological characters.

The ability of the storage scab isolates to continue the production of conidia on malt agar at room temperature is a feature which has not generally been reported for cultures of the scab fungus. Isolations made by various workers lost this ability to produce conidia when subcultured at room temperature on malt agar. Aderhold (3) in his classical work on apple scab found that isolates in early spring and summer from green leaves and fruits sporulated richly, and made little mycelial growth. With the second subculture, and those following, the cultures lost their sporiferous nature and became vegetative. Wilson (116) reported that at 24° C., with cultures on malt agar, conidia transfers gave increased production of mycelium, while the conidia were produced abundantly at 10 to 16° C.

The size of the conidia varies for the different storage scab isolates, but the average for several isolates was about 22-24 X 6-7 microns.

Aderhold (3) shows the conidia to average 16-25 X 4-6 microns; Schwarze (98) shows the average to be 30 microns, and Wiesmann (115) reports a range of 16-27 microns. These may be culture or strain differences.

The germination of conidia from artificial cultures, from fresh leaves and fruits, and from scab infested fruits and leaves held at low temperatures is known to vary considerably. The older spores of many fungi do not germinate so readily as the younger ones, whether they be in culture, or from mature fruits; and Johnstone (58) finds that many will not even germinate at all. This may be due in part to the age of the spore, or to the thickening of the outer protective membrane around the conidium. The change in temperature in the cold storage room also reduces the rapidity and amount of germination, which may result from a physical and chemical alteration of the cell contents.

Temperature plays a very important role in spore germination. The storage scab isolates were able to germinate at 1° C. in 48 hours; the same culture of spores at room temperature germinated in only 12 hours. Some workers believe that scab conidia will not germinate at storage temperature, e.g. Wiesmann (113). On the other hand, Rothe (88) found conidia to germinate at low temperatures, and Ewart (31) found that conidia exposed to temperatures of 5 to 16° F. would germinate when brought into room temperature, although not so readily as those not exposed to this temperature. Faes and Staehelin (33) found the range of spore germination to be from 3 to 30° C. with the optimum at 17° C. Harvey (53) found them to germinate in 8 hours at 50° F. Aderhold (3) found the minimum temperature for spore germination to be 11° C.

The germination of conidia has been shown to proceed at the low temperatures that are common to cold storage rooms although the fungus

apparently failed to infect the fruit under storage conditions. Aderhold (2) found that when conidia were taken from green leaves and inoculated to green fruit on the tree he obtained only 2 per cent infection, but when conidia were taken from fruit and inoculated to fruit the infection was even less. Johnstone (58) was unable to produce infection on fruits in storage by inoculation. The same results were obtained by Staehelin (103) regardless of whether or not the fruit was wrapped in paper after inoculation. Wiltshire (117) finds, too, that it is hard to infect mature fruits even while on the tree. Bratley (8), however, was able to inoculate mature fruits on the tree and obtain a high degree of infection in storage provided he kept the inoculated surface of the fruit moist for 40 hours.

The inability of the fungus to cause infection on harvested fruit is not well understood. The fungus may be unable to penetrate the thickened cuticle on the older fruits, or it may be an inability of the fungus to penetrate under the adverse storage conditions. The appressorium, which was observed on the germ tubes from conidia germinating at 1° C. from the storage scab cultures may be so altered by the cold temperature, or the presence of a toxic principle in the older cuticle, that it is unable to function properly in assisting germ tube penetration. Wiltshire (118), however, believes that the cuticle plays no part in resistance of the fruit to infection by the scab fungus, since he saw hypha embedded in the cuticle at the edge of a mat of fungus tissue.

The washing experiment was designed to answer two questions: First, whether the chemicals used in commercial washing of fruit would have a disinfecting action, and secondly, whether the chemicals may alter the cuticle so that spore germination and germ-tube penetration may be aided. The results, however, showed that neither was accomplished, since pre-storage

scab lesions increased in size in storage after the washing treatment, and healthy fruit remained free from scab under the same treatment. Checks that were not washed, but otherwise were given similar treatment, would show about as much scab infection as the treated fruit. The effect of commercial washing on the spread and growth of scab in storage has not been reported before. Faes and Staehelin (33), Staehelin (103), and Wiesmann (113) used various weak disinfectants on fruits before placing in storage, however, and found it not effective in preventing the appearance of new scab lesions, or the increase in size of the pre-storage scab lesions.

The prevalence of scab in an orchard during early summer conditions the amount of storage scab development. Where scab is abundant on leaves and fruits in the orchard, there is a greatly increased chance for secondary scab infection with favorable weather conditions in the late summer and early fall. The primary infection in the spring may take place anywhere on the fruit, while on the leaves it is greatest on the under surface. As the season progresses, and secondary scab infections spread, the leaves become infected on the upper surface to a greater degree. The fruits show infections more on the calyx end than on any other portion of the fruit. This was found to be the case by actual counts made on about 40,000 of the freshly harvested fruits examined for distribution of the pre-storage, or early summer, scab lesions on the fruit surface.

The increase in number of new lesions in storage is greatest toward the stem third of the fruit. Since the experiments reported in this paper indicate that scab does not spread from one fruit to another in storage, and inoculation experiments show that stored fruit is not infected

by this means, there is but one idea left which may help to explain the differences in the appearance of the pre-storage and the storage scab lesions on the fruit surface. The storage scab lesions appearing in storage are the direct result of late season infections while the fruit is still on the tree. These are from secondary late summer conidia that have been washed off the leaves from above the fruit. These conidia fall with the water drops onto the fruits below, striking the stem third of the fruit first, where they adhere, germinate, and penetrate the cuticle. This fruit is carried into the storage bin with the invisible infection present. The fruit in its early development on the tree has its calyx end upward or outward. As the fruit grows older, it becomes heavier, thus bending the stem so that the calyx end of the fruit points downward thus leaving the stem end of the fruit exposed to the direct drippings of the scab infected leawes from above. There is also the fact to consider that in orchards which are heavily infected with scab, the fruit when picked at harvest and stored, will tend to develop more new storage scab lesions than fruit from orchards that have been well sprayed and cared for.

With the cold temperature of the storage chamber the fungus develops very slowly and it may require some three to six months for the fungus to grow large enough under the cuticle to become visible to the eye. Very heavy spore inoculum must be present to produce the 200 or more storage scab lesions which were found on one fruit. Staehelin (103) reports finding as many as 400 new scab lesions, while Wormald (120) found over 500 storage lesions on a single fruit.

There are many interesting features of the host-parasite relationship with both the pre-storage scab and storage scab types of lesions. There

has been but little investigation on the latter, pre-storage scab lesions having received the greater consideration. It is well known so far that the center of a pre-storage scab lesion is very apt to be free from the scab fungus, the bordering parts of the lesion containing the living fungus. This condition is a result of the corking nature of the host in response to invasion by the fungus parasite. A cross-section through a callus of this sort will reveal the presence of definite cork-like cells in the affected host tissue. Sorauer (101) reports the formation of cork under the scab spot in 1875. Aderhold (3), however, failed to mention it in his early work, although in the same year Corbett (25) reports that the flesh and skin of old scab lesions become corky. Clinton (20) observed it and believed it served the purpose of affording protection to the cells below against evaporation, and the entrance of other organisms. Cooke (24) believed this cork formation aided in throwing off the disease. Marsh and Walker (78) observed similar formation of cork tissue in the apple twig affected with scab, although in this case the cork extends well down into the phellogen and cortical cells, often causing their death and destruction.

Storage scab lesions are apparently free from this cork-like tissue reported for the pre-storage scab lesion. Wormald (120), and Fischer (36) observed no cork in storage scab lesions, although Faes and Staehelin (33) often referred to cells that had been altered by the presence of the fungus as being suberized and necrotized. The observations of the writer reveal no cork formation from phellogenic tissue where there are distinct storage scab lesions. Crushed lesions, which have lost their contents, and became dried out thereby taking on a cork-like appearance, may be what Faes and Staehelin (33) were referring to as suberized tissue in storage scab lesions.

Many writers on scab refer to the fungus as growing between the cuticle and the epidermis of the fruit. Even as late as 1931 Johnstone (59) states, that in susceptible varieties of apples the fungus is able to establish itself beneath the cuticle. Wallace (109) noticed that the germ tube bores through the cuticle and continues to grow between the cuticle and epidermis. This scab tissue may become very thick and actually stretch the cuticle, but where the cuticle is thick and old it is more difficult for the fungus to penetrate, or to break through the surface. This may be the condition responsible for the storage scab lesion's failure to break through the cuticle, and causing the plates of mycelium to grow in the cuticle more or less parallel to its outer surface.

The findings of this investigation show that the mat of parenchymaticus cells of the scab fungus not only grows between the cuticle and the epidermis, but also in plate-like fashion in the cuticle as well. It is not confined to these regions alone, but may penetrate into the cortical tissue below the epidermis to a distance of 8 to 10 cells. In entering this region certain injury will result to the cells under the influence of the invading fungus. The upper cortical cells become somewhat crushed where there is a considerable amount of scab tissue above. Some of the host cells are no doubt killed and necrotized. This may be due to the toxic materials liberated by the fungus itself, or by the affected host cells as expressed by Faes and Staehelin (33).

Arthur (7) observed that the cells in the "peel" and epidermal cells of the McIntosh apples are dead in early November when in storage. Could this account for the fungus penetrating the cortical region, and not pushing through the cuticle in the storage scab lesions? Cortical invasion was observed in some and not in other storage scab lesions when the tissue

below was badly bruised and sunken. The death of the cells in this tissue must not be a factor in the intercellular cortical growth of scab hypha. Tetley (106) shows that the cuticle of certain varieties of apple fruit tends to increase in thickness while in storage.

Only three other investigators definitely refer to scab as penetrating into the tissue below the epidermis. Voges (107) refers to the scab organism as being endophytic and growing in the cork cells. Rudloff and Schmidt (91) state that the mycelium in the cortex has been observed. Their observation is not thorough and convincing. Faes and Staehelin (33) report the appearance of scab in the cortex, but their source of material and discussion is not clear regarding the type of scab lesion to which they refer.

It has been clearly shown in this investigation that the fungus in the storage scab lesions is endophytic. Why it grows in this tissue instead of the region between the cuticle and epidermis is hard to determine. Hamilton (52) noted that when ascospores penetrate the leaf, the mycelium grows between the cell walls. The fungus must be able to sustain itself in the region of the middle lamella, as there were no haustoria observed on the mycelial strands either in the host or in culture. The chemical change in the middle lamella during the storage period of the fruit may account for the intercellular growth of the fungus in the cortical region. Appleman and Conrad (6) have shown that tomatoes have an increased amount of pectin over protopectin in the ripened fruit, and the following year, they report a similar condition in the ripening Crawford peach. Tetley (105) finds with the Bramley's Seedling apple that the change of protopectin to pectin is a slow, gradual process. Aderhold (3) finds that scab conidia germinate better in calcium pectate than in pectic acid. It is reasonable to believe

that the middle lamella of the cells of the ripe apples, held in storage for several months, could be altered in its chemical nature to the extent that the scab hypha may grow more easily between the cortical cells. Since protopectin, the cementing material between the cells, is more abundant in the green than in the ripe fruit, it is expected that the early season (pre-storage) scab lesions would show no penetration into the cortex, but would be more active in its vegetative and spore production in the cuticle and epidermal layers. This is the appearance of the scab spots that most of the investigators have noticed. Any subsequent growth of the scab lesion in the ripened fruit would take place in the region through which it could grow the easier, and with the cuticle increasing in thickness on stored fruit, the scab fungus becomes confined to the region below the cuticle.

Storage scab mycelium is found to be intracellular as well as intercellular. In this growing condition it soon occupies the cellular cavity and tends to cause the affected cell to swell. The pits found in the upper cortical cells as seen in Figure 44, and reported by Tetley (106), may serve as an avenue of entrance for the fungus into the cells, and between the adjoining cells from where it grows into the middle lamella.

SUMMARY

The investigations reported in this thesis are concerned with storage scab lesions on apple fruits, and with pre-storage scab lesions to the extent that they influence the amount, and alter the appearance of the scab spot on the fruit when placed in the environment of the cold storage chamber.

Pre-storage scab lesions continue to increase in size at the cold storage temperature (33° F.), although the rate of growth is greatly reduced, and the nature of the growth is much altered. The smaller pre-storage scab lesions increase in size more than the larger ones. The activity of the fungus is confined to the margin of these spots in the form of black, zonated growth under the cuticle.

Typical storage scab lesions appear as small brown to black smooth, shiny lesions growing under the cuticle. With some conditions of the fruit these lesions may be slightly rough on account of the abundant growth of fungal tissue under the cuticle. These two types of lesions do not break through the cuticle, nor do they produce conidia. Another type of storage lesion may have a dusty gray black surface, which is rough in appearance. Numerous small islands of conidia may be seen on this surface. For the most part the development and growth of storage scab lesions are confined to the region under the cuticle.

Many isolates of the scab fungus from storage scab lesions of several apple varieties were made on malt agar. The cultures grew readily, and most isolates produced an abundance of conidia at room temperature. No isolate was entirely vegetative from such lesions. The production of conidia continued from the storage scab isolates on malt agar at room

temperature, while other isolates obtained from various investigators lost this character under the same treatment and became vegetative.

Several cultural and morphologically different types of the scab organism were isolated from the same, and from different apples.

The rate and percentage of germination of conidia was greatly reduced when the fruits and leaves containing the abundantly sporulating scab lesions were placed in the environment of the cold storage room for three months. Different lesions on several fruits of the same variety of apple varied greatly in their percentage of germination.

Twenty day old conidia from isolates of storage scab lesions on fruits that were in cold storage from 2 to 4 months germinated in 12 hours at room temperature. By the end of 48 hours many branched germ tubes were observed. Similar conidia placed at temperatures approximating those of cold storage (1° C.) germinated more slowly, and the germ tube did not show branching in 96 hours. The percentage of germination was about the same for the two temperatures at the end of four days. Appressoria, or functionally equivalent structures, were observed at the base of the germ tube to a greater extent at the colder temperature.

Inoculation and washing experiments on harvested fruits that were treated and stored at once, gave negative results. The unwashed and the washed fruit, when inoculated with conidia suspension, produced new scab lesions to about the same degree. Fruits that were apparently scab free when washed and inoculated remained free from scab after four months in storage. Any increase in the number of new scab lesions was not attributed to the results of inoculation.

Pre-storage scab lesions continued to increase in size in storage after being washed with weak acid, and soap.

Early summer scab lesions on Williams Early Red and Delicious were found most abundantly distributed on the calyx portion of the harvested fruit. These lesions continued to increase in size in storage.

Storage scab lesions were more abundant on the stem third of the stored fruit. Fruit apparently free from scab selected from orchards heavily infested with scab, when placed in storage without being artificially inoculated, developed numerous storage scab lesions. The infection must have resulted from late season inoculum in the orchard. The position of the apple on the tree at this time is such that any water dripping over the scab infested leaves above the apple, will accumulate conidia and carry them to the upper or stem third of the fruit, where they germinate and cause infection.

When heavily scab infested fruit is stored together there is a greater increase in number of new lesions and a greater increase in size of pre-storage lesions, than when lightly scabbed fruit is stored together.

Scabby fruit placed in the same basket with apparently scab free fruit collected from well sprayed orchards, resulted in no new scab lesions being produced on the healthy fruit which contacted the scabby fruit.

Fruit apparently scab free from well sprayed orchards remained practically free from scab in storage.

Thin cross-sections through a typical pre-storage scab lesion with its characteristic storage scab developed margin, as a result of being held in cold storage for three or four months, will show three or more distinct zones of fungal activity. The center of the lesion shows callus

formation with cork meristem below, and with remnants of the scab fungus scattered over its surface. The gray zone around the callus center zone contains the conidia borne on thick mats of parenchymaticus scab tissue, which ruptures the cuticle, and gives this zone its gray colored frayed appearance. Very slight to abundant cork formation may be found in the host tissue in this zone. In the outer black zone, the region which grew in storage, the fungus is confined under the cuticle as a thick mat of parenchymaticus fungus tissue. It may be found growing intercellularly in the cortex to a depth of from 2 to 3 cells. The cortical cells below may appear distorted.

Thin sections through storage scab lesions show the presence of a thick mat of fungus tissue under the cuticle. The host cells including the epidermis and the upper cortical cells directly below are crushed and become necrotic. There is no evidence of cork meristem tissue in these lesions.

In storage scab the fungus not only grows between the epidermis and the cuticle, but grows also in the cuticle layer in a plate-like fashion parallel to the fruit surface.

The mycelium in the storage scab lesion readily penetrates into the cortical tissue of the host to a depth of 8 to 10 cells. Where the thin strand of scab mycelium penetrates the host there is little distortion of the host cell. Crushed cortical cells that are found in bruised fruit do not show the presence of the intercellular scab mycelium to any greater degree than do sound cortical cells.

After entering the fruit cortical tissue the fungus grows between the cells in the region of the middle lamella in a wedge-like manner and separates the adjacent cell walls. In ripe and stored fruit with the protopectin changing to pectin the cementing property of adjacent cell walls

is minimized. The fungus may move from cell to cell through the intercellular spaces in the cortex.

In some storage scab lesions the fungus has been found growing intracellularly, and the cell becomes packed with the fungus hypha.

In cleared tissue preparations thin sections reveal the presence of pits in the upper cortical cells in the Stayman apple. These may serve as an avenue for the scab fungus to enter the region of the middle lamella, or through which the fungus may establish itself intracellularly in the host cells.

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Explanation of Plates

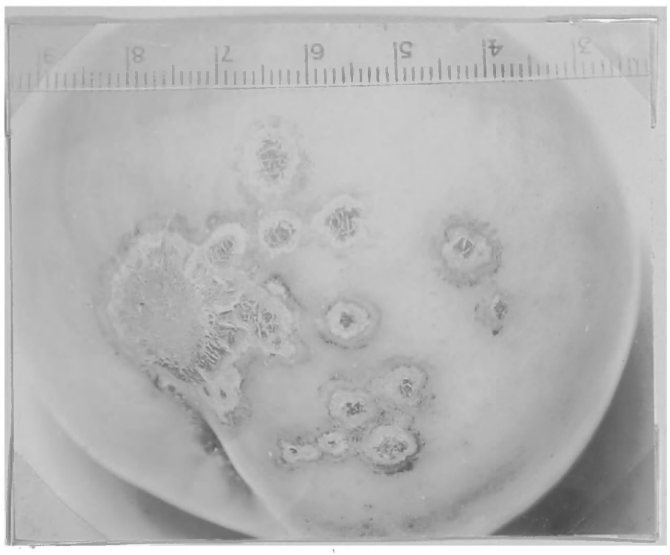
Figures 1, 2, 3, 4, 5, 11, 12, 13, 14, 15, 18, 20, 21, 22, 23, and 24 were made with a Bausch and Lomb Protar lens attached to a Zeiss photomicrographic camera. Figures 16, 17, and 19 were made with the aid of Gordon's Photomicro-Camera extension tubes attached to a Zeiss photomicrographic camera. Figure 5 was made with a Bausch and Lomb Auto lens in a Tele-photo Poca A camera. Figures 8 and 25 were made with the aid of a Spencer 16mm. achromatic objective and 15X compensating ocular. Figures 6, 7, 9, 26, 27, 28, 29, 30, 32, 33, 34, 38, 39, 40, 41, 42, 43, and 44 were made with a Spencer 4 mm. achromatic objective and 15X compensating ocular, and figures 31, 35, 36, and 37 with a No. 55 Queen 1.35mm. fluorite oil immersion objective and 15X compensating ocular. All photomicrographs were made with a Zeiss photomicrographic camera, using a Zeiss lamp as the light source. Wratten K3 #9, K2 #8, yellow and green filters were used while making photomicrographs. Wratten and Wainwright Process Panchromatic and "M" Plates were used for the photographs. Magnification was determined by the aid of an accurately calibrated Spencer Filar micrometer, and for very low magnifications a Fisher Bacteriological celluloid scale was used.

Plate I

Figure 1. Development of storage scab around a pre-storage scab lesion on a Black Twig apple held in a home cellar storage for two months. X 1.2.

Figure 2. Storage scab development around a pre-storage scab lesion on the same fruit more highly magnified to show the detailed structure of the lesion. X 3.3.

PLATE 1



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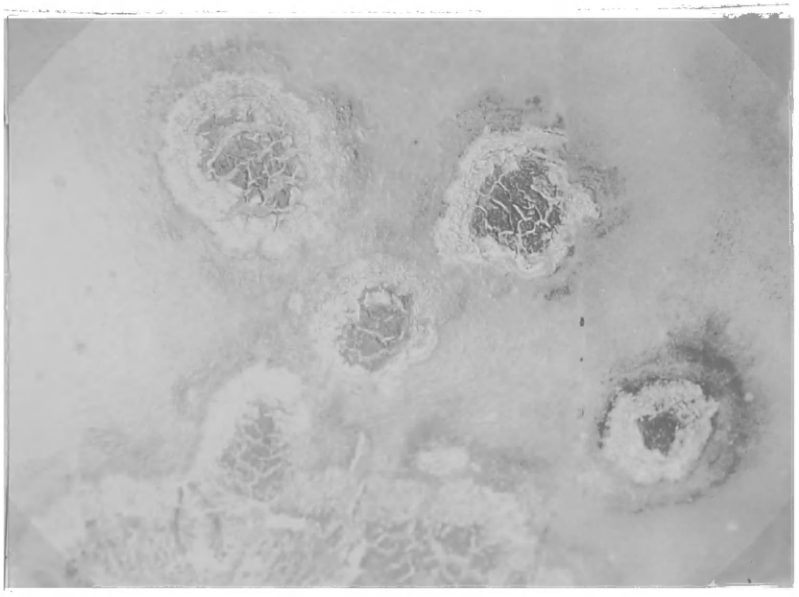

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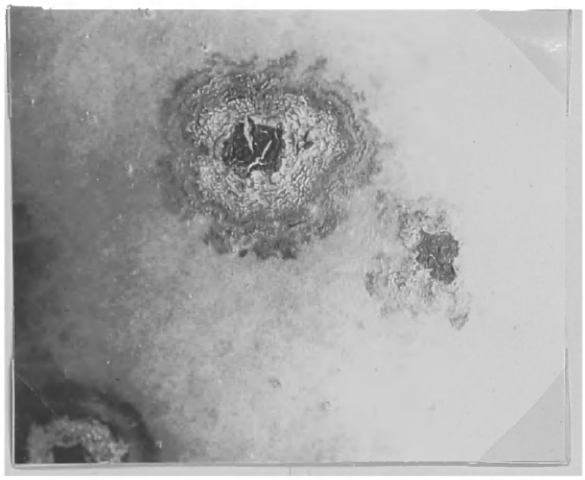
Plate II

Figure 3. Storage scab development around a pre-storage scab lesion, showing the black irregular margin and the definite zonation of the lesion. X 3.3.

Figure 4. Storage scab appears here as a gray-brown halo around a pre-storage scab lesion on a Stayman apple kept in a home cellar storage for two months. X 5.0.

Figure 5. A black erumpent storage scab border around a pre-storage scab lesion on a Stayman apple held for two months in a home cellar storage. X 5.0.

PLATE II



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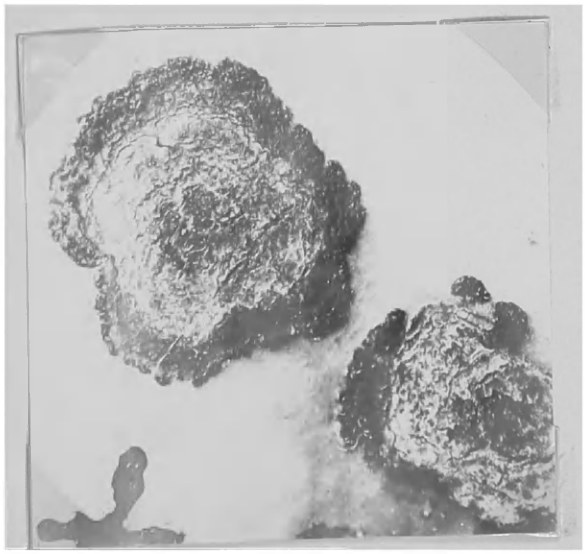

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Plate III

Figure 6. A cross-section of a typical pre-storage scab lesion on a Williams Early Red apple showing the cuticle pushed up and the conidia separated from the mycelial mat below. Note the dark mass of disintegrated cork cells immediately below the cushion of mycelium. Phellogen cells are found below the cork, and normal cortical cells appear from the middle to the bottom of the photograph. X 395.

Figure 7. Pre-storage scab lesion on a Delicious apple with the cuticle in place, and a mass of conidia above. A single row of cork cells is seen immediately below the scab stroma. The small phellogenic cells are seen below the cork layer. X 445.

PLATE III

6

7

Plate IV

Figure 8. Pre-storage scab lesion on Williams Early Red apple showing that the influence of the presence of the scab fungus has spread beyond the lesion on both sides to produce cork-like tissue. X 115.

Figure 9. Very old cork-like tissue in a pre-storage scab lesion on a Black Twig apple. Just the remnants of the scab mycelium remain to indicate scab was present at one time. Note the dark thin line that is made up of dead, crushed cork cells at the top of the photograph. The phellogen appears below, and the normal cortical tissue is beneath it. X 265.

PLATE IV

8

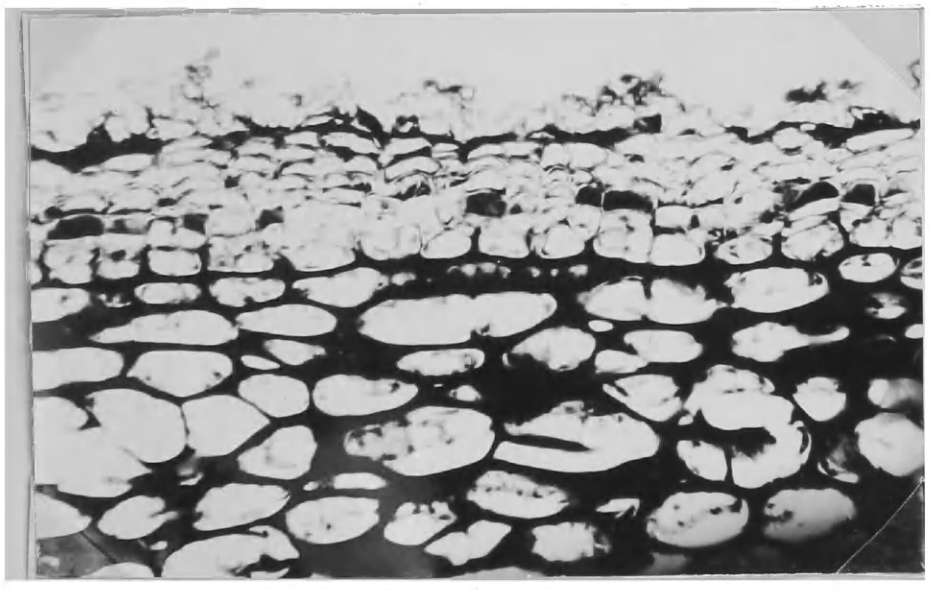

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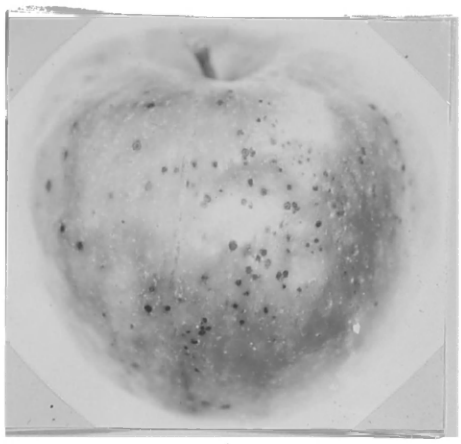
Plate V

Figure 10. Black, shiny, pinhead storage scab lesions with rough surfaces appearing on Stayman apples held for two months at 33° F. X .88.

Figure 11. Portion of the same fruit more highly magnified to show the granular nature of these lesions. X 3.3.

PLATE V



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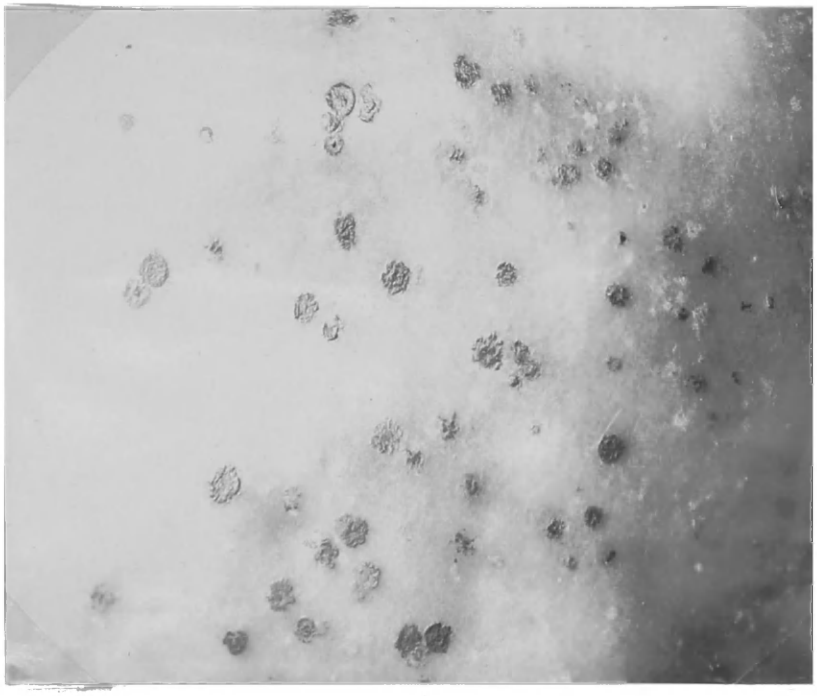

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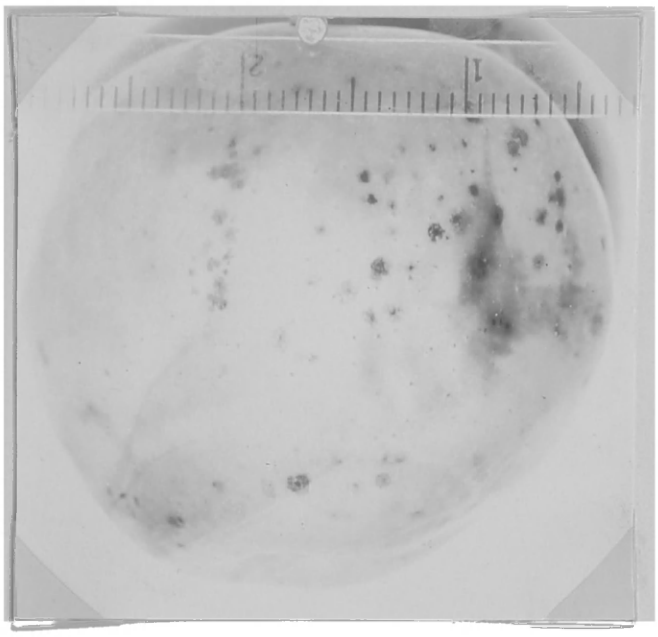
Plate VI

Figure 12. Storage scab as brown, shiny, submerged lesions on Stayman fruit held for two months at 33° C. X 1.2.

Figure 13. Same lesions highly magnified to show the rhizoid to filamentous appearance of these submerged storage scab lesions. X 3.3.

PLATE VI



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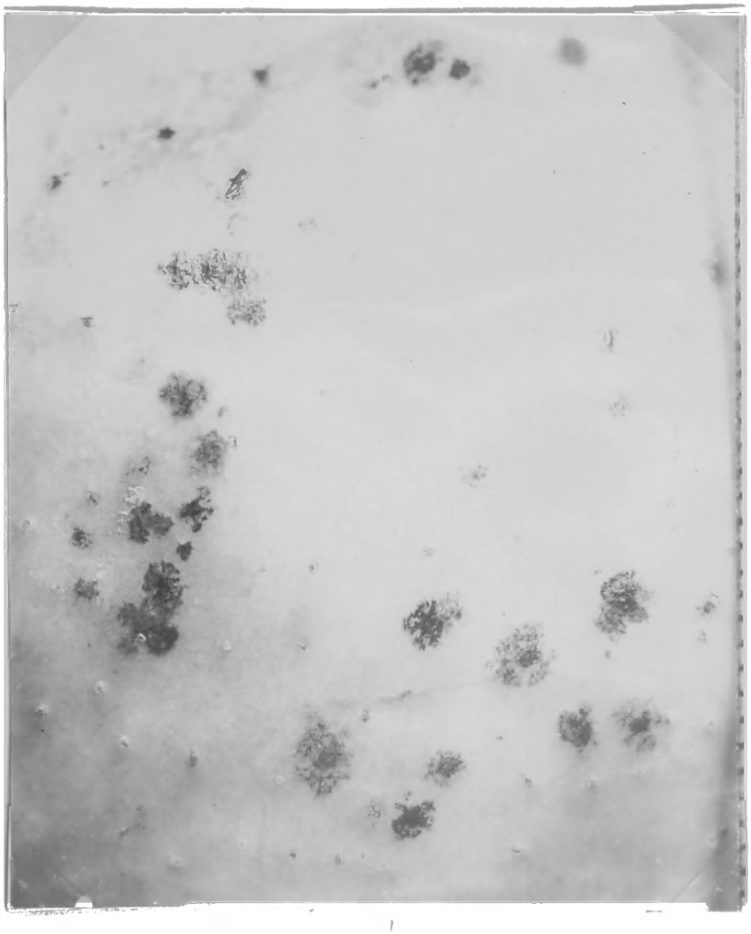

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Plate VII

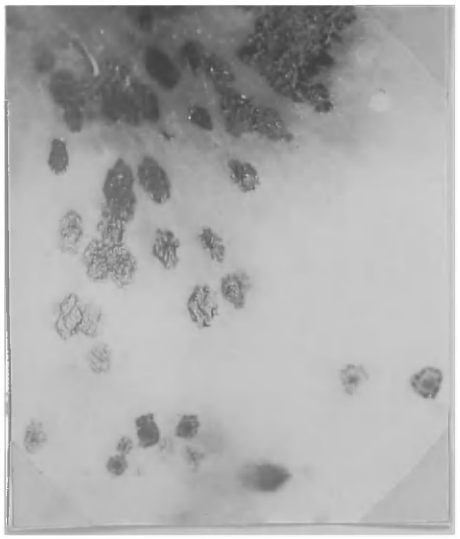
Figure 14. Rough surface, black storage scab lesion on the stem end of a Stayman apple held in a home cellar storage for four months. X 3.0.

Figure 15. Black, irregular, rough storage scab lesion on a Black Twig apple held in a home cellar storage for two months. Note the inactive pre-storage scab lesions above. X 3.5.

Figure 16. Minute storage scab lesions on a Stayman fruit held in a home cellar storage for four months. X 3.0.

Figure 17. A portion of the same fruit to show the off-center nature of these tiny storage scab lesions. X 6.0.

PLATE VII



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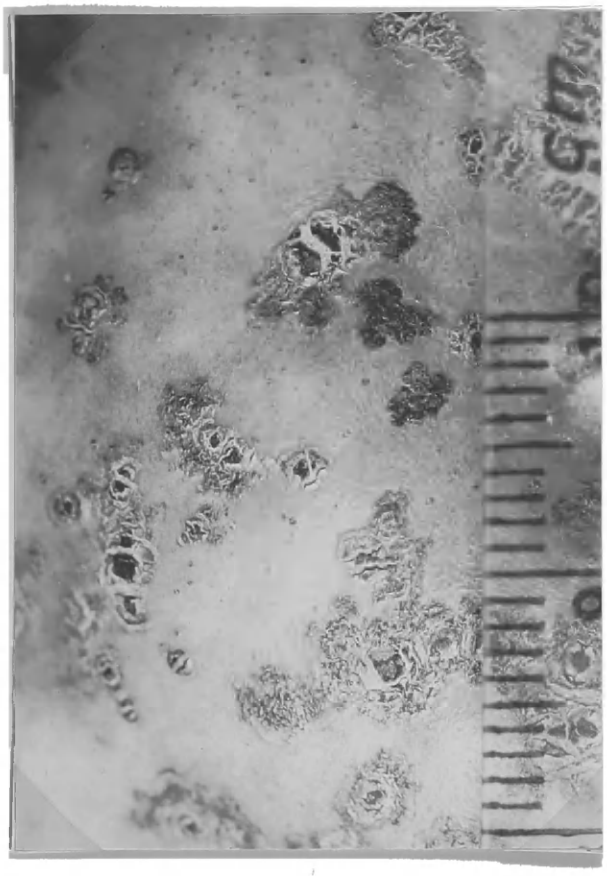

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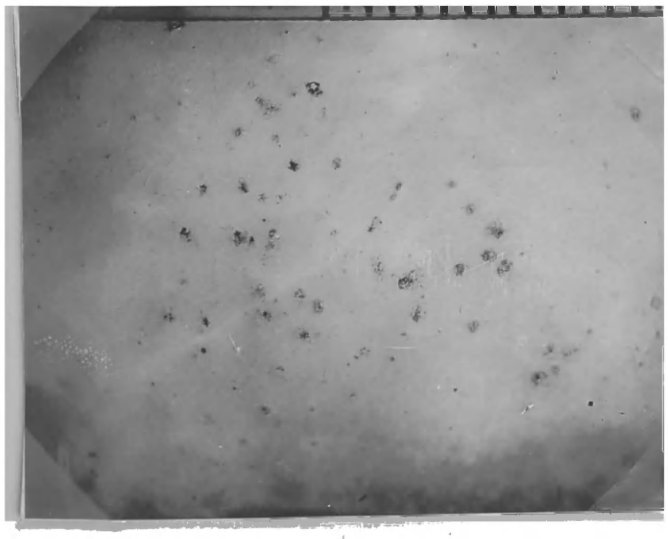

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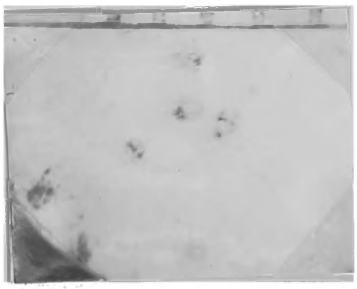

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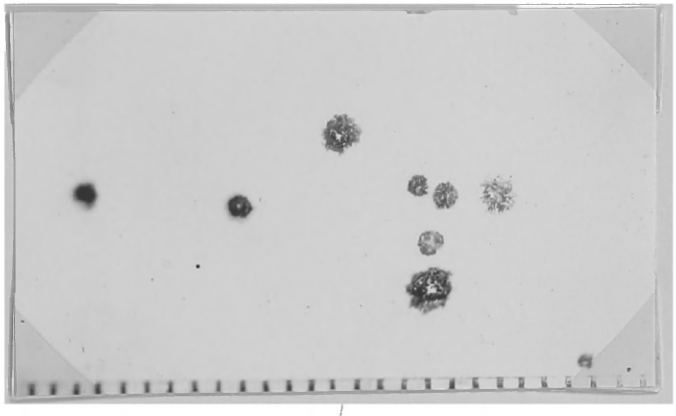
Plate VIII

Figure 18. Storage scab on a Stayman apple stored in a home cellar storage for two months. X 3.3.

Figure 19. The same lesions magnified to show four types of storage scab lesions on the same fruit. There is a filamentous black type; a smooth, entire brown type; a black, finely granular type; and a black, lobate type. X 6.3.

Figure 20. Storage scab on a Stayman apple held at home cellar storage temperature for two months, and then at room temperature for two months. Note that the black, submerged lesions have not broken through the cuticle. X 3.0.

PLATE VIII



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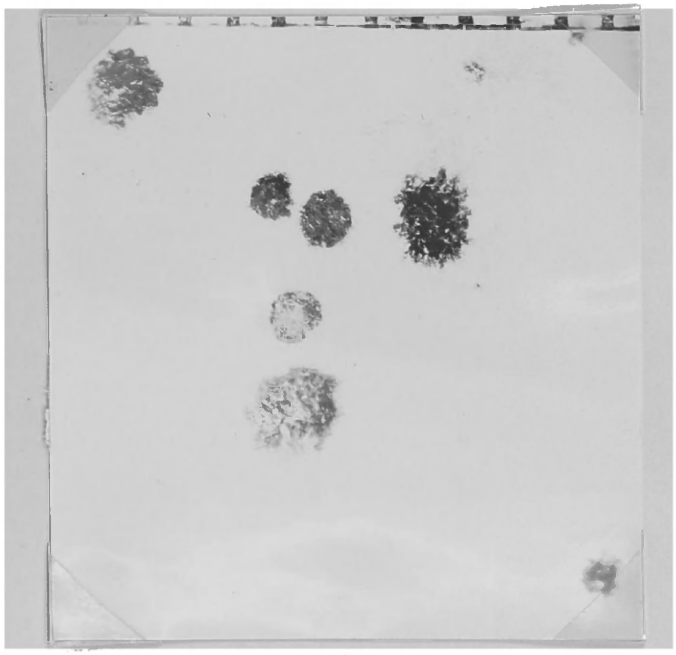

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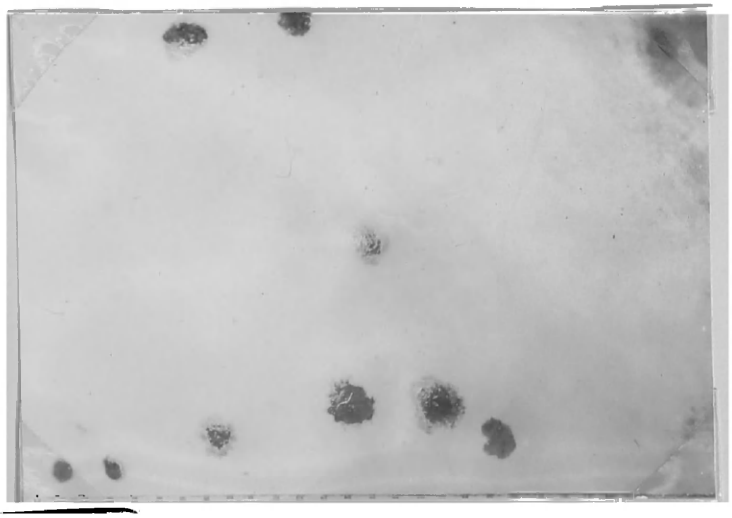

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Plate IX

Figure 21. Pre-storage scab lesion on a Stayman apple, center of photograph, surrounded by a brown halo of storage scab development. Note the numerous storage scab lesions nearby that are only about 1 millimeter in size. X 3.5.

Figure 22. Black storage scab lesions on a Winesap apple of the type which breaks through the cuticular surface. This fruit was stored at 33° F. for four months. X .85.

PLATE IX

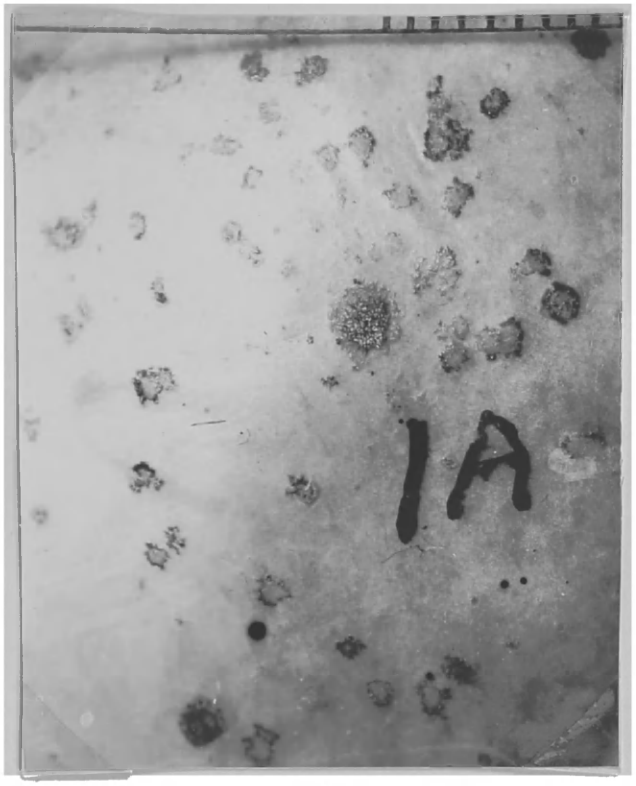

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Plate X

Figure 23. Isolates #1N and #1M from brown storage lesions on Stayman apples on malt agar. Two tubes to the left are isolate 1N, and the two to the right are isolate 1M. The tubes on either end are 50 days old; those in the center are 30 days old. The culture to the left is rough, filamentous and produces abundant spores. The culture to the right is smooth and compact and has few conidia. X 1.2.

Figure 24. Tubes of the same age culture as figure 23 showing smooth culture on the left and a rough culture on the right. Two tubes to the left are isolate 1D2 from a black storage scab lesion from a Stayman apple. The two tubes to the right are isolate 1DD from a brown storage scab lesion from the same apple. X 1.2.

Figure 25. A ten day old culture of isolate 1G3 from a storage scab lesion on a Stayman apple showing an abundance of spores. Some are still attached to the hypha, and some spores are germinating. X 258.

PLATE X

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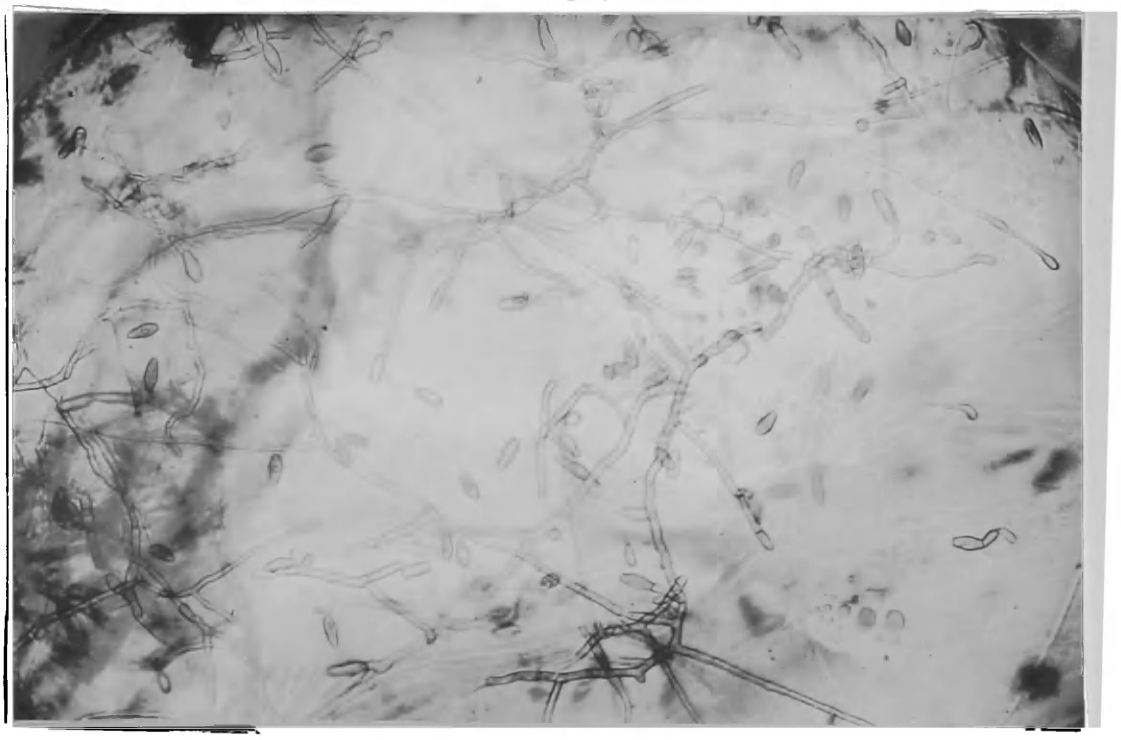

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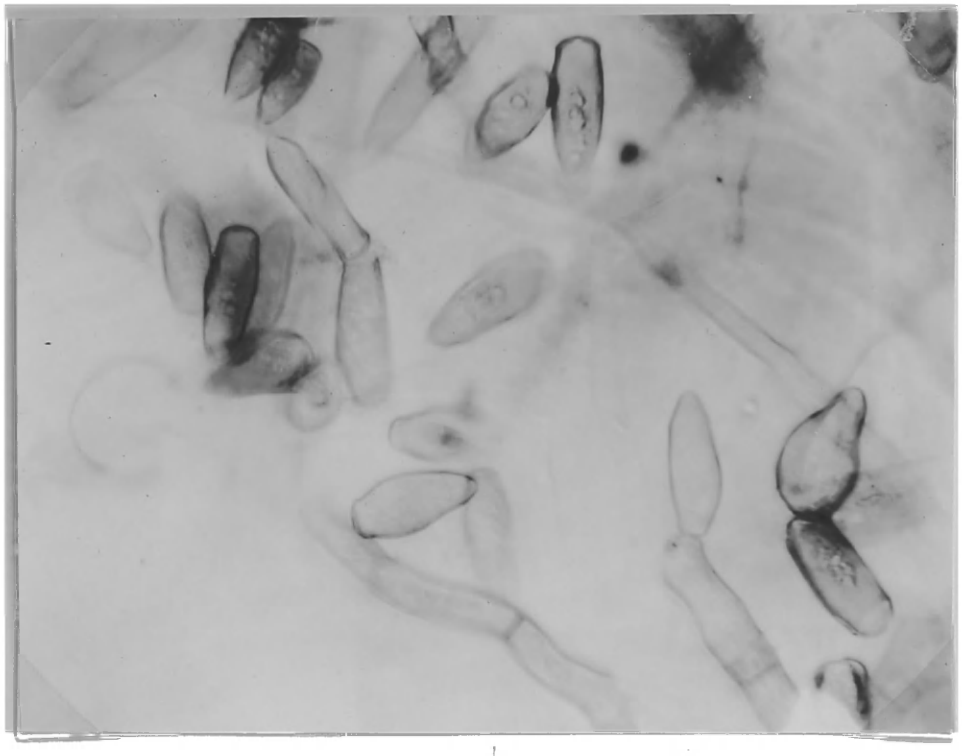
Plate XI

Figure 26. A 10 day old malt agar culture of isolate 1G3 from a black storage scab lesion on a Stayman apple held in a home cellar storage for two months to show the abundant spore production. X 1120.

Figure 27. Growth of hypha from a 10 day old malt agar culture of isolate 1G3 after 48 hours in tap water. Note the germination of the individual spore to the right, and the production of a terminal spore. Other terminal conidia to the left have produced smaller conidia at their apex. X 1120.

PLATE XI



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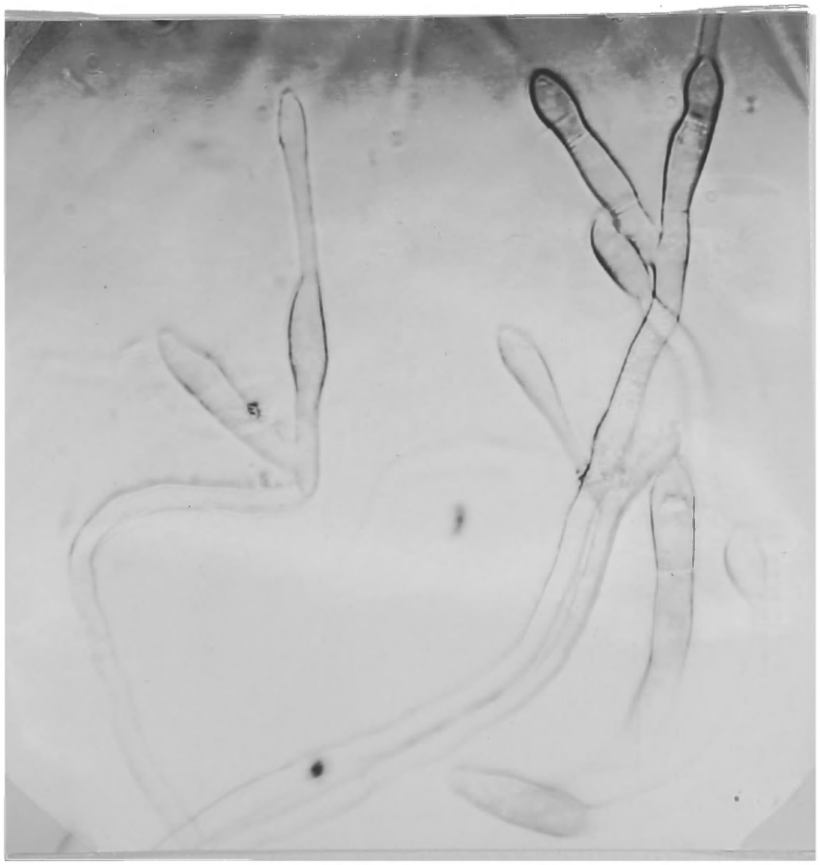

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Plate XII

Figure 28. A portion of a 10 day old fruiting hypha with two conidia attached. X 1120.

Figure 29. Mycelium from the center of a 75 day old malt agar culture of isolate 1J3. X 615.

Figure 30. Conidia obtained from the younger growing outer margin of a 75 day old malt agar culture of isolate 1J3 from a black storage scab lesion on a Stayman apple. Note the pointed appearance of these spores. X 615.

PLATE XII

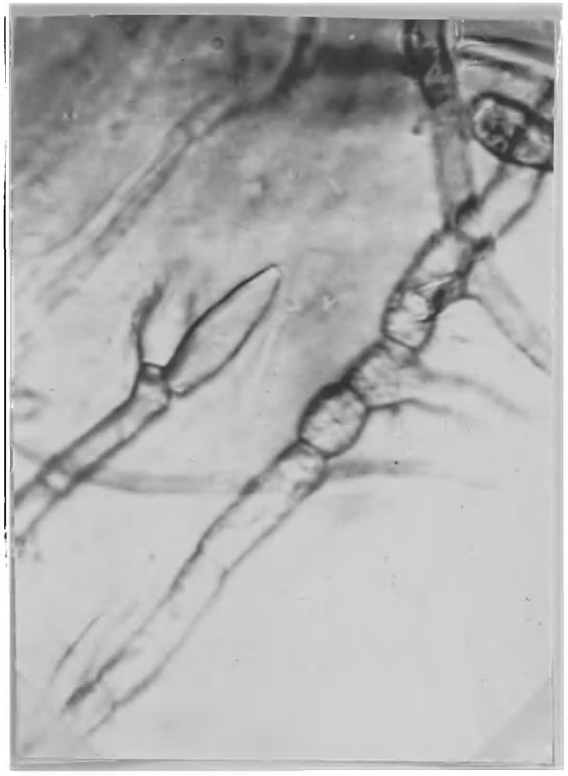

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Plate XIII

Figure 31. Conidia and mycelium from the outer margin of a 75 day old malt agar culture of isolate 1J3, stained with Mayer's Haem-Alum to show uninucleated cells. X 1500.

Figure 32. Mycelial development in the black storage scab zone of a pre-storage scab lesion on a Delicious apple held in storage for two months at 33° F. The fungus stroma is in the cuticle, and around some epidermal cells. It enters into the host cortex to a depth of only two cells. X 420.

PLATE XIII

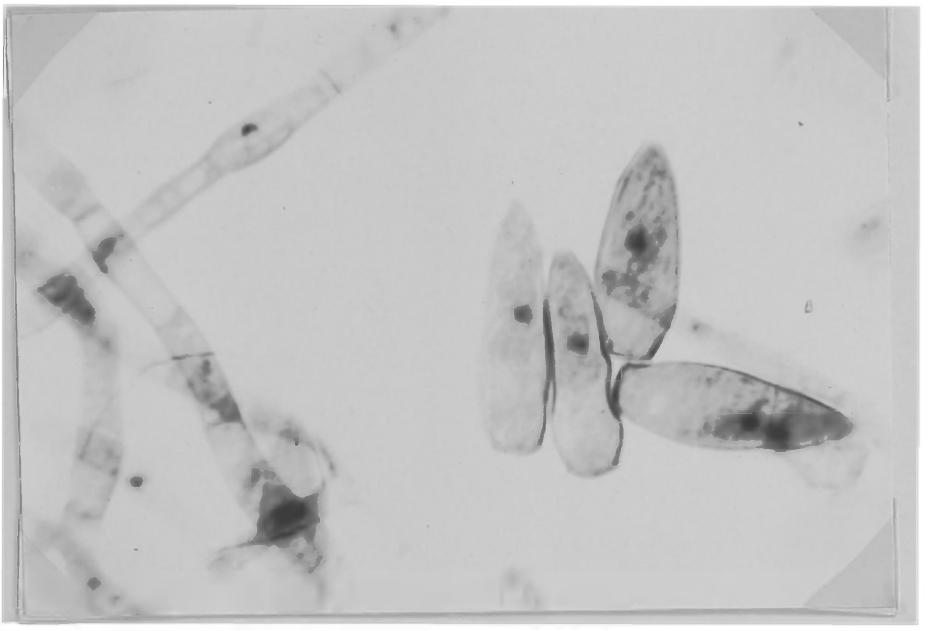

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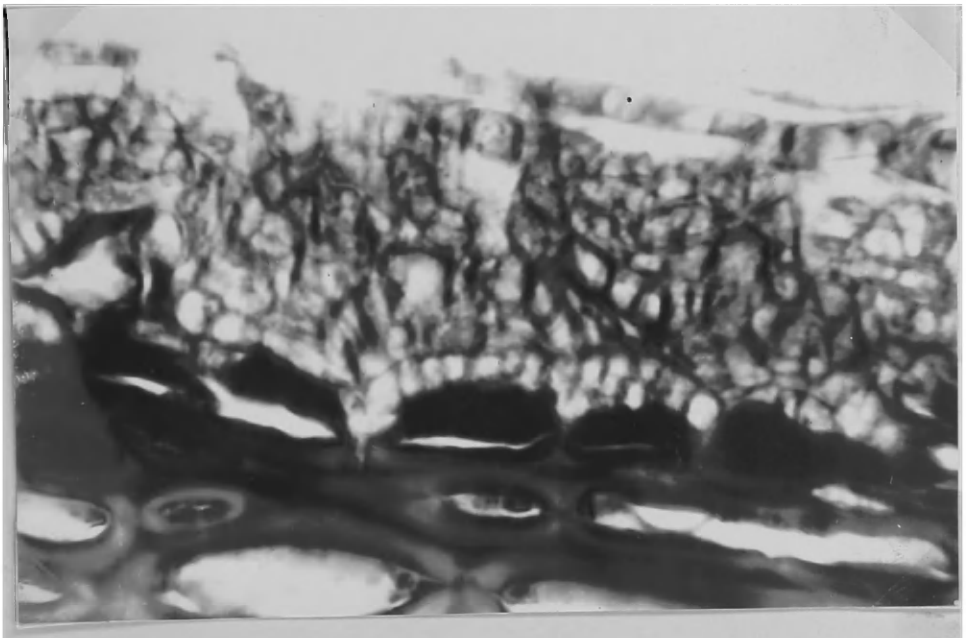
Plate XIV

Figure 33. Storage scab mat of mycelium penetrating an intercellular space in a Stayman apple as it grows between two cells. Note the mycelial plates growing parallel to the outer cuticle layer. X 935.

Figure 34. Growth of the fungus in the extreme outer margin of the black storage scab zone of a pre-storage scab lesion. The fungus is penetrating the middle lamella of the cells immediately below the epidermis. There is no mycelium in the cuticle above. X 800.

PLATE XIV



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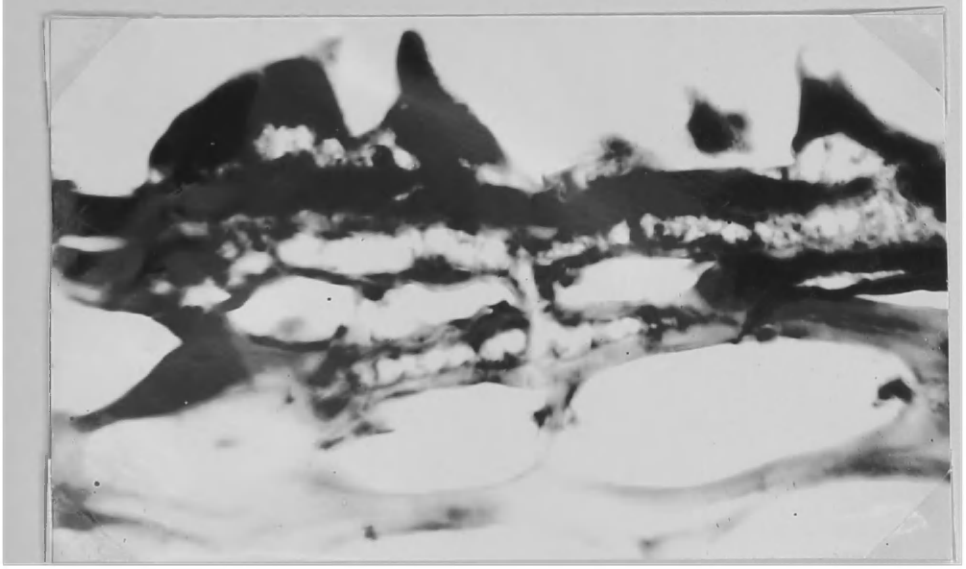

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Plate XV

Figure 35. Mycelium from a storage scab lesion growing into the intercellular space of a cortical cell in a Stayman apple stored for two months in a home cellar storage. X 2540.

Figure 36. Mycelium from a storage scab lesion spreading out in several directions after entering the intercellular space of the cortex of a Stayman apple. X 2644.

PLATE XV

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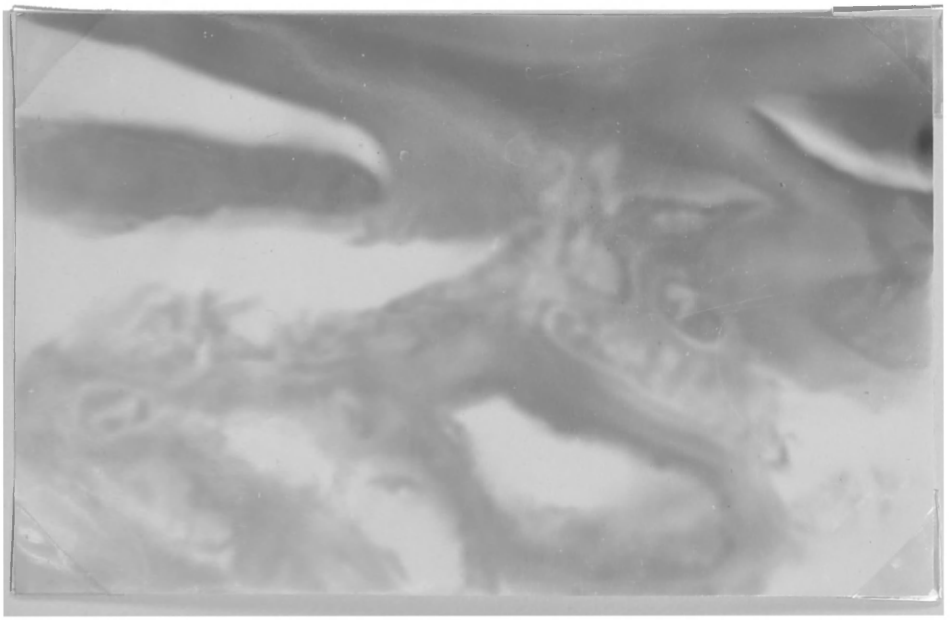

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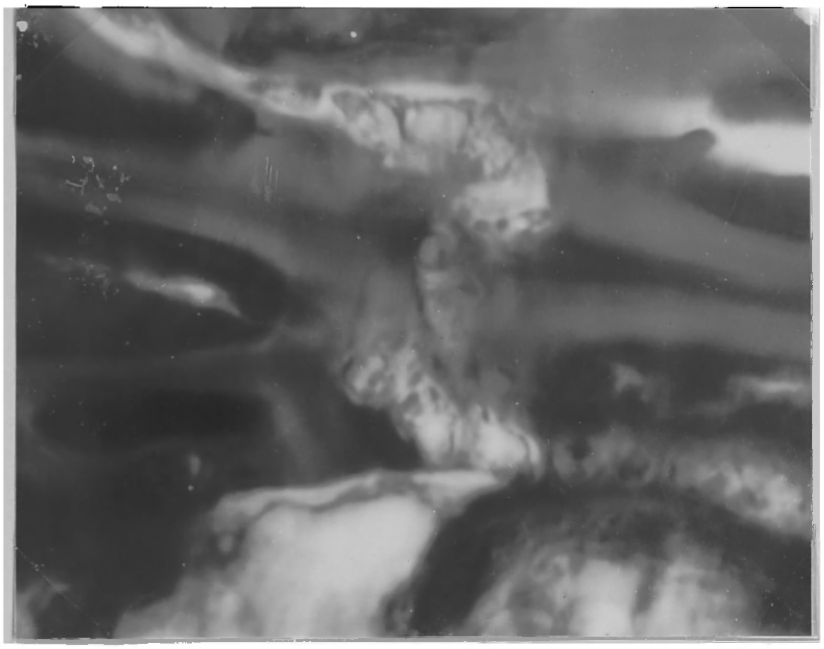
Plate XVI

Figure 37. Growth of mycelium in a storage scab lesion from the middle lamella of one cortical cell above to another cell below. Growth is taking place between the ends of adjoining cells. X 2035.

Figure 38. Storage scab developing in the middle lamella of the upper cortical cells of a Delicious apple. The host cells are greatly crushed below the mat of mycelium. X 540.

PLATE XVI



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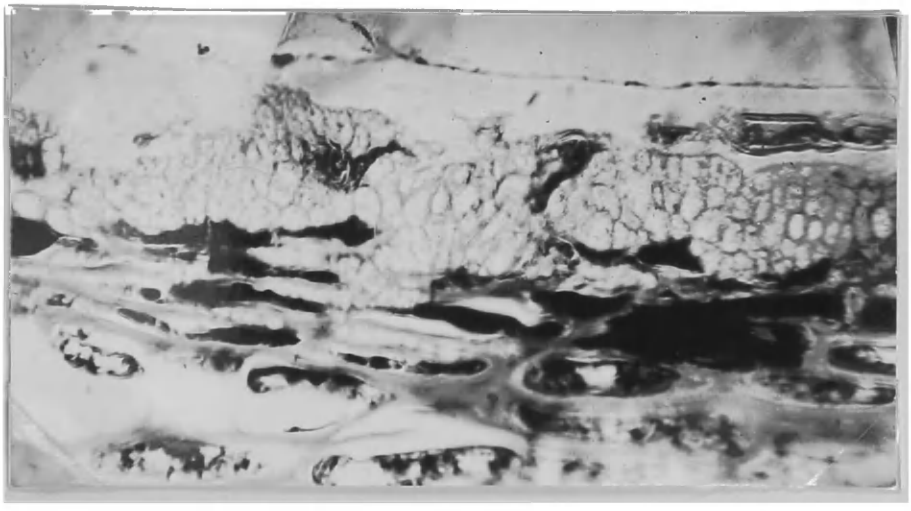

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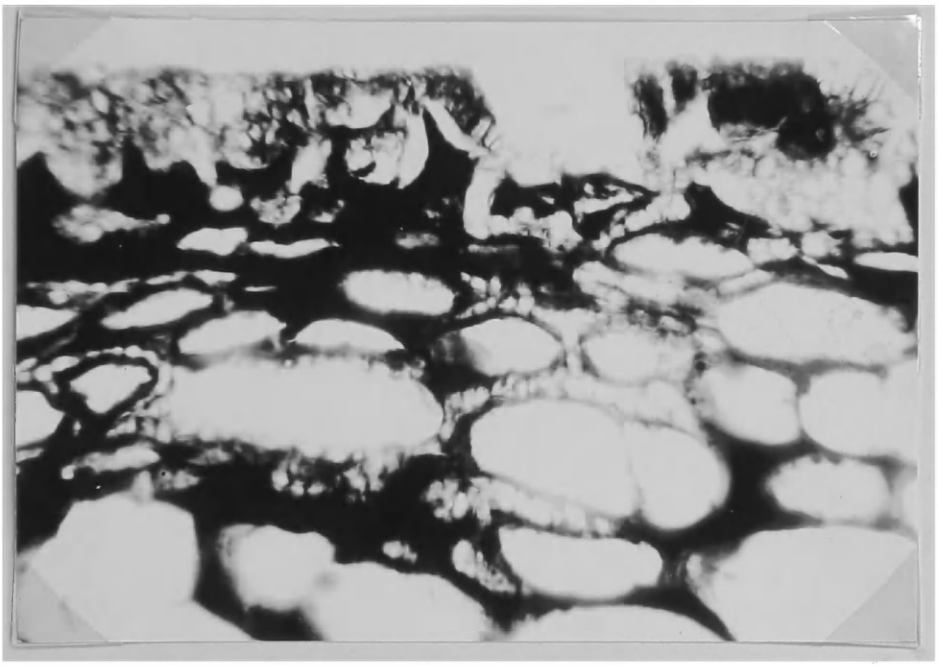
Plate XVII

Figure 39. Storage scab mycelium developing between the cell walls of adjacent cells in the cortex of a Delicious apple. The fungus cells are appearing between the fifth and sixth cells below the epidermal cells. X 470.

Figure 40. Mycelium of storage scab from a Stayman fruit penetrating the cortex to a depth of eight cells below the epidermis. The growth of the mycelium here is both intercellular and intracellular. X 445.

PLATE XVII



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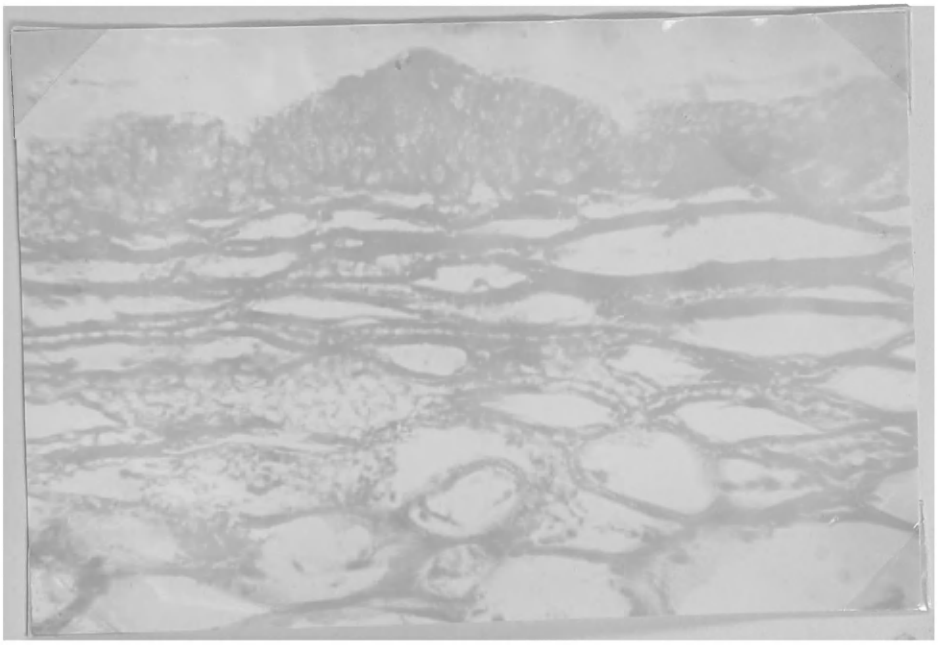

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Plate XVIII

Figure 41. Storage scab lesion on a Stayman apple with the mycelium growing between the walls of the adjacent fifth and sixth cortical cell below the epidermis. Cell differentiation of the fungus is not distinct. X 800.

Figure 42. Mycelium mat of a storage scab lesion from a Stayman apple. Note the horizontal growth of a plate of the mycelium in the outer edge of the cuticle. X 756.

PLATE XVIII

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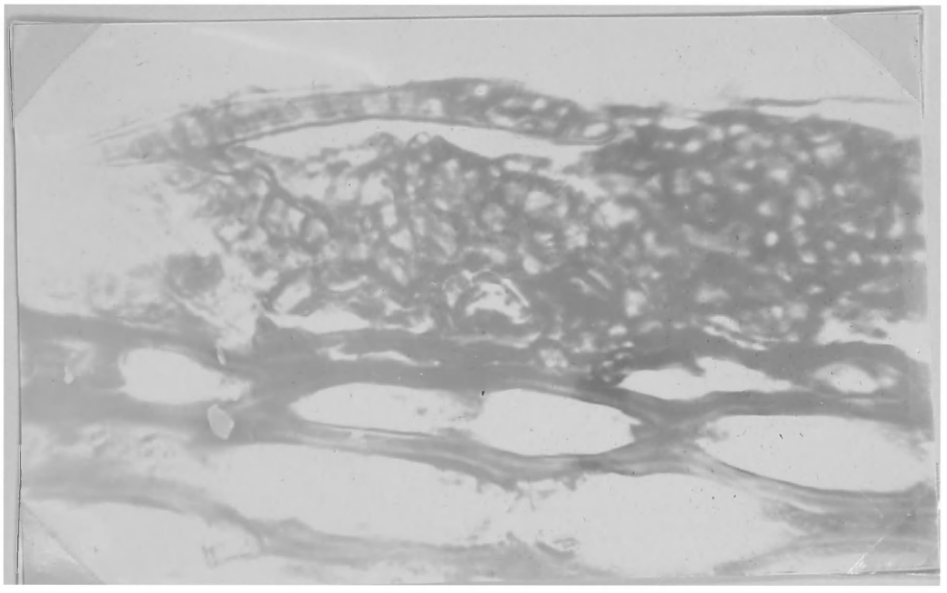

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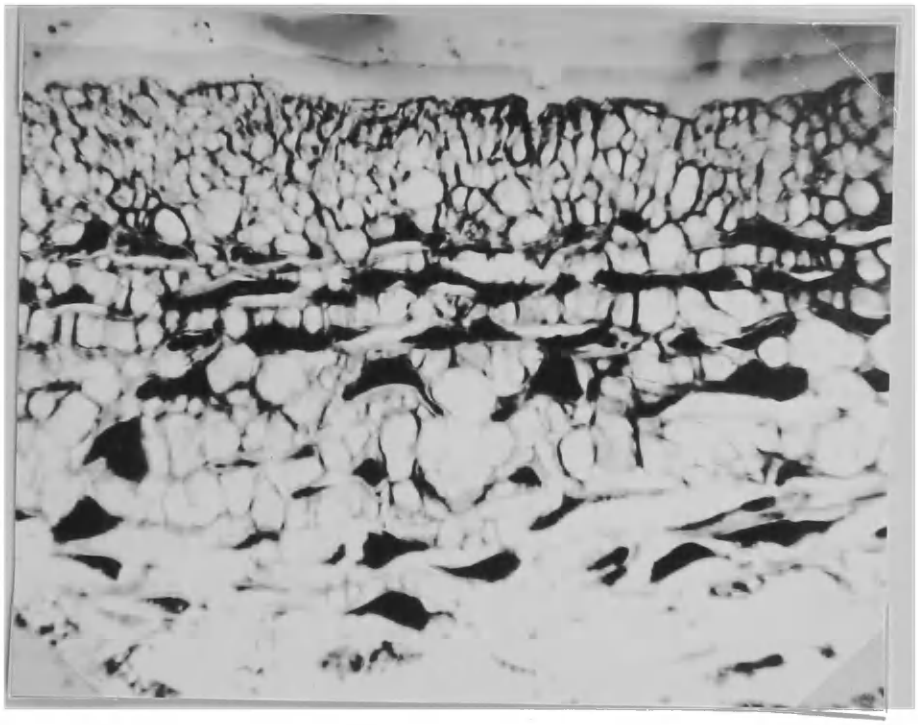
Plate XIX

Figure 43. A storage scab lesion on a Williams Early Red apple held in storage at 33° F. for five months. Note the great depth to which the fungus has penetrated the cortical region. The host cells are crushed, and many have disintegrated. The cuticle remains intact above the dense mat of the scab mycelium. X 615.

Figure 44. A section through the uppermost layer of cortical cells of a Stayman apple cut only three microns thick, showing pits in the cell walls. This material had been treated previously with KOH to swell the cell walls. X 756.

PLATE XIX



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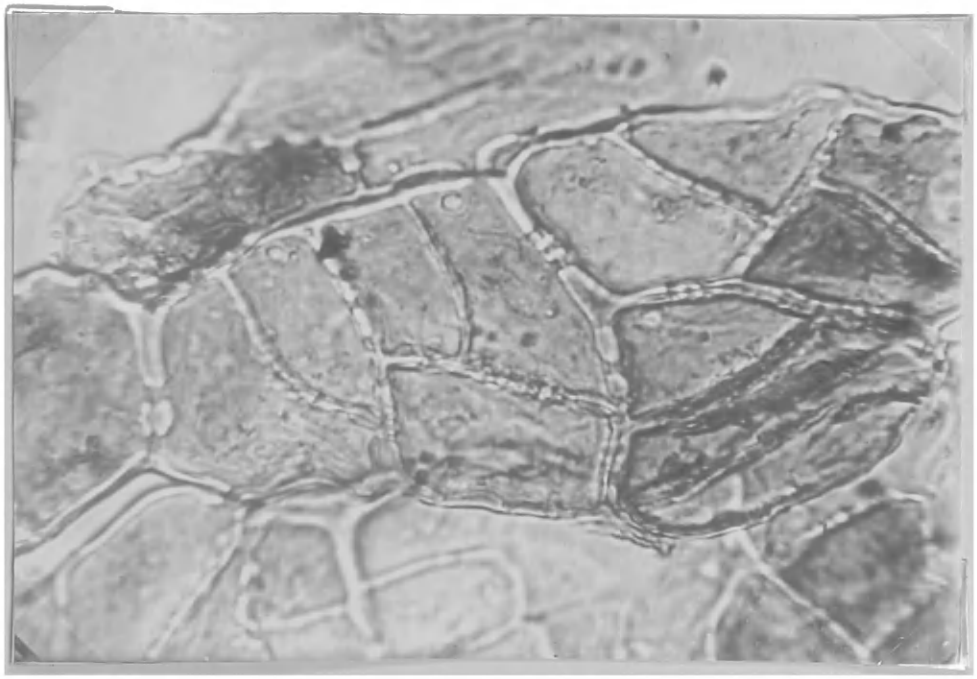

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