STUDIES ON THE OVER-WINTERING AND MODES OF INFECTION OF THE FIRE BLIGHT ORGANISM

A Thesis Submitted in Partial Fulfillment
of the Requirement for the Degree of
Doctor of Philosophy in the
Michigan State College
of Agriculture and Applied Science.

Edgar Cecil Tullis A.B., M.A.

East Lansing, Michigan,
August 1927.

Table of Contents

	Page
Economic Importance	I
History of the Disease	3
Review of Literature	4
Scope of the Investigations	5
Over-wintering	6
Dissemination by Insects	I5
Dissemination by Meteoric Water	27
Infection	32
Examination of Prepared Material	43
Migration of the Organism	45
Normal Anatomy of the Apple Leaf	46
Control Measures	49
Discussion	53
Summary	54

STUDIES ON THE OVER-WINTERING AND MODES OF INFECTION OF THE FIRE BLIGHT ORGANISM

Economic Importance of Fire Blight in Michigan

Fire blight in Michigan ranks among the more important of the diseases of the apple and pear and periodically at least causes heavy losses to the grower. These losses are not merely a reduction in the current year's crop as is the case with scab but probably more important yet is the loss of fruiting wood caused by the removal of spurs, twigs and limbs which have been invaded by the fire blight organism. This means that at least several years will elapse before a severely blighted tree will replace the lost parts with new growths and reach the stage of production possessed before it becomes diseased. In severe cases of course it becomes necessary to remove the tree itself.

The development of the pear industry in this state is largely limited by this disease. Certain regions in the eastern and southern part of the state have been forced to cease growing this crop because of the losses caused by blight. The remains of many of these old orchards may still be seen in various parts of the state.

The apple as a rule has not suffered the severe and rapid effects of the disease as has the pear but even so in cases of severe attack many apple orchards have sustained very severe losses. Apparently this loss has been more severe in the past few years.

Records taken at the Huron Farms Company orchard during the summer of 1926 showed that of a block of 1508 apple trees, 166 had been so badly damaged by blight, over a period of three years, that they had to be removed. Root and crown injury in this same block was so severe that a number of trees had to be bridge grafted. Sixty-one trees had been bridge grafted in the spring of 1925. These trees were girdled and approach grafts were set in 1926, because it was found that this treatment was more satisfactory than attempting to clean up the cankers and make bridge grafts to the roots. One hundred fifty-nine additional trees were girdled and bridge grafted in the spring of 1926.

Losses in one block of 325 pears in this same orchard amounted to about 20 percent of the trees. These trees were so badly blighted that though every attempt was made to save them, they were lost because so little was left after the diseased limbs had been removed. A small pear orchard of 30 trees was observed during the summer of 1927, on the west side of the state, south of Sagatuck. Fully 90 percent of the trees were blighted. Twenty-five of these trees were removed the following spring.

While the orchards described above represent some of the more severe infestations and losses which have been observed during the past three years, they are by no means isolated, rare examples of the ravages of blight. The disease is present and causes throughout the state a certain amount of damage in a considerable number of orchards every year.

The following data obtained from the Plant Disease Reporter for the years 1921-1925 inclusive, represents the estimated percentage losses of the apple crop in Michigan, caused by fire blight and other diseases.

TABLE I

Percentage losses of the apple crop due to fire blight and other diseases

YEAR	BLIGHT	SCAB	ALL OTHER DISEASES	TOTAL
1921	5	2	1	8
1922	0	16	0	16
1923	5	1	1	7
1924	5	12	2	19
1925	8	2	3	13
AVERAGE	5.6	6.6	1.4	12.6

This table shows that losses due to fire blight are estimated by collaborators of the Plant Disease Survey to have been on an average approximately one percent lower than those due to scab for this period.

History of the Disease

Fire Blight has been known for about one hundred fifty years. The disease apparently is indigenous to North America and no doubt occurred on wild hosts previous to the introduction of the apple and pear into this country. Among the wild hosts listed in North America are wild crab, hawthorne, service berry, the red berried California holly, mountain ash and the loquat. The disease has also been reported from Japan, New Zealand and Italy. It was first described in I794 but had been observed almost fifteen years earlier by William Denning in the Hudson River Highlands. The disease spread westward and southward into the Mississippi valley and it was not until I900 that it had reached California and in I9I5 it had become a serious disease in central Washington.

Nothing was known as to the cause of the disease for almost one hundred years after the disease was described. Various explanations were made in an attempt to account for its appearance. Among the causes were listed, electricity, sunscald, frozen-sap, old age, over-nourishment, under-nourishment, fungi, insects and other factors.

Review of Literature

In I878 Burrill (5) then botanist at the University of Illinois advanced the theory that fire blight was due to a bacterial infection. This theory was substantiated by his experiments which he reported in I88I and by the work of Arthur (I) in I885. Waites (28) contribution in I898 regarding the transmission of blossom infection by bees, wasps and other nectar seeking insects has been followed and supplemented mainly by the work of Gossard and Walton (I2). Other investigations by Jones (I6), Stewart and Leonard (27), Merrill (I8) and Burrill (4) have established the importance of various species of chewing and biting insects in the dissemination of twig infection. Jones (16), Lathrop (17) and Burrill (4) have also made contributions concerning aphid transmission of this phase of the disease. Contributions to our knowledge of the over-wintering of the organism have been made by Sackett (23), Whipple (29), Brooks (6) and Nixon (19). Miss Bachman (2) and Nixon (19) have shown the manner of intercellular migration of the organism in the invasion of the host. The importance of wind blown rain has been demonstrated by Stevens (25) and his co-workers, Gossard and Walton (12) and others. Heald (15) first recognized the fact that leaf invasion produced a leaf blight. Day (9) has worked out efficient disinfectants and Reimer* has found and is testing out resistant pear stocks and our present commercial varieties also in an attempt to determine which of them are most satisfactory.

Scope of the Investigations

The investigations reported in this paper were begun in the fall of 1925 and have continued through the summer of 1928. The writer was able through arrangements with the Huron Farms Company of Ann Arbor Michigan, to spend five months during the summer of 1926 and the greater part of the

* Reimer, F.C.: Blight resistance in pears and characteristics of pear species and stocks. Ore. Agr. Exp. Sta. Bul. 214: 1-99. 1925.

summer of 1927 in the Company orchards near Ann Arbor. Trips were also taken into various parts of the southern half of the state to examine blighted orchards. The remainder of the work was done at the Michigan State College, Department of Botany.

The problems under consideration in this paper relate to over-wintering of the causal organism, <u>Bacillus amylovorus</u> (Burr.) Trev., dissemination of the disease by aphids and by meteoric water, certain problems of infection, and the production of twig blight.

Over-wintering

It is a well established fact that the fire blight bacterium over-winters in the cankers produced on the limbs and trunks of apples, pears and quince trees. The percentage of cankers in which the causal organism remains alive through the winter in Michigan orchards has been found to vary considerably in different seasons and under different conditions of vigor of the trees. Time of infection and weather conditions during the late summer and fall also seem to exert an important influence at times. It has been commonly reported and experimental evidence seem to show that the region of over-wintering is along the edge of the canker or in apparently healthy tissue just in advance of the canker edge.

However cankers have been observed in which the spring exudate comes first from the more central part of the diseased area of the bark. These observations have led to an attempt to determine whether the organism may not in some cases remain alive in bark which is either dead or which contains many dead cells.

Two types of cankers have been observed on both apple and pear. The first may be called the determinate type of canker in which a definite margin is very evident. This is caused by the formation of a layer of cork by the host in advance of the canker edge. The second may be called the indeterminate type, for cankers which fall into this category have no definite edge and determination of the extent of the affected area is only possible by cutting through the outer bark. Cankers which have not been checked by drought or other factors are most commonly of the second type. These may result from infections of the late summer or fall. Cankers formed as a result of spring and early summer infections usually are of the determinate type. Some of these cankers seem to remain alive during the greater part of the summer and fall season and in some the bacteria seem to renew activity in the fall after a period of slow development during the summer.

In making incisions into a number of pear and apple cankers of the indeterminate type, it was found during the fall of 1926 that there were certain areas outside the cambium layer in such cankers, which had a rather dark and water soaked appearance. Closer observation indicated that these areas contained bacteria in enormous numbers. Sections of this tissue gave floods of organisms similar to those obtained from recently blighted twigs or bark. It was thought worth while to mark a number of cankers of the type described above and make isolations at regular intervals through the winter and spring to watch for earliest signs of bacterial activity in the spring.

Accordingly during the winter and spring of 1926 and 1927, observations were made on the over-wintering of the blight organism in pear and apple cankers.

Isolations were made under microscopic observation by a modified capillary tube method, which served very satisfactorily to obtain cultures of the organism. In only a few cases did contamination result. Bits of tissue, removed aseptically, were mounted on a slide in sterile water. Capillary tubes were drawn from 2mm. soft glass tubing and fastened in a modified Barber manipulator; the capillary tube was then maneuvered until the tip was observed to be over a mass of the organisms as they exuded from the tissue. The tube was then lowered into the suspension of the organism and quickly withdrawn. The portion of the tube containing the bacteria was then broken off by means of sterile forceps and dropped into a tube of boullion. Clouding usually occurred within forty-eight hours. These cultures were then inoculated into the host by puncture. The plants were incubated in the chambers as shown in figure 3.

Isolations were made during the months of January, February, March and April. The first exudate in the spring of 1927 was observed April 30 on pear at Ann Arbor. Cankers of apple and pear were used in making the isolations and are so designated in the table which follows. On March 8, three cultures were obtained twelve inches back of the canker edge from an indeterminate type of apple canker. The canker was on a limb about one inch in diameter and was about three feet long. Again on April 8, three more isolations were made from a pear canker similar to the type just described on apple. One of these cultures produced infection when inoculated into young succulent apple shoots. Two of 26 cultures made from pear on April 29 also produced infection.

The results of these inoculations, with the date of isolation of the organism, host and the region of the canker from which the isolations were made are given in the following table.

TABLE 2

Results of inoculations of cultures obtained from dormant cankers

DATE OF ISOLATION	NUMBER OF CULTURES OBTAINED	HOST	REGION OF CANKER FROM WHICH ISOLATIONS WERE MADE	RESULTS OF INOCULATIONS OF YOUNG APPLE TWIGS WITH CULTURES FROM CANKERS
Jan. I2	7	Pear	-----	5+, 2-
Febr. 6	5	4 Pear I Apple	I/4 In. back of edge	I+, 3- Negative
Febr. I5	4	Apple	I/2 In. back of edge	I+, 3-
March 3*	3	Pear	Center of small canker around spur	Negative
March 5	3	Apple	I/2 In. back of edge	3+
March 8	3	Apple	I2 In. back of edge	3+
April 5	3	Apple	I/2 In. back of edge	I+, 2-
April 8	3	Pear	I2 In. back of edge	I+, 2-
April 9	3	2 Pear I Apple	2 In. back of edge	I+, I- Negative
April 29	26	Pear	Advance of discoloration	2+, 24-

* The isolations on March 3 were from material which was collected at Ann Arbor in January and February and had remained out-doors in the snow until the time of isolation as indicated in the table.

This table shows that ten of 45 pear cankers and that seven of 15 apple cankers contained fire blight bacteria. The percentage of active cankers may be higher earlier in the winter but it would be necessary to work with larger numbers of cankers before any conclusions on that point might be reached. It is probable that occasionally at least, the organism may live over in the dead areas considerable distances back of the advancing edge of the canker especially in the indeterminate type of canker.

Each spring during the course of this work, blighted orchards have been kept under observation for evidences of over-wintering of the blight organism in twigs and blossom spurs. Undoubtedly the chief source of spring inoculum in an average season is from the cankers on limbs of more than an inch in diameter and from cankers on the trunk of the affected trees. There seems to be very good evidence however, as shown by Sackett (25) and later by Brooks (6), that the blighted twigs may in some seasons serve as a limited source at least of spring inoculum. The desirability of a full knowledge of the percentage of twigs in which the blight bacteria will live through the winter is evident since this has a direct bearing on practices of control by sanitation. Some of the evidence obtained during the course of this work indicates that twigs may in exceptional cases be a relatively important source of spring inoculum.

During the fall of 1926 a young Pippin tree was observed which had a number of blighted twigs. On May 23, 1927 these twigs were cut off and examined to determine the number in which over-wintering of the organism had occurred. Invasion of the healthy tissues in advance of the canker edge had already begun, so examination was made microscopically. No isolations were made from this material. The following table gives the results obtained from examination of this material.

TABLE 3

Over-wintering of the organism in twigs

SIZE OF TWIG	3/16	4/16	5/16	6/16	7/16	10/16
NUMBER OF TWIGS FROM WHICH FIRE BLIGHT BACTERIA WERE OBTAINED	4	1	3	5	3	0
NUMBER OF TWIGS FROM WHICH NO FIRE BLIGHT BACTERIA WERE OBTAINED	0	18	0	6	0	2

This shows that over-wintering may occur in small twigs and it is possible that such twigs may occasionally serve as a source of inoculum in the spring.

A large Fall Pippin in the college orchard has been observed to have cankers on some of the large limbs for the past two springs of 1927 and 1928. The spring of 1927 it blossomed very heavily and about 90 percent of the blossoms were blighted. Five or six cankers in the inside of the tree were observed to have become active about the middle of May in 1928. The few blossom clusters which were produced, became blighted. Several isolated cases appeared in a crab tree adjacent to this tree. Numerous blossom infections also appeared on another neighboring tree, the limbs of which almost interlace. The blighted blossoms appeared on those limbs closest to the Pippin tree.

Twig infection was observed near an active canker in a King in which no blossom blight had appeared. This canker was located about three inches above and to one side of the infected twigs.

One infection was secured on an apple seedling in the spring of 1928 from inoculum taken one-fourth of an inch back of the canker edge from apparently dead bark from a Northern Spy canker. Another case was observed in the college orchard in which over-wintering had occurred in a twig seven-sixteenths of an inch. The development of the canker in the spring had cut off the terminal bud shortly after it had produced leaves.

The pear orchard discussed on page 2, in which 20 percent of one block of 323 trees was lost, was due to the failure of the operator to remove a single canker on a limb about one-half inch in diameter.

Observations were made in an orchard at Morrice Michigan, the first week in July 1928. In eight isolated trees blossom blight had occurred. In seven cases over-wintering had occurred in twigs no larger than a lead pencil.

It is quite obvious from the results of these observations and the observations of others that small twigs may at times serve as a primary source of inoculum and so the necessity of the removal of these twigs should not be minimized.

It was also shown that over-wintering of the blight organism might occur in dead tissue some distance back from the canker edge.

Whipple (29) reports that the pear is probably the most important host for the over-wintering of the fire blight bacterium in Colorado, and Sackett (23) succeeded in recovering the organism from 21 of 85 blighted pear twigs. Reinking was unable to isolate the organism from apple during the following fall, winter or spring in Wisconsin. Brooks (6) finds that over-wintering occurs in the apple in twigs as small as three-tenths inches in diameter. He also reports that the organism was isolated as far as one inch in advance of the canker edge in dormant cankers.

Nixon has observed that pockets are formed in the tissues of the bark, along the margin of the canker. These pockets are filled with masses of jelly-like substance, in which the organisms are embedded. He considers that these masses or "cysts" represent the organism in its over-wintering condition. The work of different investigators seems to show that these "cysts" may not be necessary. These so called "cysts" have in the past been referred to by most investigators as "pockets" and were supposed to be the seats of over-wintering.

Dissemination by Insects

The dissemination of the fire blight organism in the orchard is one of the phases of the fire blight problem which has been given most attention by investigators. Rain spatterings and wind blown rain are undoubtedly very important in dissemination as are insects of various kinds.

Among the insects are listed, the tarnished plant bug, (Lygus pratensis Linn.), aphids, (Aphis mali Fab.), (Aphis avena Fab.),apple leaf hopper, (Empoasci mali LeBaron), curculio, (Anthonomus quadrigibbus Say.), shot hole bark boring beetle, (Scolytus rugulosus Ratzeburg), Campyloma verbasci Mey., Orthotylus flavosparsus Sahlberg, Poeciloscytus basilis Reuter, Adelphocoris rapidus Say., Plagiognathus politus Uhler, and bees, flies and wasps.

Blossom Blight

Blossom Inoculations by Nectar Seeking Insects.

Experimental evidence and observations on the matter of blossom infection by insects includes dissemination by nectar seeking insects and aphids. These experiments were carried on at the Huron Farms Company orchard at Ann Arbor and in the college orchard at East Lansing.

During the early spring of 1926, the apple and pear orchards at Ann Arbor were very thoroughly inspected in an attempt to remove all blight cankers from the tops of the trees. The trunks and crowns of all the trees were also examined. Three cankers were found after the trees had come into leaf. One was found in the pear block and two trunk cases were found in the apple block. Both the trunk cases in the apples were just above the surface of the ground and accordingly little transmission, if any, could have been possible from either of these sources. The canker in the pear was in the top of the tree in such a location that rain which fell during the early part of the blossoming period, could spatter from it to blossoms in the vicinity.

Because of past unproductiveness of the pear orchard in question, it was decided to place bees in the orchard to aid pollination. Seven hives were placed under the trees in row six figure I. The hives were placed under every third tree numbering from the bottom to the top of the chart. A bee yard was established at the same time about a hundred yards outside the main orchard, figure 2 opposite X.

The pear block consists of Lawrence, Boussock and Bartlett pears. The trees represented in Figure I are largely Lawrence and Boussock. The Lawrence predominating in rows IO-I4, and Boussock in rows I-IO.

The first pear blossoms of the various varieties opened between May I4 and May 20. Precipitation occurred on May I4-I5-I9-2I-22. The Boussocks began to blossom on May 20 and rains of .04 inches on May 2I and .47 inches on May 22 fell during the early part of the blooming period of the Boussock variety. The canker which was found in the pears was in a Boussock tree and it is very interesting to note that the heaviest blossom infection occurred in this variety.

A survey of about half the pear block showed that many trees were badly diseased. After a thorough search only one canker which showed evidence of having been active before the main outbreak of blight took place, was found in this section of the orchard. Evidence indicates that this canker was the original source of infection which took place as shown in figure I, which gives the results of the survey. It shows the tree in which the canker was found and gives an approximate idea as to the severity of the infection. The squares or portions which are cross hatched represent I-IO percent of infected blossoms, the solid black represents IO-60 percent infection. By far the heaviest infection occurred in the Boussock block and isolated Boussock trees showed quite heavy infection.

The whole orchard showed quite a heavy infection and it took two men about two weeks to remove the blighted parts from the affected trees over the whole I20 acres. The whole orchard was gone over twice during the spring of I927 and any canker which had developed, after the summer cutting had been discontinued, were removed. One active canker on a Thompkins King tree was found later and one trunk canker on a pear. However neither of these trees showed any blight during the summer of I927.

May I3 and I4 blossom inoculations were made by by spraying a water suspension of the organism into the blossoms. These inoculations were made as a rule around the periphery of the tree and not over five or six feet from the ground.

The following table shows the variety inoculated, the number of blossom clusters in each variety, and the number of these inoculations which produced infection. One blossom cluster was inoculated in each of the trees shown in table 4.

TABLE 4

Results of spray inoculations of blossom clusters

VARIETY	BLOSSOM CLUSTERS INOCULATED	BLOSSOM CLUSTERS INFECTED
Rhode Island Greening	I4	8
Wagener	I7	7
Wealthy	I7	4
Westfield	I4	2
Grimes	7	I
Duchess	3	2
North Western Greening	5	3
Jonathan	2	0
McIntosh	2	I
Fameuse	6	4
Total	87	32

Nine of the 32 trees in which the blossoms were infected, showed further blossom blight. From these 32 possible sources there were II0 trees infected. The writer had by examination of other apple and pear trees in the vicinity ascertained that no other sources were at hand. Some of the trees were a quarter of a mile from any other infected trees, so it seems most probable that nectar seeking insects, probably bees, were agents of transmission.

The weather during the blossoming period in the spring of 1927 was quite cool and a great many rainfall periods occurred during this time. The activity of bees and nectar seeking insects was no doubt below the normal and this may have accounted for the rather limited amount of spread as shown by figure 2. The largest losses occurred in the Wealthy variety which was just coming into blossoming when these inoculations were made and was just opposite the bee yard.

It should be noted that the Jonathan, North Western Greening, Rhode Island Greening, Wealthy and Grimes trees had at this time not more than half of their blossoms open. The heavy infection on the Wealthies cannot therefore be said to have occurred because of the fact that this was the only variety still in blossom.

It has been noted that years in which severe blight epidemics occur, heavy blossoming occurs also. This has been noted to be the case in a Fall Pippin tree in the college orchard. Several cankers were observed in this tree in the fall of 1926 and again in the fall of 1927. The tree blossomed heavily in the spring of 1927 and about 90 percent of its blossom clusters were blighted. Severe twig blight appeared also in this tree during the summer of 1927. One, of the half-dozen blossom clusters produced in the spring of 1928 became blighted. No twig blight appeared in this tree.

It is evident that nectar seeking insects, during seasons of favorable weather conditions, are a considerable factor in establishing primary sources of infection in the blossoming tree. The height of their activities are no doubt best recorded in the very severe blossom epidemics which are apparent from time to time. These results corroborate the findings of Waite (28), Crandall (8), Jones (16), Gossard and Walton (12) and others, that bees and other nectar seeking insects serve as agents in the dissemination of blossom infection. Gossard and Walton (12) have also shown that these infected blossoms may serve as the primary source of infection in the tree.

Hutt (14) again in 1903 proved experimentally that bees may act as agents in blossom infection. Gossard and Walton (12) show by experimental evidence and corroborate the evidence of Waite (28) that the bees, after visiting blossoms which have become contaminated, transmit the disease to the next blossoms visited. It is quite evident that other nectar seeking insects may transmit the disease in a like manner.

Gossard and Walton (12) also show that the organism may live in the honey in the hive seventy-two hours but they believe that this approaches the limit. Güssow (15) however cites data to show that the organism lives as long as forty-five days when inoculated into sterile honey. Gossard and Walton (I2) have evidence to show that bees may carry the organism in the pollen baskets as well as on the mouth parts. Blossoms of peach, plum and cherry when inoculated with the organism, yielded the organism up to and including the fifth day. It is possible that blossoms so inoculated may serve as a source of spread to apple and pear if these trees be interplanted or in adjacent plots. They report that it was observed that initial infection in a young pear orchard in which the blossoms had always been removed each year, occurred through failure to remove the blossoms.

Blossom Inoculations by Aphids

Some investigators have reported that aphids when hatching near the rough bark of the canker edge have crawled through the exudate and so have become contaminated with the blight organism. It is possible under such conditions that these aphids may crawl into the blossom and cause primary infection in the spring.

The following experiments were carried on to determine to what extent the aphids might be of importance in this respect.

Twigs were cut on which large numbers of aphids were found. These twigs were then allowed to wilt so that the aphids would cease to feed and start to crawl off the twig. These aphids were removed by means of a camels hair brush to a moistened piece of bark from an active canker.

They were allowed to crawl on this bark for fifteen or twenty minutes so that their feet and perhaps mouth parts might become contaminated. Inoculation was then made by placing one aphid on the style of an apple blossom. The results of these inoculations are given in table 5.

TABLE 5

Inoculation of apple blossoms by contaminated ahhids

PLACE	BLOSSOMS INOCULATED	BLOSSOMS INFECTED	BLOSSOMS NOT INFECTED
College Orchard	85	7	78
Huron Farms Co. Orchard	89	21	68
Total	174	28	146

This table shows that aphids may be the cause of primary infection in the tree, but only with a certain combination of weather conditions could this occur. It is probable that relatively few aphids would become so contaminated in any season.

Twig Infection by Aphids

For several years it has been generally believed by fruit growers as well as by a number of plant pathologists and horticulturists that aphids are very important in spreading blight from tree to tree.

It did not seem at the beginning of the investigations reported in this paper that the evidence, presented by Jones (16), Merrill (18) and Burrill (4) as discussed later, was extensive enough to justify very definite conclusions regarding the role played by these insects.

The importance of this question from an economic standpoint alone would seem to justify the expenditure of considerable effort in an attempt to evaluate the importance of these insects in the spread of fire blight. During the past three years a considerable part of the time spent in fire blight work at East Lansing and Huron Farms has been devoted to different angles of the spread of blight from blossom to twig and from twig to twig. The study of the importance of aphids in this connection has necessarily been one of the major portions of this work.

With this idea in mind the following experiments regarding aphid transmission were carried out during the summer of 1927 and the spring of 1928. Both green and rosy aphids were used in these inoculations.

Green aphids

Three aphid covered, blighting twigs of a Wagener tree were observed in the Huron Farms Company orchard, on August 1, 1927. The signs of the disease had just begun to appear on these twigs even though exudation had occurred six inches back from the tip of the twig. It has been observed that under orchard conditions aphids do not remain on the diseased portions of the twig after the tissue becomes water soaked or browned. The aphids at the time of removal from the twig had ceased feeding and were crawling around on it. The aphids on healthy twigs were not crawling about.

The aphids on these twigs were transferred to three succulent healthy twigs of the same variety. No disease appeared in these twigs. On August 9, aphids were removed from blighting twigs of Winter Banana, and were placed on 15 rapidly growing, healthy shoots of the same variety. Five aphids were put onto each twig. At the same time aphids were removed from similar blighting twigs of a Rhode Island Greening tree and transferred to 35 healthy shoots of the same variety. Five aphids were placed on each shoot as in the previous experiment. The inoculated twigs remained healthy.

On July 9, 1928 an experiment was carried out at the nursery of the Horticulture department of the Michigan State College in a further attempt to determine how efficient aphids might be in transmitting the disease to twigs.

Twigs infested with green aphids were brought into the laboratory where they were allowed to wilt. After the a aphids had crawled off they were allowed to crawl through a water suspension of the organism. Fifteen aphids were then placed on each of 120 succulent Borsdorf twigs. Forty checks were used. These were inoculated on July 11. Twenty of these were inoculated by puncture and on 20, a water suspension was put onto the tip, leaves and the stem. These checks were inoculated during a rain which lasted several hours. Three weeks after the inoculations were made, two of the aphis inoculated shoots and nine inoculated by puncture became blighted, the rest remained healthy.

Rosy aphids

Similar experiments were carried out in the spring of 1928 in the greenhouse and in the nursery at the Michigan State College. The rosy aphid was used in all these experiments.

Twigs infested with rosy aphids were brought into the laboratory where they were allowed to wilt. After the aphids had crawled off, they were placed on blighting shoots of Transcendent Crab from which the leaves had been stripped. The blighting shoots were kept in water and the aphids transferred to them by means of a brush. These aphids were observed to feed on the diseased twigs. The twigs were then placed in the foliage of potted Borsdorf seedlings in the inoculation chambers in the greenhouse to make certain that they would keep dry. The aphids crawled off the blighted twigs and colonized on 14 of the Borsdorf shoots. At the end of twenty days no blight resulted on any of these twigs. Seven days is the usual time required for symptoms of blight from puncture inoculations to become evident under the conditions of these experiments.

Other aphids were allowed to crawl through a suspension of the organism in water. The suspension was made from macerated twigs of Borsdorf and Transcendent Crab. Five of these aphids were placed on each of 22 shoots of potted seedlings where they colonized and produced young. These plants were also put into inoculation chambers in order to have the foliage remain free from water which might otherwise fall on the leaves in watering the other plants in the greenhouse. These plants were free from blight at the end of twenty days.

Another similar experiment was carried on in the nursery of the Horticulture department on Borsdorf and Ben Davis seedlings. Three series of inoculations, I5, 80 and 55 twigs respectively were inoculated by placing five aphids on each twig. The aphids used had previously been allowed to crawl through a suspension of the fire blight organism. These twigs were all bagged with glassine bags to keep out rain. After three days the bags were removed and the aphids killed with Black Leaf Forty.

Two days later 450 more inoculations were made in the same manner as described above except that the inoculated twigs were not bagged and the aphids were killed about twenty-four hours after they were placed on the plants. One twig of the second series of bagged twigs was blighted on July 2.

In all about 4500 aphids were used in these experiments and about 800 twigs were inoculated. Six check shoots inoculated by puncture on June 20 became blighted at the end of ten days. It cannot be said as a result of these experiments that aphids are not a factor in the dissemination of the disease to twigs. It is however quite evident that under the conditions of the experiments, very little infection was secured. Two hundred aphids were put onto one seedling to determine if possible whether the number of aphids used might influence the results of the inoculations, but this seedling also remained healthy. The results of these inoculations seem to indicate that under certain conditions at least aphids are not a considerable factor in the dissemination of the disease to the twigs.

In as much as wind blown rain is a factor in dissemination of the causal organism as was shown by Stevens (25) Gossard and Walton (I2) and others the results obtained by Burrill are open to criticism. His experiments were carried on in the field and he reported that a twig outside of his cages became infected so it is entirely possible that infection was present in the trees in the vicinity of the inoculated shoots. He does not state that infection was not present. The infections secured might presumably have come from the outside, especially if rain had fallen, irrespective of the aphids.

Merrill's experiments were made in the open and he notes that rain fell during the period that the inoculations were made. The aphids then might have been only passive carriers of the organism and not active in the production of infection.

Experimental evidence as reported by Stewart (26), Stewart and Leonard (27), Lathrop (I7) and others leave little doubt as to the effectiveness of the other chewing and sucking insects in the dissemination of the disease from twig to twig.

Dissemination by Meteoric Water

The importance of Meteoric water as a means of dissemination of bacterial diseases of plants has been quite thoroughly worked out in the case of some diseases. While this source of dissemination may be only relatively important, it is probably true that in an average season it is the most consistent of all the factors which might bear on the dissemination of the blight organism. It has been noted by numerous investigators that periods of blight infection may be often correlated with rain fall periods.

Because of the fact that insect transmission has been stressed in the past and because of the prevalence of these reported insects in the orchard in any normal season, exact data on spread of fire blight by wind blown rain or rain drip will be almost impossible to secure. It is true however, that careful observations of conditions, in the orchards of Michigan under observation, may aid materially in the interpretation of conditions as they have been observed to exist. Such observations were made in the early summer of 1926 at Ann Arbor and in the field at the Michigan State College in the spring of 1928.

The first symptoms of blight appeared on June 14, 1926 in the pear planting of the Huron Farms Company orchard and by June 16, the symptoms had appeared in abundance in the apple trees adjacent to the pear trees. 1.03 inches of rain fell on June 14, and .09 inches fell on June 15, with .22 inches on June 21 and .07 on June 25. These rains should have been sufficient to spread the organism from the blighting blossoms to the twigs. It should be noted too that while a few rosy aphids and leaf hoppers had been observed on May 30, the orchard as a whole was almost free of these until well along into the summer when only the green aphids were present but not in sufficient numbers to justify the use of nicotine in attempting to control them.

Summer cutting of blight in the apples was begun during the last week of June and in the trees nearest the pears it was observed that twig infection often occurred beneath infected blossom clusters. A record was kept for two days of the instances in which these infected twigs were observed to occur beneath infected blossom clusters.

On the first day 4I cases of twig blight were found beneath infected blossom clusters. On the second day II cases of blossom infection alone were found and 38 cases of twig infection below blighted blossoms. Eleven blighted twigs were found during this period in trees which had no infected blossoms, these trees it was observed were adjacent to trees in which blossom blight occurred and it would not be impossible as shown by Faulwetter (II) that wind blown rain, or even spatters of rain from infected blossoms of neighboring trees, might have been the source of inoculum.

The twigs when removed were blighted back ten to twelve inches from the tip which is about the average distance for twigs, two weeks after inoculation, as compared to conditions found in experimental inoculations.

On July 8, all the blighted twigs and blossoms were cut out of two rows of Wagener and Baldwin apples. Just after the two rows were completed it began to rain and sprinkled the greater of the afternoon and well into the night. A week later on July 15, in cutting over these same two rows, 19 new twig infections and seven fruit infections were found. The latter had developed by the movement of the organisms from infected spurs out into the fruits. These spurs no doubt served as sources of inoculum from which the twig infection occurred, as rain fall had occurred on three successive days, on July 8-9 and 10 after the first cutting was done.

The following observations were made on some small Transcendent Crab trees at the Michigan State College. Nine, seven, fifteen and sixteen blighted twigs, respectively, had been removed on May 18 from four trees. On May 25, one week later these trees had five, seven, two and five infected twigs respectively. Rain had occurred during the week of May 11 to May 18. No aphids or leaf hoppers were present in these trees.

Other inoculations were made by fastening blighted twigs into the tops of some of the young apple and pear trees. These trees were then sprayed by an oscillating sprayer over night. The trees had also been sprayed before the inoculations were made; for one-half hour.

Three twigs of one pear tree so treated became blighted after 20 days. Four twigs of one apple tree and one of another of three similarly inoculated trees also became blighted.

From the results of observations during the past three years regarding twig infection, it seems that a close correlation exists between rainfall periods and the appearance of twig infection. Infected blossom clusters no doubt serve as the source for primary twig infection and then the first infected twigs serve as a source of inoculum for further twig infection during the summer. It has been observed that higher percentages of twig infection occur in trees which have severe blossom blight. It is hardly probable that insects might serve as carriers in this primary twig infection as they do not attack the less succulent tissues as are found in the blighted blossoms and blossom spurs.

Gossard and Walton (I2) show by experimental evidence that cheese cloth bagged blossom clusters of late blossoming varieties in the vicinity of infected trees showed just as large a percentage of blight as those not bagged. So from this one might presume that insects are not the only agents of dissemination but rain too may be a considerable factor in the spread of blossom infection. Rain was also proved by them to be a most important carrier after centers of infection had been established in the blossoming tree. From 50-90 percent of the blossom infection may be accomplished by rain.

Stevens (25) and others found that wind was a factor and because they do not make any definite statement regarding rain, it has been taken by some investigators to mean that the exudate drues and becomes powdery and that this powder is blown by the wind to other trees where infection occurs. It is presumed that they refer to wind blown rain.

Faulwetter (II), working on angular leaf spot of cotton, Rolfs (2I), on black spot of peach, Wolf (30) on citrus canker, Rapp (22), Sackett (23), and Edgerton (IO) on bean blight and Carsner (7) on angular leaf spot of cucumber all suggest that meteoric water may be an important factor in the spread of these bacterial diseases.

Some of the investigators mentioned above have found rain and dew to be an important factor in the dissemination and others suggest it as a possible means of spread. It seems quite probable that dissemination of diseases of this type may be accomplished by wind blown rain or dews. This is very evidently the case in the dissemination of fire blight.

Infection

The Relative Importance of Wounds and Natural Openings

The work of various investigators on infection of the apple and pear by the blight organism has raised the question, as to the relative importance of wounds made by insects, man, wind and other agencies and the natural openings in the plant tissue.

It is a well known fact that usually both types of infection occur in any blighted orchard, yet which of these may be the most important is difficult to determine.

Blossom infection occurs through the nectaries of the flowers, and examination of blossoms which have been inoculated by spraying a water suspension of the organism into them, soon show signs of the disease. It was observed that such blossoms showed water soaked spots after three to four days, and on the second or third day following these spots had become dark brown to blackish in color. On about the tenth or twelfth day a water soaked condition could be seen on the outside of the calyx cup and drops of exudate appeared several days later.

The organisms are at first located among the cells of the nectary but on multiplication spread throughout the tissue of the torus and ovary and then move downward into the pedicel through the intercellular spaces. Invasion of the fruit spur follows and many times the young fruits are invaded from the spur through the pedicel.

In the apple the invasion of the spur usually results in canker formation at its base. This is not always true as large cankers often result from spur infection of this type on the more susceptible varieties. In the pear however, if conditions are at all favorable, these spur infections usually result in invasion of the limb which spreads to the scaffold parts and causes large limb cankers and even occasionally results in invasion of the trunk.

Twig infection as reported by numerous investigators occurs near or at the tip of the twig, at least the symptoms appear first in this region. The further invasion of the organism involves the basal part of the twig and the bark of the limb in the immediate vicinity of the base of the twig. In many cases the progress of the organism is checked at this point at least temporarily and a quite definite line appears due to the formation of a large amount of cork by the host tissue. Often however, the organism is not entirely cut off from the bark tissues by this layer and further invasion follows. These periodic waves of advancement leave more or less distinct ringed markings on the bark. These concentric markings are even more marked often times in the inner than in the outer bark.

Insect wounds are often found to be the seats of infection in the fruits. Several investigators have recorded this for curculio wounds and I2 such cases were observed in the Huron Farms Company orchard in 1926. No doubt injuries caused to the fruit by worms likewise serve as seats of infection. Wounds on leaves caused by the wind whipping them against branches and fruits also may serve as sources of inoculation. Six cases were observed in the spring of I928 on small Transcendent Crab trees in which leaf injuries of this type became infected and twig blight resulted in all the cases.

Limb injury brought about while pruning or removing blight during the growing period have become infected and injuries brought by pickers to limbs and crotches have se served as a source of entrance of the organism. Wounds on the trunks from saw cuts, removal of suckers, and injuries from discs or harrows in cultivated orchards often times become infected and start trunk cankers. An orchard of pears near Sodus, Michigan, which was examined in the early summer of 1928 proved to have very nearly five percent of the 2000 trees infected in the trunk and the majority of these cases could be traced to disc injury on the trunk. Cultivation too close to the tree is also very likely to wound the roots and infection through these wounds results in the death of the tree in the majority of cases.

Ten, eighteen year old pear trees at the Huron Farms Company orchard were inoculated at the crown by wounding the tissues in this region with a spade, only two or three wounds were made on the crown of each tree. Two gallons of a water suspension of the organism was poured around each of these trees. Two of the trees became diseased through these wounds. Rain drip from infected twigs and blossom clusters above is no doubt the source of inoculum from which such wounds become infected.

Twig infection, on the whole, has not it seems been satisfactorily explained. Reports by various investigators of severe twig infections in the absence of any of the insects which have been reported to disseminate the causal organism, raise the question as to what additional factor or factors may be concerned. Some experimental evidence by Merrill (18) and Burrill (4) seems to show that aphids may be a source of spread while experiments with about 4500 green and rosy aphids on 800 shoots carried out at the Huron Farms Company orchard and at the Michigan State College orchard have failed to corroborate these results, only three twigs having become infected.

Some inoculations made in the greenhouse during the early winter of 1927 on plants which had not been wounded and on which no insects could be found produced infection. The question arose as to how these infections had occurred. The first observed symptoms in these plants was a browning of the tips and discoloration of the tip leaves of the infected shoots.

From the results of inoculations and the observations of other investigators it seemed that further investigation into the matter of these leaf infections was justified.

Further experiments were therefore carried out to determine the symptoms and seat of infection in these leaf lesions. Borsdorf seedling apple trees about three years old were used. Other varieties, Ben Davis, Winesap and Jonathan were used in later experiments with the same results as the Borsdorf.

The potted seedlings were placed in the inoculation chamber as shown in figure 3. Five or six seedlings might be inoculated in this chamber at one time. The plants were first sprayed by means of the spray attachment in the inoculation chamber to wet the leaves thoroughly both on the upper and lower surface. The organism was then sprayed onto the under leaf surface in a water suspension by means of a DeVilbiss atomizer, or small pieces of diseased tissue about one millimeter square were placed in the droplets of water which collected on the under side of the leaves.

Inoculation on the upper side of the leaves gave negative results except cases in which the immediate tip of the serration was included in the drop of water in which the inoculum was placed.

The plants were kept in the inoculation chamber for twenty to twenty-four hours, and the leaves were kept wet by occasionally turning on the spray. The attachment was turned at an angle so that only a fine mist filled the inoculation chamber without the force of the spray touching the leaves. The plants were then removed to the bench and examined several times daily for the appearance of the symptoms. The period of incubation varied from two to five days depending on greenhouse conditions. Temperature seemed to affect this more than any other factor.

The lesions when they first appear on the leaves are tiny black specks which are visible only by reflected light, within three or four hours these specks have increased several times in diameter and have become water soaked in appearance. Soon they occupy the area between adjacent veinlets making an angular spot. Figure 4 represents a leaf in which the spots have reached the water soaked stage. Shortly after, the edges of the spot become browned and the progress of the organism is checked as these veinlets are reached as shown in figure 5. It is possible that the arresting of the organism at this point is mechanical as the intercellular spaces of the tissues surrounding the veins are very small in comparison to the size of those of the mesophyll. Within several hours upon penetration of this tissue, the water soaked condition will appear along one of the veins leading to the midrib and invasion of the shoot from the leaves follows. Figure 6 shows such invasion from two infected leaves.

Invasion of the shoot has been observed to occur within 24 hours after leaf infection became visible under greenhouse conditions, and four days is the shortest time under field conditions.

Early investigators described fire blight as a disease primary of the cambium and up to the time of Nixon's (I9) publication this view was held by most plant pathologists. Nixon showed by his work that the organism under optimum conditions progressed most rapidly in the outer region of the cortex and that the invasion of the cambium and medullary rays occurred last. Miss Bachmann (2) found that occasionally the younger xylem elements were invaded also.

Examination of a number of cankers in Pippin and Thompkins King trees at the Michigan State College orchard apparently bore out the findings of Nixon. The findings of the writer bore out those of Nixon relative to the nature of the matrix in which the organism is embedded.

Aseptic cultures were made on August I5 and I9, I928, by stripping back the bark toward the canker region. Fifty cultures were made from several twigs and small branches. The material was taken from the cambium from two regions; (I) just under the most advanced point of the canker edge, (2) one inch in advance of the canker edge.

One-third of the cultures from series one and one-tenth of the cultures from series two developed bacteria which produced typical lesions when inoculated into green apples and pears.

It seems that further investigation along this line is justified if the organism may be present as far as one inch in advance of the visible canker edge in August when the cankers were not developing rapidly.

This fact may have an important bearing on the matter of over-wintering of the organism and may explain why it is possible to secure cultures an inch or so in advance of the canker edge in dormant cankers as has been shown by Brooks (6) and others.

Leaf spot may also result from stomatal infections as is shown in figure 7. These spots had not developed further, three weeks after the appearance of the symptoms.

The results of the inoculations on Borsdorf and other seedlings showed no varietal differences and so no attempt will be made to differentiate between the varieties in recording the results in the following table which gives the results of these inoculations as obtained in the greenhouse during the winters of 1927 and 1928.

TABLE 6

Leaf inoculation of apple seedlings without wounding

DATE OF INOCULATION	PLANTS INOCULATED	PLANTS INFECTED	PERCENT OF INFECTION	REMARKS
I927				
March 3	9	7	77	
March 7	5	3	60	
March I5	3	0	0	
March 20	2	2	I00	
Oct. I7	3	0	0	
Oct. 24	I	I	I00	
Oct. 25	4	2	50	
Oct. 28	4	2	50	
Oct. 29	4	2	50	
Oct. 3I	4	0	0	
Nov. I	I	I	I00	
Nov. 2	4	0	0	
Nov. 8	2	2	I00	Pure culture
Nov. I0	3	I	33	
Nov. I3	3	I	33	
Nov. I7	2	I	50	
I928				
Febr. 7	4	2	50	
Febr. 8	4	0	Samples taken for sectioning	
Febr. 9	4	2	50	
Febr. II	4	2	50	
Febr. I8	4	I	25	
March I	4	3	75	
March 7	5	5	I00	
March 8	2	2	I00	
March I0	4	4	I00	
April I8	3	2	66	
April 24	3	I	33	Aphid free
April 30	4	4	I00	"
May I	5	4	80	"
May 4	5	4	80	"
May 6	4	3	75	"
May I0	II	7	63.6	"
Total	I24	7I		

These tables show that an average of 57 percent of the plants inoculated became infected. The largest number of infections observed on a single leaf was 28 and the average number in the different series varied quite widely with different conditions in the greenhouse at the time the plants were in the inoculation chambers.

The green peach aphid was present in considerable numbers on some plants which were inoculated. However the wounds produced by these insects in feeding are very characteristic and may easily be determined by use of a hand lens or microscope. Only one case of over 200 lesions examined was observed in which infection might possibly have occurred through one of these wounds. A series of inoculations were also made on aphid free plants and are so noted in table 6.

Field inoculations on Transcendent Crab and pear were made as follows: the plants to be inoculated were first sprayed by means of an oscillating spray for varying lengths of time. The inoculum was put onto the young rapidly growing twigs in an aqueous suspension. One or two drops were used for each twig. The spray was then turned on again and allowed to run over night. These plants were free of aphids and leaf hoppers at the time the inoculations were made.

The field inoculations have given very divergent results. One set of inoculations, in which four small Transcendent Crab trees were used, gave 24 percent positive inoculations of a total of 273 inoculated twigs. A similar set of inoculations on three trees with 89 susceptible twigs gave .07 percent of positive inoculations.

Of 200 inoculations on pear twigs only five twigs became infected. Another set of inoculations of seven pear and I7 Transcendent Crab trees gave no positive results except where the organism gained entrance through an injury on the apple leaves, ten such inoculations occurred.

It is possible that the treatment before inoculation was the factor influencing this divergence in the results. The plants which gave the highest percentage of infection were sprayed for five hours before inoculation. In the experiment the following day in which less than one percent infection was secured the plants were sprayed for only one-half hour before being inoculated.

In the last experiment due to the fact that the trip mechanism of the sprayer failed to work, only three trees were sprayed very thoroughly before being inoculated.

The results, as obtained for the pear in these inoculations in the field, seem to show that less twig infection occurs in the pear than in the apple. Field observations for the three years the work was in progress also corroborated this evidence.

The evidence on insect transmission as presented by the various investigators does not seem adequate to explain twig infection under the varied conditions following which it appears. As has been pointed out twig infection frequently occurs in the absence of insects which are supposed to transmit it. It is of course entirely possible and indeed it is very probable that some of these infections take place through wounds other than insect punctures or bites on the leaves and stems.

Examination of early stages of leaf invasions have shown that infection occurs on healthy sound leaves and these infections occur through the stomata of the leaf while it is in certain definite stages of development. It seems quite likely that this type of infection occurs in a large percentage of the observed twig infections in this state.

Examination of Prepared Material

Microtome sections of the various stages of leaf infection proved that infection was without question through the stomata as was suggested by Heald (I5). Figure 8 is a photomicrograph of a cross section through a young leaf. It will be noted that the sub-stomatal chamber alone is filled with bacteria. A little later stage of infection is shown in figure 9 which is a camera lucida drawing of the surface view of a spot which has just come to occupy the area beneath two stomata. This appeared as a very small water soaked spot on the leaf.

Invasion then proceeds downward through the intercellular spaces of the mesophyll which are quite large as is common in most of the sun type of leaves. It has been noticed that invasion to the petiole frequently only includes the tissues on one side of the midrib, which is itself often not invaded.

Sections have been observed in which the advancing edge of the zoogloeal mass is wedged shaped and tapers off quite gradually. The advance is always along a vein and it is quite possible that the organism moves in this region because of the fact that more water is available. Figure IO shows the advancing edge of an invading mass of bacteria on the left side of the vein.

Burrill (5) in I880 writes of twig infection that "the leaves however are invaded from the bark, or as it seems may be the starting point of the infection. I have no direct experiment upon this point but have found infected and dying leaves on healthy stems." Experiments in which he painted the underside of the leaves of apple and pear with a suspension of the organism gave only negative results.

Arthur (I) in I885 reported that the tips of apparently healthy young pear leaves, became infected when smeared with a culture of the organism. Stedman (24) in I893 suggested that the organisms were carried by wind blown rain or perhaps by insects to the leaves through the stomata. Duggar in his text makes the following statement regarding twig infection. "Nevertheless it is also true that infection may result through growing twigs, injuries and sometimes perhaps even water pores may be the seats of infection. In general however it is certainly true that the germs upon the surface of healthy tissues would not result in the production of disease in those parts."

Heald (I5) in I9I5 reported that observations at North Yakima showed lesions on leaves of Bartlett pear which were advancing from the edges or tips. Such lesions on the following morning after a rain showed drops of bacterial exudate. Similar lesions were found a few days later in the vicinity of Spokane on apple leaves. Further collections of similar material were made at Walla Walla, Kennewick and Prosser from both pear and apple trees. He observes that the region of initial infection "in the majority of cases start at the margin and are either lateral or terminal, although central lesions have been found in some cases on apple leaves." Brooks (6) was unable to produce stomatal infection.

Migration of the Organism

A study of the migration of the organism in the leaf and stem tissue was also made in connection with the production of twig blight from infections in the leaf. The findings of Nixon (I9) were corroborated in this study. The matrix in which the organisms are embedded is jelly-like as Nixon describes it, and more dense apparently than Miss Bachmann (2) believed it to be. She concluded from her studies that invasion was accomplished by the movement of the organism in a liquid which she believed to be withdrawn from the lumina of the adjacent cells causing them to lose their turgidity.

Stained microtome sections of infected leaves showed the organism to be embedded in a substance which stained very definitely with most of the common stains such as gentian violet, acid fuchsin, eosin, iron alum haematoxylon, Delafields haematoxylon, methylene blue, and licht grun. Nixon used Fleming's triple stain and the matrix then stained a light blue with the organisms a dark red color. He refers to these masses as zoogloea and his interpretation in this respect is probably correct. Beijerinck (3) and other investigators have demonstrated that certain bacterial organisms can build up from the simpler sugars certain pectic-like compounds and from a few tests it seems that the matrix is pectic in nature. Microtome sections of diseased leaves when stained with Ruthenium Red, a specific stain for pectic-like substances, give a positive reaction in the matrix while other cellulose tissues fail to take the stain. That diseased twigs have a decidedly higher sugar content than normal twigs was shown by the increased reduction of Fehlings solution with equal amounts of macerated healthy and diseased tissues. The tests as made were not quantitative nor specifically qualitative but may indicate in a measure at least the nature of the matrix.

Normal Anatomy of the Apple Leaf

During the experiments on infection of apple seedlings it was observed that quite a close correlation existed between the age of the leaf and the degree of susceptibility to infection and it seemed that a study of the normal anatomy of the apple leaf might aid in interpretation of some of the results obtained by the inoculations.

Superficial microscopic examination and microtome sections showed that the leaf as it emerges from the bud is composed of a meristematic-like tissue in which the veinlets and veins have been differentiated. As it increases in size the palisade, mesophyll, and epidermis at the apex begin to mature. The increase in the size of the cells of the upper epidermis is accompanied by an increase in the size of the palisade cells. On the lower surface stomata are formed by the division of epidermal cells, and shortly after, the formation of the intercellular spaces of the mesophyll occurs. Hydathodes or stomata which are slightly larger as a rule than the normal stomata may be found on the upper surface of the leaf at the tips of the serrations. There may be as many as six or eight of these but usually not more than three or four. These hydathode-like structures develop very near to the end of the vascular strand which terminates in the serration tip.

Figure II shows the maturation of the tissue at the tip of a serration which is entirely comparable to the condition observed in the leaf tip proper. Stomata are shown in various stages of development, the youngest being the most distant from the tip. Note the increase of size of epidermal cells in the region of the mature stomata.

The guard cells as soon as formed increase rapidly in size and in so doing tend to bow outward forming a sub-stomatal chamber. The subsequent development of the leaf allows the guard cells to assume the normal position which is approximately level with the surrounding epidermal cells.

The maturation progresses from the tip of the leaf and the tip of the next lower serrations downward, and inward to the base of the leaf. Figure I2 shows a series of leaves taken from a young shoot. Table 7 gives the length of leaf, approximate age in days and the region in which stomata are found in this series of leaves. The determination was made microscopically.

TABLE 7

Leaf Development

NUMBER OF THE LEAF COUNTING FROM TIP DOWNWARD	LENGTH OF LEAF IN MM.	APPROXIMATE AGE IN DAYS	DISTANCE STOMATA FOUND DOWNWARD FROM TIP
I	8	I-2	I/2mm.
2	IO	3	Imm.
3	I6	4	2 I/4mm.
4	22	7	5mm.
5	35	IO	I3mm.
6	45	I4	Whole Leaf

The mature region of a young leaf may be quite accurately determined by the color. The mature region is dark green, the immature region is a greenish yellow color. The mature region shows microscopically, mature stomata and intercellular spaces in the mesophyll. The immature region is composed of small closely compacted cells which in section are cubical to slightly round.

This study of the normal anatomy of the apple leaf has shown that susceptible leaves may be quite easily determined and this may aid in practical methods of control as trees in which no new shoots or young leaves are found may be regarded as not susceptible to twig infection. The conditions which cause leaf spot may also be explained by this study of the anatomy of the leaf. If stomatal infection occurs on young leaves which have not attained their full size and in which the intercellular spaces are still small the development of the zoogloeal mass will, because of lack of sufficient water, be stopped before a vein of sufficient size is reached and the infection will never pass the leaf spot stage.

Control Measures

Control measures recommended for fire blight are sanitation, the use of resistant varieties, and the practice of proper cultural methods. Exclusion of the organism by means of sprays has not yet been proved to be of any great benefit.

Sanitation in the form of removal of diseased parts is at present the most practicable of control methods for Michigan. Removal of diseased limbs and cankers may be done either during the dormant or growing season, or as is practiced by some growers, removal of diseased parts during both the dormant and growing seasons.

The time of removal of the diseased parts must necessarily be determined by the conditions existing in the orchard. It has been recommended that under Michigan conditions removal may, usually best be accomplished when the trees are pruned during the dormant season. If few cankers are known to be present in the orchard, the removal of these cankers, not only is a small matter but also insures the grower against the possibility of a severe blossom infection the following spring as often times occurs.

In heavily fertilized orchards occasionally the spread of infection following heavy blossom blight becomes unusually severe and the removal of diseased parts during the growing season seems desirable but the expense incurred in doing this is almost prohibitive for the small grower. This practice has a more important place in pear than in apple orchards because of the more rapid invasion in the pear. The possibility of spread by the tools used in removal of the diseased parts is much greater in the case of the pear than the apple and this means that the operator must exercise great care in the removal of diseased parts. With the exception of very late falls during which much precipitation occurs, late infections in the apple in this state are not of much importance.

The use of resistance stocks, which at present is still of questionable value due to the scarcity of material of the varieties recommended, may be of considerable value in that the trunk and scaffold parts of the trees will be saved in case of infection. It is true that even though this part is saved, severe infection will mean the loss of the greater part of the top in many cases. The necessary top working will reduce the productiveness of the tree for a period of years.

The increased cost of the trees when they are introduced commercially will probably be a factor which will discourage many growers from planting these resistant varieties. Other such cases have been recorded and there is no reason to believe that this may be an exception. Reimer's work does show that some of these varieties hold considerable promise for use as stocks for root and top working. At present no very resistant apple varieties are known which might be used for the scaffold parts.

The present practice of some growers of using susceptible varieties of apples as fillers in the orchard is of questionable value. This is particularly true of the Crab apples and the Yellow Transparent and Tolman Sweet.

The Wealthy and Duchess are very susceptible to fire blight once infection occurs. The value of the Wealthy as an early apple in this state however, overbalances to some extent its undesirability due to its blight susceptibility while on the other hand the Duchess even though susceptible usually blossoms so early that only in exceptional cases does it become severely infected in the state of Michigan.

From observations of orchards in the past three years the advisibility of planting Wagener trees as fillers is questioned, as the tree is very susceptible to infection. Crown and root infections were observed to have been very abundant in the Huron Farms Company orchard on this variety and the removal of many trees was due to blight.

It seems advisable to separate pear and apple plantings as widely as possible so that infection in either one may not so easily be spread from one to the other.

Cultural methods may go far to aid in control of the disease. Most Michigan orchards unless fertilized or heavily cultivated do not make much late growth and so are not subject to late twig infection. Twig cankers from these late infections in the apple it seems are one source of overwintering of the organism. Due to the differences in rate of invasion in apple and pear, the apple rarely becomes infected in the scaffold and trunk from blossom or twig infection unless through spurs or shoots on these parts, while infection at the tips of branches or branch spurs of pear often results in invasion of the larger limbs and trunk.

Late growth if allowed to develop will serve as a source for further infection and so successive waves of twig blight will carry active canker formation over into the late summer and fall. Quick early growth with terminal bud formation of the shoot should aid materially in checking the spread of the disease. The production of a smaller amount of growth which is subject to the rapid invasion of the organism would it seems give fewer cankers and less over-wintering.

Discussion

A considerable amount of work remains to be done on various phases of the fire blight problem. As has been pointed out the matter of the over-wintering of the organism has not been investigated thoroughly enough. If the organism over-winters in small twigs in a fairly large percentage of the cases in any given year it will mean that much more careful work must be done by the average operator in removing blighted twigs and cankers from the orchard during the dormant season. Old, apparently dead cankers must not be left in the tree as they too, as has been shown, may serve to aid in over-wintering.

The evidence at hand seems to show that bees and other nectar seeking insects cause more damage through the spread of blossom infection from tree to tree than do the other insects which are reported to be of importance.

Meteoric water is no doubt much more effective, in spreading the organisms to both blossoms and twigs from the primary infections, than any of the insects. The nectaries of the flowers and the stomata of the young developing leaves are in a very receptive condition during rainy periods. As was shown by Gossard and Walton (I2), the blossoms are not liable to infection 72 hours after pollination. It is likewise true that young leaves reach a stage of development at which time infections are not produced through the stomata. The leaves however are in a susceptible condition over a longer period, as a rule, during a normal season than are the blossoms. The production of new growth over a long period as is the case in excessively rainy springs and summers, keeps the twigs in a continued susceptible condition.

Stomatal infection as determined, may aid in the interpretation of many cases of twig infection for which a logical explanation has heretofore been lacking.

Field inoculations have corroborated the evidence obtained in the greenhouse concerning stomatal infections.

Twig infection it seems results from stomatal infection of the leaf in this state. This is perhaps due to the slow growing conditions which are brought about by the fairly low temperatures during the early part of the growing season. These tend to hold back the leaf development and so allow for the penetration of the zoogloeal mass into the petiole and shoot.

The relationship which exists between the age of leaf and ease of infection enables an observer to determine whether or not the growth produced by any given tree may be susceptible to infection.

Summary

I. Fire blight is one of the most important diseases of apple, pear and quince in Michigan.

2. Over-wintering of the causal organism usually occurs in the marginal region of the cankers. However in

two cases the organism has been found to remain alive from fall to the following spring in dead tissue as far as twelve inches back of the canker edge. The percentage of cankers in which this may occur has not been determined.

3. Dissemination of the causal organism may be brought about by a number of different species of insects. Bees no doubt are a factor in establishing sources of infection in the blossoming tree. Rain is no doubt an important factor in spreading the disease to other blossoms after these primary sources have been established. Attempts to duplicate twig infection by aphids as reported by other investigators have given negative results on all but three of 800 twigs. About 4500 aphids were used in these inoculations.

4. Periods of twig infection may be correlated rather closely with rainfall periods.

5. Infection of healthy twigs does occur. Examination of stained microtome sections of infected leaves shows that primary infection in the leaf occurs through the stomata. Experiments in the greenhouse and in the field have given 75 to 25 percent of infection respectively under optimum conditions.

6. Wounds of leaves, fruit, limbs and trunk if fresh no doubt serve as a considerable source of infection during rainfall periods.

7. Stomatal infection usually results in twig blight if the infection occurs in very young leaves of

growing twigs. Leaf spot results if the infected leaf is nearly mature at the time of inoculation.

8. The movement of the organism in the tissue seems to be one of mass action, the organism being confined to the jelly-like matrix in which they are embedded. In no case in any of the preparations examined were bacteria observed to be in advance of this matrix.

9. The most satisfactory control measures for fire blight consists of, (I) sanitary measures in the elimination of blighted parts, (2) the use of cultural practices which will induce a moderate twig growth, and (3) the use of resistant varieties as stocks.

Acknowledgements

The writer wishes to express his thanks to Dr. C.W. Bennett for valuable suggestions given during the progress of the work and also to Dr. E.A. Bessey and Dr. G.H. Coons for criticism of the manuscript; to F.C. Bradford and H.A. Cardinell for materials used and suggestions; to H.A. Cardinell and W.E. Underdown through whose efforts arrangements were made with the Huron Farms Company which enabled the writer to carry on the investigations during two summers that the work was in progress.

LITERATURE CITED

(I) Arthur, J.C.: Diseases of the pear. Pear blight. N.Y. (Geneva) Agr. Exp. Sta. Rept. 3:357-367. I885. Pear blight. N.Y. (Geneva) Agr. Exp. Sta. Rept. 5: 275-289. I887.

(2) Bachmann, Freda M.: The migration of Bacillus amylovorus in the tissues of the host. Phytopath. 3: 3-I7. I9I3.

(3) Beijerinck, M.W.: Verzamelde geschriften. Vijfde Deel: 89-II0. I922

(4) Burrill, A.C.: Insect control important in checking fire blight. Phytopath. 5: 343-347. I9I5.

(5) Burrill, T.J.: Pear blight. Trans. Ill. State Hort. Soc. II: II4-II6. I878. Fire blight. Trans. Ill. State Hort. Soc. I2: 77-8I. I879. Anthrax of fruit trees. Proc. Amer. Assoc. Adv. Sci. 29: 583-597. I88I.

(6) Brooks, A.N.: Studies of the epidemiology and control of fire blight of apple. Phytopath. I6: 665-696. I926.

(7) Carsner, Eubanks.: Angular leaf spot of cucumber: dissemination, overwintering, and control. Jour. Agric. Res. I5: 20I-220. I9I8.

(8) Crandall, C.S.: Fire blight and other plant diseases. Colo. Agr. Exp. Sta. Bul. 4I: I-I4. I898.

(9) Day, L.H.: Experiments in the control of cankers of pear blight. Phytopath. I4: 478-480. I924.

(10) Edgerton, C.W.: The rots of the cotton ball. La. Agr. Exp. Sta. Bul. 137: 1-13. 1912. Citrus canker. La. Agr. Exp. Sta. Bul. 150: 1-10. 1914.

(11) Faulwetter, R.C.: Wind blown rain a factor in disease dissemination. Jour. Agric. Res. 10: 639-648. 1917.

(12) Gossard, H.A. and Walton, R.C.: Dissemination of fire blight. Ohio Agr. Exp. Sta. Bul. 357: 81-126. 1922.

(13) Güssow, H.T.: Report of the Dominion botanist. Ont. Agr. College Rept. 1922.

(14) Hutt, W.N.: Pear blight. Utah Exp.Sta. Bul. 85: 45-52. 1903.

(15) Heald, F.D.: Preliminary note on leaf invasions by Bacillus amylovorus. Wash. Agr. Exp. Sta. Bul. 125: 1-7. 1915.

(16) Jones, D.H.: Bacterial blight of apple, pear and quince trees. Ont. Agr. College Bul. 176: 1-63. 1909.

(17) Lathrop, F.H.: Leaf hoppers injurious to apple trees. N.Y. (general) Agr. Exp. Sta. Bul. 451: 183-200. 1918.

(18) Merrill, J.H.: Notes on apparent relations between aphids and fire blight. Jour. Econ. Entomol. 10: 45-47. 1917.

(19) Nixon, E.L.: The migration of Bacillus amylovorus in apple tissue and its effect on the host cells. Penn. State College Bul. 212: 1-16. 1927.

(20) O'Gara, P.J.: Organization and methods of control of plant diseases with special reference to pear blight. Proc. Wash. State Hort. Soc. 9: I20-I44. I9I3.

(2I) Rolfs, F.M. A bacterial disease of stone fruits. N.Y. (Cornell) Agr. Exp. Sta. Memoir 8: 38I-435. I9I5.

(22) Rapp, C.W.: Bacterial blight of beans. Okla. Agr. Exp. Sta. Bul. I3I: I-39. I920.

(23) Sackett, W.G.: Holdover blight in the pear. Colo. Agr. Exp. Sta. Bul. I77: 2-8. I9II. Some bacterial diseases of plants prevalent in Michigan. Mich. Exp. Sta. Bul. 230: 205-2I2. I905.

(24) Stedman, J.M.: Fruit tree blight in general. Ala. Agr. Exp. Sta. Bul. 50: 6-I0. I893.

(25) Stevens, F.L., Ruth, W.A. and Spooner, C.S.: Pear blight wind borne. Science N.S. 48: 449-450. I9I8.

(26) Stewart, V.B.: The fire blight disease in nursery stock. Cornell Univ. Agr. Exp. Sta. Bul. 329: 3I4-3I7. I9I3.

(27) Stewart, V.B. and Leonard, M.D.: The role of sucking insects in the spread of fire blight bacteria. Phytopath. 5: II7-I23. I9I5. Further studies in the role of insects in the dissemination of fire blight. Phytopath. 6: I52-I58. I9I6.

(28) Waite, M.B.: Cause and prevention of pear blight. U.S. Dept. Agr. Yearbook I895: 295-300. I896. Pear blight and its treatment. Proc. Eastern N.Y. Hort.

Soc. I897: 779-790. I898.

(29) Whipple, O.B.: Western slope fruit investigations. Pear blight. Colo. Agr. Exp. Sta. Bul. II8: 7-9. I907.

(30) Wolf, F.A.: Citrus canker. Jour. Agric. Res. 6: 69-99. I916.

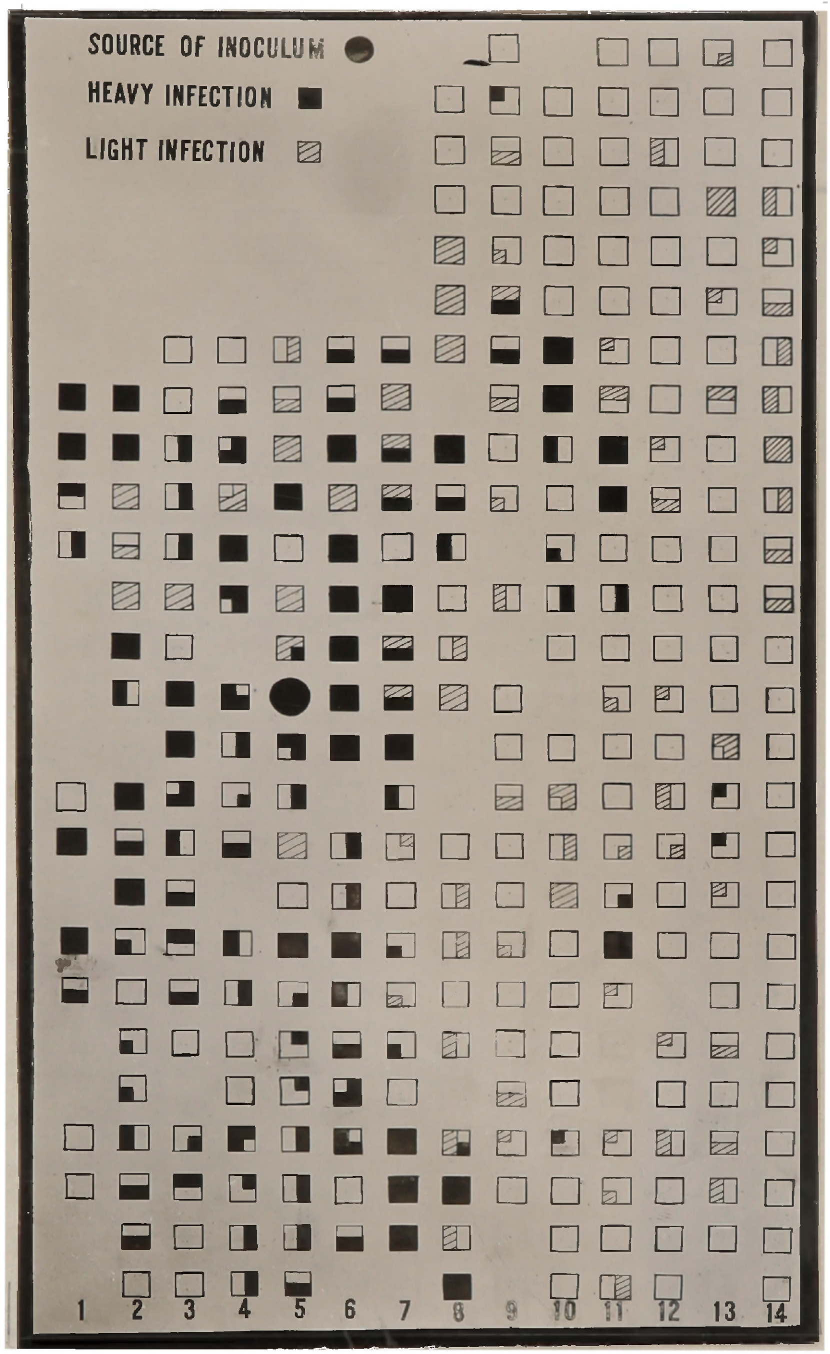

Figure I. Chart of part of the pear orchard of the Huron Farms Company at Ann Arbor Michigan. This chart shows the source of inoculum for the other infections in the orchard during the season of 1926. It also shows the approximate severity of infection in the trees in this block.

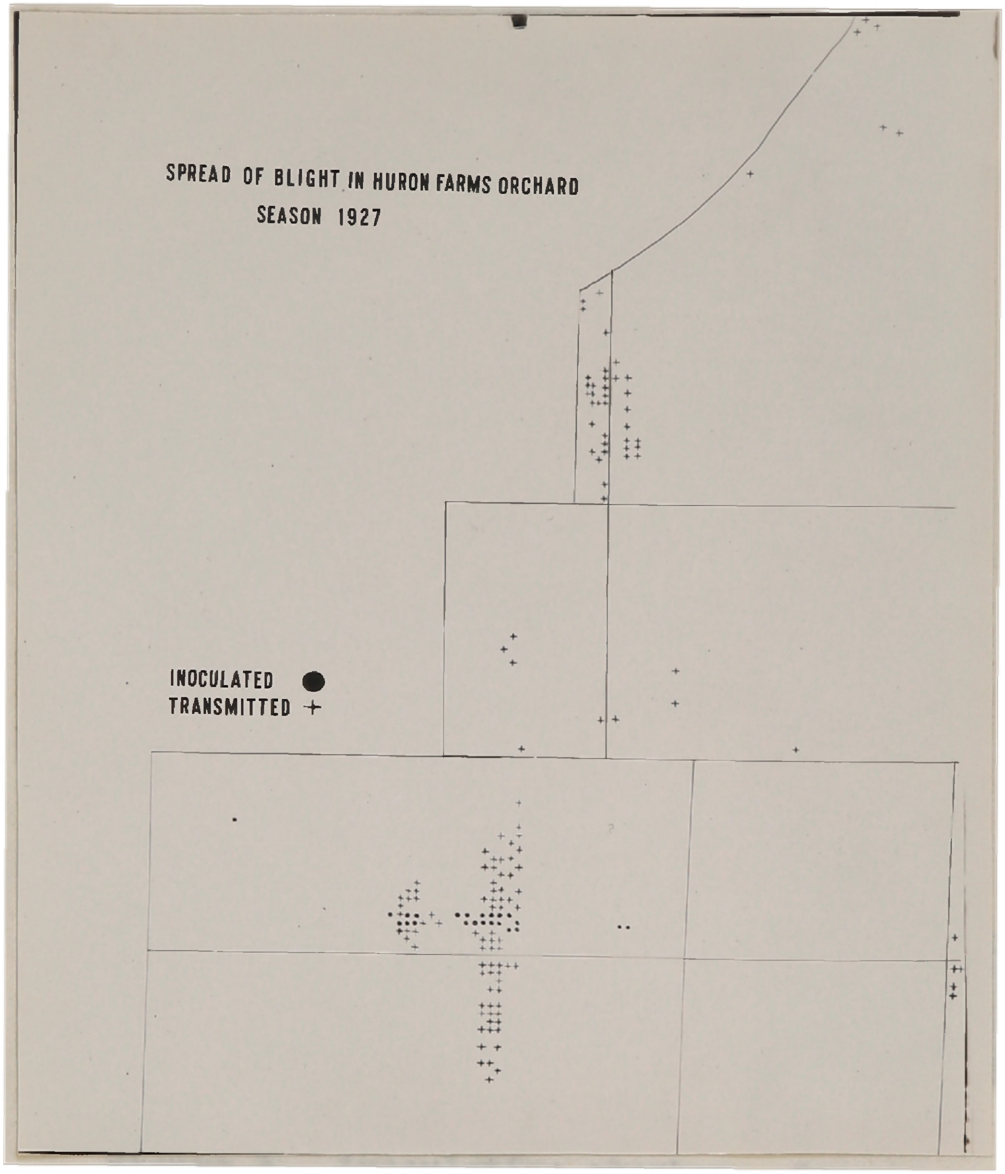

Figure 2. Chart of the entire orchard of the Huron Farms Company at Ann Arbor Michigan. This chart shows the spread of blight in 1927, from the trees in which blossom clusters were inoculated.

Figure 3. Inoculation chamber. Greenhouse inoculations were made in this type of inoculation chamber.

Figure 4. Early stage of infection produced by stomatal infection. These spots were water soaked but not discolored.

Figure 5. Later stage of infection than shown in figure 4. Note the dark margins of the spots. This condition follows that shown in figure 4.

Figure 6. Twig blight produced by leaf infections. The third and fourth leaves were the seats of the invasion which progressed into the twig. Note the discoloration along the veins.

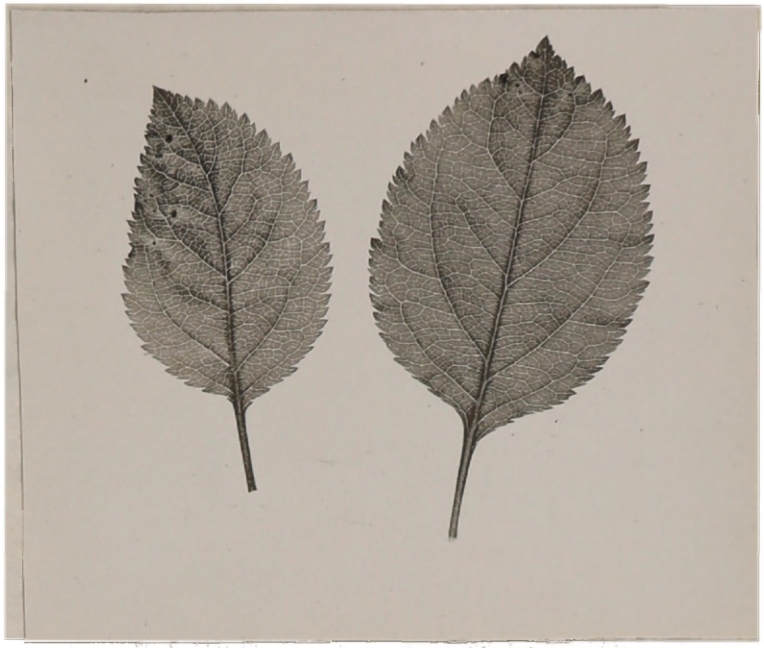

Figure 7. Leaf spot produced by the fire blight organism. These spots had developed no more, three weeks after inoculation.

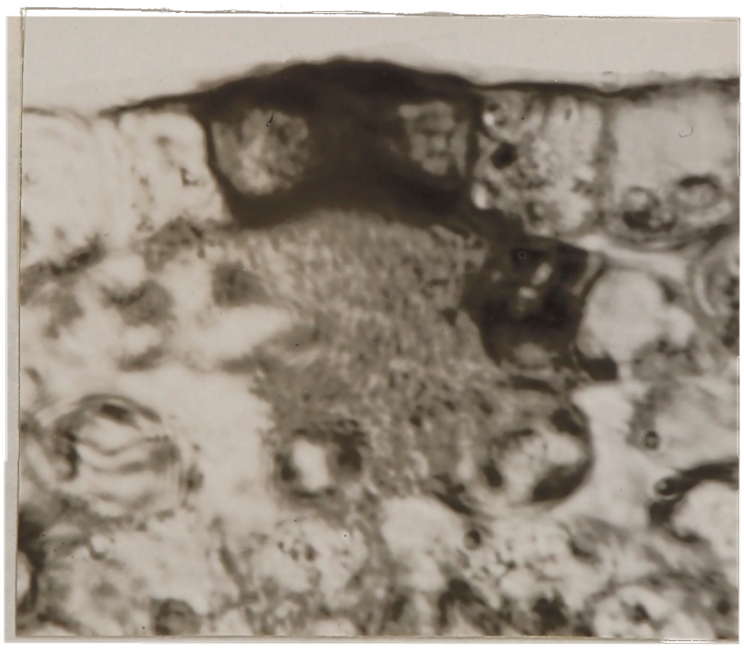

Figure 8. Stained microtome sections of an infection which had just come to occupy the stomatal chamber. Note the small size of the intercellular spaces in this region and the globular shape of the cells in the mesophyll.

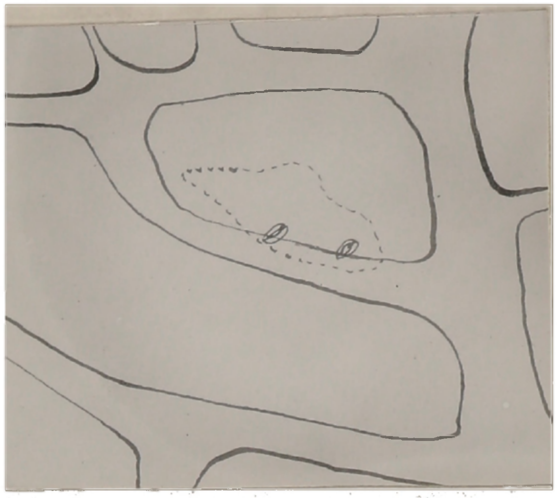

Figure 9. A camera lucida sketch of an early stage of infection in which only the region beneath two stomata had been invaded.

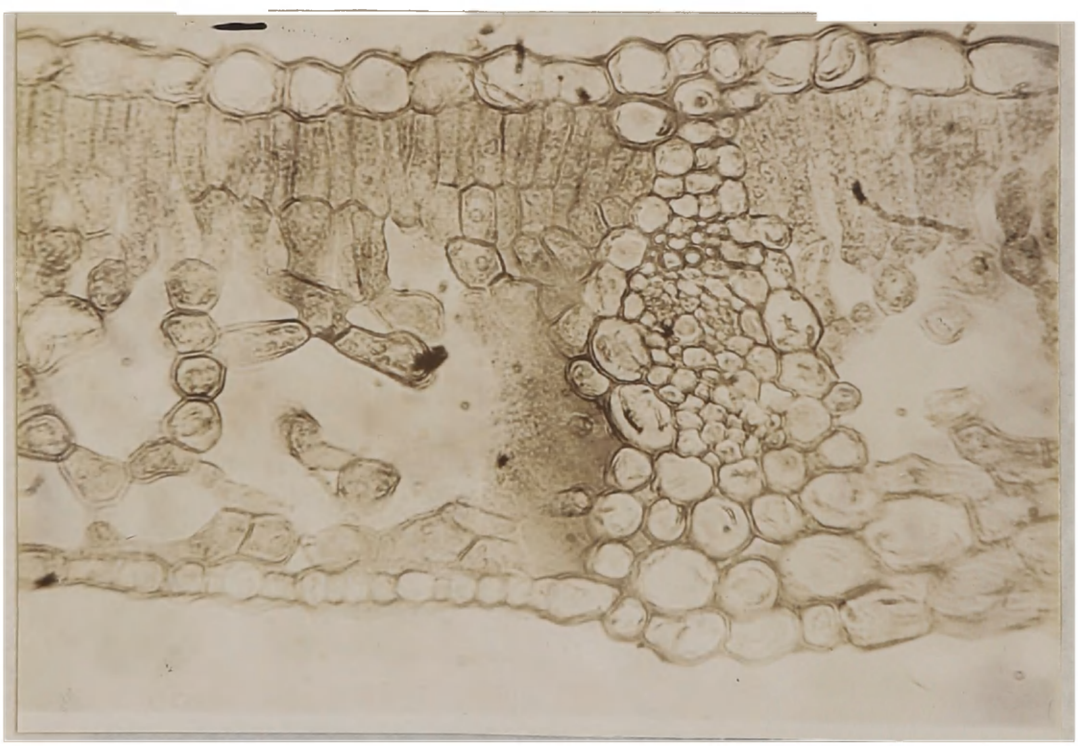

Figure IO. Invasion of the leaf by the zoogloeal mass. A portion of the wedge shaped invading edge is seen along the left side of the bundle. This is one of the large lateral veins.

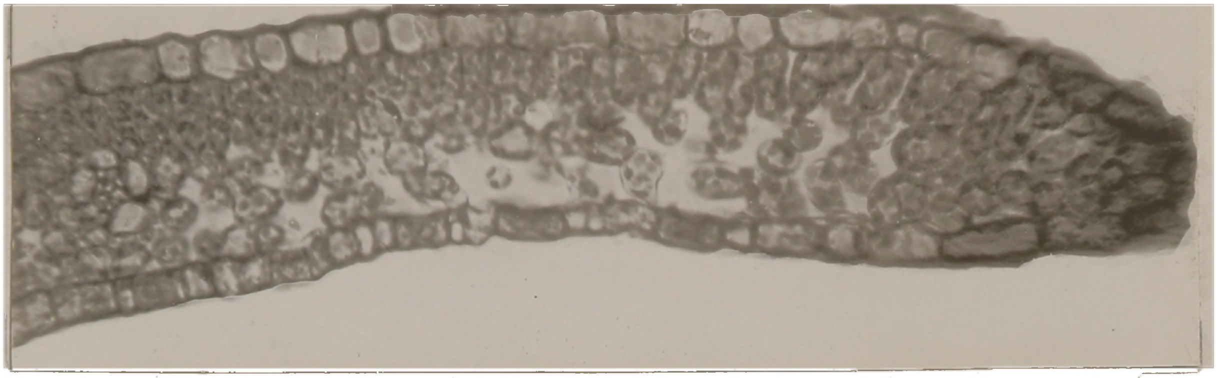

Figure II. A section of the tip region of one of the serrations a short distance back from the tip of a healthy apple leaf. The stomata, five of them, are seen along the lower surface of the leaf. The three at the left are immature. Note the small intercellular spaces above them as compared to those above the two stomata to the right. The cells of the lower epidermis are smaller, with the exception of the stomata, than those of the upper epidermis.

Figure I2. A series of leaves from the tip of a young Borsdorf shoot. The relative conditions of maturity of these leaves are shown in table 7.