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The Overwintering of the Fireblight Bacteria Outside of Living Tissue in Utah

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THE OVERWINTERING OF THE FIREBLIGHT BACTERIA OUTSIDE OF LIVING TISSUE IN UTAH

by

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Sherman V. Thomson

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The Overwintering of the Fireblight

Bacteria Outside of Living

Tissue in Utah

by

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The purpose of this investigation was to determine the sites of overwintering of Erwinia amylovora (Burrill) Winslow et al. outside of living tissue in Utah. Bacterial isolates were taken from the surfaces of blighted twigs and mummified fruits remaining on the tree; also from internal dead tissue of blighted twigs and mummies, and from soil beneath blighted trees. Each isolate was tested for agglutination with antiserum specific for Erwina amylovora, susceptibility to Erwinia amylovora bacteriophage, production of symptoms in pear seedlings and development of bacterial ooze on green pears.

Both virulent and avirulent isolates were taken from all sites tested. There was also a slight increase in the number of Erwinia amylovora isolates taken as spring approached. There was no apparent difference in apple or pear trees as overwintering hosts.

(42 pages)

INTRODUCTION

During the 1967 growing season, Utah had a heavy outbreak of fireblight in apple and pear orchards. Extensive damage was done to the pear crop, and future production was reduced due to the loss of trees.

A low incidence of fireblight in previous years and the extremely rapid increase of the disease to epidemic proportions, suggests sources of inoculum present other than that from holdover cankers. These other sources could be: soil, fruit mummies, surfaces of stems and fruits, or other sources.

Several authors have studied the overwintering potential of Erwinia amylovora (Burrill) Winslow et al.; however, no work has been reported on the overwintering of E. amylovora in Utah. In other localities, E. amylovora has been found to overwinter on the margin of dormant cankers (Hildebrand, 1936; Tullis, 1929) just beneath the surface of blighted twigs (Tullis, 1929; Brooks, 1926) in mummified fruit (Goodman, 1954; Anderson, 1952), and in beehives (Rosen, 1930). Not all of the above mentioned places of overwintering have been consistent in the localities studied.

Early investigators (Ark, 1932; Rosen, 1929, 1936; Thomas, 1930) used the development of symptoms in young pear shoots or green pear fruit as a criterion of survival. These methods identify only virulent strains of the bacteria. Baldwin and Goodman (1962) have shown there are numerous strains of the fireblight pathogen that vary from virulent to avirulent. Furthermore, Goodman, Shaffer, and Baldwin (1962) have been able to demonstrate a reversion of E. amylovora from avirulent to virulent in the

laboratory by using an aphid extract as a growth medium. Therefore, the early investigators might have overlooked a possible source of inoculum by not testing for the avirulent strains of the pathogen.

The purpose of the following investigation is to determine possible sites of overwintering of E. amylovora outside of living tissue in Utah. The sites to be investigated will be the surface of mummies and stems, inside dead host tissue, such as stems or mummies, and in the soil. Methods of identifying both virulent and avirulent strains of the bacteria will be used.

REVIEW OF LITERATURE

Hildebrand (1936), Brooks (1926), Tullis (1929), Parker (1936), and most other investigators agree that virulent bacteria can overwinter in the margin of dormant cankers. In the spring, under environmental conditions of high humidity and high temperature, these cankers begin to ooze a sticky mass of bacteria and are considered one source of primary inoculum (Parker, 1936). However, Rosen (1929) stated that the bacteria are able to overwinter in the margins of cankers, but that large limbs and body cankers, are not major sources of primary inoculum in the spring. The bacterial oozing from overwintering cankers is not a common occurrence in most apple and pear orchards. Rosen (1929) and Brooks (1926) have been able to isolate virulent bacteria from small twigs that were killed the previous year. The size of the twig does not limit overwintering potential according to Brooks (1926). He found that the organism overwintered in 0.6 per cent to 2.5 per cent of the blighted twigs.

Baldwin and Goodman (1963) isolated E. amylovora from 40 per cent of the healthy buds taken from fireblighted trees in January. Tullis (1929) was able to isolate the pathogen within 12 inches of the canker edge on large limbs.

Rosen (1930) claimed to have successfully isolated the fireblight organism from beehive material and bees throughout the summer, winter, and early spring. His work was done in Arkansas where winter conditions are mild. Pierstorff and Lamb (1934), however, reported that they were unable to demonstrate the presence of E. amylovora on combs, frames or honey taken from hives 24 hours after inoculation. They concluded that

The beehive is not a probable source of inoculum in the spring under Ohio conditions. Hildebrand (1936), after four seasons of studies in New York, concluded that the pathogen is incapable of overwintering in the beehive or in association with the honey bee. The above conflict might be resolved by noting the different geographical areas of investigation, and consequently the different climatic conditions.

Anderson (1952) suggested pear mummies as a possible source of primary inoculum for spring infections, in the absence of holdover cankers. Goodman (1959) followed this suggestion and isolated virulent cultures of E. amylovora from mummified apple tissue in February.

Pierstorff (1931) indicated that all attempts to isolate the fireblight bacteria from the soil were negative. Ark (1932) used a new method of isolating gram negative bacteria from the soil using Patel's oxgall medium to inhibit the growth of the gram positive bacteria. He had two phases of experimentation, reisolating the organism from inoculated soils and isolating the organism from naturally infested soil. He found that the maximum length of time that viable bacteria could be isolated from inoculated soil was 54 days. The other phase of his investigation was to periodically take soil samples from infected orchards and test them for the presence of virulent E. amylovora. The latest date in the year that he found soil with infective bacteria was November 10. He concluded that the fireblight organism could remain viable at least several weeks in soils.

Certain environmental conditions are conducive to extended periods of bacterial quiesence. Rosen (1936) collected hardened bacterial exudates and stored them in the laboratory. Periodically he tested them for infectivity of Bartlett pear shoots. When air dried exudates from blighted twigs were exposed to laboratory air at Fayetteville, Arkansas, the bacteria were found to be dead at the end of 1 year. When similar exudates

were kept under the same conditions, but with the humidity approaching 0 per cent, the bacteria were still infectious 1 year after they had been confined (Rosen, 1936).

Rosen (1938) followed up his previous experiments mentioned above with more exacting controls. He found that the bacteria were infective after 1 year at low temperatures (16 C) and with a relative humidity of 45 per cent. The bacteria were also alive longer than 1 year at temperatures up to 40 C with a lower humidity (35 per cent). At the conclusion of his investigation, bacteria kept at 0 per cent humidity and room temperature, were still infective after 610 days. He concluded that low temperature and low humidity are conducive to long life. A combination of low humidity and low temperature, which are prevalent in Northern Utah, would greatly increase the longevity of the fireblight bacteria.

Almost all of the early investigators who worked with the overwintering of the fireblight organism, including Ark (1932), Brooks (1926), Hildebrand (1936), Parker (1936), Pierstorff (1931), Rosen (1929), Thomas (1930), and Tullis (1929), used the development of symptoms in pear seedlings or green pears, as a criterion of survival of the pathogen. This method only identified virulent strains of the micro-organism.

Ark (1937), Baldwin and Goodman (1962) and Goodman et al. (1962) have shown that there are strains of the fireblight pathogen that vary from virulent to avirulent strains. This added knowledge would indicate that the early investigators might have overlooked the avirulent strains of E. amylovora in their overwintering studies due to identifying only the virulent bacteria with the production of symptoms. Baldwin and Goodman (1963), found that of 1,324 cultures isolated from overwintering apple buds, 523 (40 per cent) were classified as E. amylovora by phage typing. The interesting point is that all of these isolates were

avirulent. The presence of avirulent isolates only, from apple buds, was of interest to Baldwin and Goodman (1963), who suggested:

> . . . infected buds may carry the pathogen over winter in an avirulent form. The avirulent form may persist in infected buds by virtue of their superior survival capacity during periods of food, temperature, and moisture stress. Reversion of the pathogen to the white-smooth virulent type may reflect the return of those factors which favor its development, e.g., temperatures of 70-85 F, high intercellular relative humidity, and perhaps a more conducive concentration of sugars.

Goodman, Shaffer and Baldwin (1962) were able to produce this reversion process in the laboratory by taking avirulent isolates of E. amylovora from healthy apple buds and exposing them to aphid extracts for 72 hours. If these bud isolates were mixtures of predominantly avirulent bacteria, then the aphid extract may have simply acted as a selective medium which favored growth of the virulent form. This area of question is still under investigation by Goodman and others.

Elrod (1941) and Elrod and Starin (1941) isolated five strains of E. amylovora which were morphologically and physiologically distinguishable, and on the basis of agglutination tests and agglutin-absorption experiments, no antigenic difference among any of the isolates could be detected. They concluded that serologically, E. amylovora, is an exceedingly homologous species.

MATERIALS AND METHODS

Materials

Fireblighted Bartlett and Anjou pear, Wealthy and Jonathan apple twig samples, mummified fruit remaining on the tree, and soil samples from beneath tress infected with fireblight were taken periodically throughout the months of February, 1968 to July, 1968. Most of the samples of twigs ranged from one-fourth inch to 1 inch in diameter, with fireblight symptoms exhibited somewhere on them. All showed a definite margin between living and dead tissue (Figure 1). The mummies collected were those infected late in their growth and therefore were usually quite large (Figure 2). Collections were taken from three orchards in different locations in Cache County, Utah.

Media

The composition of the media used in this investigation was as follows:

Medium A -	Bacto-Beef extract	0.3 per cent
	Bacto-Peptone	0.5 per cent
	Bacto-Agar	1.5 per cent
	Yeast extract	0.5 per cent
	Dextrose	1.0 per cent
Medium B -	Bacto-Beef extract	0.3 per cent
	Bacto-Peptone	0.5 per cent
	Yeast extract	0.5 per cent
	Dextrose	1.0 per cent

Figure 1. Section of fireblighted pear stem showing definite margin between living tissue on the left and dead tissue on the right.

Figure 2. Stem with attached pear fruits which have been mummified by fireblight.

Isolation

A pear or apple twig was surface washed directly over the canker margin with medium B. The washing suspension was put in a sterile tube and incubated for 24 hours at 28 C.

A pear or apple mummy was put into a sterile bottle of 20 ml of medium B and shaken for 5 minutes on a Burrill wrist-action shaker. The mummy was removed and the bottle of medium B was incubated for 24 hours at 28 C. A loopful (3mm diameter) of each washing broth suspension was streaked on an agar plate of medium A and incubated for 24 hours at 24 C.

The blighted twigs were surface washed with a 10 per cent Clorox solution and the bark on the canker margin was removed with a sterile scalpel. Another sterile scalpel was used to obtain a section of tissue from the exposed margin area. This tissue was placed in 10 ml of medium B and incubated for 24 hours at 28 C. The same procedure mentioned above was used to obtain a distal section of dead tissue 3 inches away from the canker margin on the dead side. A loopful of each incubated broth suspension was streaked on a plate of medium A and incubated for 24 hours at 28 C.

Mummified fruits remaining on fireblighted twigs (Figure 2) were collected periodically from February through June. The fruits were surface washed with a 10 per cent Clorox solution, placed in 100 ml of sterile distilled water, and chopped finely with a Waring blender which had previously been autoclaved. Several inoculating loopfuls were transferred to 10 ml tubes of medium B and incubated at 28 C for 24 hours.

Soil samples from under and near blighted trees were taken during the month of March 1968. A small amount (0.05g) was placed into 10 ml of medium B and incubated for 24 hours at 28 C. A loopful of each suspension

was streaked on a plate of medium A and incubated for 24 hours at 28 C.

Representative colonies from each of the above streak plates were then transferred to 10 ml of medium A and incubated for 24 hours at 28 C, and after incubation stored at 2 C.

Testing

The antiserum specific for E. amylovora was produced in rabbits. The tree strains of bacteria (33, 36, 39) were heat killed in a water bath at 80 C for 30 minutes and the titer was adjusted.[1] The antiserum was produced according to the following injection schedule:

Day no.	Method	Amount (ml)
1	intraperitoneal	5.0
2		
3	intraveinous (ear)	0.25
4		
5	intraveinous (ear)	0.25
6		
7	intraveinous (ear)	0.25
14	titer checked and rabbit bled	

On the fourteenth day the titer was checked and if it was sufficiently high, 20-50 ml of blood was drawn using a cardiac puncture.

The blood was placed in the refrigerator at 5 C and allowed to form a clot. The serum was drawn off and centrifuged at 1500 rpm to extract any remaining cells. A titer check was made and the serum was diluted to the lowest titer which produced agglutination. The antiserum was put in sterile bottles and stored at -20 C. The agglutinating antigen was prepared by inoculating agar slants of medium A with each isolation and incubating for 24 hours at 28 C. A bacterial suspension was prepared by

[1]This was done by comparing the optical density to a standard McFarland Tube number 3.

spraying 0.5 ml of sterile 0.8 per cent saline over the culture with a syringe. One drop of the suspension and one drop of antiserum specific for E. amylovora were placed on microflocculation slides and rotated for 4 minutes on a TekTator rotator. A control for each sample was run using the bacterial suspension plus 0.8 per cent saline. The tests were viewed microscopically for agglutination of the bacterial cells, at a magnification of 100 times. If agglutination occurred, it was considered a positive test.

Bacteriophage isolated 33, 36, and 39 were obtained from W. H. Shaffer, Jr., University of Missouri, Columbia, Missouri. These phages were used because of their wide spectrum of lytic activity. Inoculations from each of the collected isolates were made into 10 ml tubes of medium B, and allowed to incubate for 24 hours at 28 C. (These bacterial broth suspensions will be referred to as the broth stock cultures throughout the remainder of the paper.) A 0.5 ml quantity of broth stock culture was then added to 5.0 ml of medium A, which had been maintained at 43 C in a water bath. The seeded broth-agar mixtures were thoroughly agitated and then spread over the surface of agar plates containing a 20 ml base layer of medium A which had been dried for 24 hours. The seeded plates were allowed to solidify and dry for 2-3 hours. The three phages, at a concentration of about 10^4-10^5 phage particles/ml along with a drop of medium B acting as the control, were then dropped into the specific areas of the petri plates with a syringe.

The plates were incubated at 28 C and examined for plaques 12, 18, and 24 hours later (Figure 3). Those cultures with plaques were considered to be positive.

Two different methods were used to determine if the isolates were virulent or avirulent.

Green Bartlett pears were punctured several times with a needle and placed in a plastic bag. Then 0.5 ml of the brothstock culture, with an adjusted titer of 10^4 bacteria/ml, was sprayed over the surface of the pear, after which the bag was sealed and incubated at 28 C. After 24, 48, 96, and 120 hours the pears were examined for the typical bacterial ooze produced by E. amylovora (Figure 4). Positive virulent cultures were those which produced the bacterial ooze, while the avirulent isolates produced no noticeable results.

Bartlett pear seedlings 1-2 years old, which had been actively growing in the greenhouse were used as a second method of testing virulence. Trees with light-green tender leaves were inoculated with 0.5 ml of each broth stock culture, with an adjusted titer of 10^4 bacteria/ml, using a syringe to inoculate the growing apex and to spray onto the leaf surface. The inoculated areas were then covered with a plastic bag to keep the humidity near 100 per cent. The trees were examined regularly for 3 weeks for the development of typical fireblight symptoms (Figures 5, 6).

Figure 3. Plate inoculated with unknown culture of bacteria collected from overwintering material showing typical plaques in the phage 33 and 39 areas. The plaques in phage 33 area were from a phage culture with low titer, thus the numerous small clear areas.

Figure 4. Green Bartlett pear fruits showing progressive development of bacterial ooze following inoculation with a culture collected from overwintering material.

Figure 5. Young pear seedling showing typical fireblight symptoms on the left stem after being inoculated with a culture collected from overwintering material. The right stem was inoculated with sterile broth.

Figure 6. Section of pear stem following inoculation with a culture collected from overwintering material, showing typical bacterial ooze.

RESULTS

Isolation Loci

A relatively small number of isolations was made from the surface of the mummies and stems; but this was sufficient to show that the fire-blight bacteria were present in the spring. Table 1 shows the number and percentage of isolates presumed to be E. amylovora identified by one or more methods of testing. It also shows the number and percentage of virulent cultures in the isolations. It should be noted that 20 per cent of all surface isolations were positive; and 15 per cent of all surface cultures were virulent. These surface cultures were made in May and June.

The majority of the isolations were made from the interior of dead tissue. The mummified tissue seemed to be an ideal location for over-wintering; yet the results show that only 19.6 per cent of the cultures tested were positive as compared to the average of 24.6 per cent positive for all other cultures tested.

Table 2 shows the percentage of positive isolates was 19.6 per cent and 8.9 per cent of the isolates were virulent.

The isolation from just under the bark showed the presence of positive isolates. Many of the distal isolations were made 1-3 inches away from the margin and yet positive isolates were readly isolated.

Table 3 indicates a higher percentage of positive samples in the distal isolates than in the marginal isolates.

When a comparison is made of the time when samples were taken, it is noticeable (Table 3) that positive isolates were present throughout the winter and the number actually increased as spring approached. Of nine

isolations made from the soil in March and April, four were positive for *E. amylovora* (Table 4).

Table 1. Cultures isolated from the surface of mummified fruit and from the surface of fireblighted twigs

	Number of isolations	Number positive[a]	Per cent positive	Number virulent	Per cent virulent
Mummy surface	9	1	11.1	1	11.1
Stem surface	11	3	27.3	2	18.2
Total	20	4	20.0	3	15.0

[a]Positive refers to the isolate being positive by any test.

Table 2. Cultures isolated from the interior of mummified fruit and from the interior of blighted twigs, both marginal and distal to the dead margin

	Number of isolations	Number positive[a]	Per cent positive	Number virulent	Per cent virulent
Mummy interior	56	11	19.6	5	8.9
Stem marginal	62	13	21.0	10	16.1
Stem distal	44	15	34.1	10	22.7
Total	162	39	24.1	25	15.4

[a] Positive refers to the isolate being positive by any test.

Table 3. Cultures isolated from mummies and stems during the months of February through June

	Mummy no. of isolates	Mummy no. of[a] positive	Mummy per cent positive	Stem marginal no. of isolates	Stem marginal no. of positive	Stem marginal per cent positive	Stem distal no. of isolates	Stem distal no. of positive	Stem distal per cent positive
February	18	3	16.6	7	0	0	26	1	3.8
March	23	4	17.4	25	4	16.0	28	10	35.7
April	6	2	33.3	6	1	18.3	3	1	33.3
May	3	1	33.3	3	1	33.3	3	2	66.6
June	1	1	100.0	8	5	62.5	2	0	0

[a] Positive refers to the isolate being positive by any test.

Table 4. Cultures isolated from the soil

	Number of isolates	Number of positive[a]	Per cent positive	Number virulent	Per cent virulent
Soil	9	4	44.4	3	33.3
Total of tables 1, 2, and 4	191	47	24.6	31	16.2

[a] Positive refers to the isolate being positive by any test.

Table 5. Number of trees from which E. amylovora was isolated during the months of February through June

	Number of trees	Trees from which positive isolates were taken	
		Number positive	Percent of trees positive
February	9	3	33.3
March	29	13	44.8
April	8	4	50.0
May	3	2	66.6
June	3	2	66.6

Testing

Even though E. amylovora is said to be an extremely homologous species, several isolates did not react with the E. amylovora antiserum. With the combination of serology, bacteriophage typing, and virulence tests on trees and green pear fruit, it is thought that most of the E. amylovora isolates were detected.

Table 6 shows the comparison of different isolations and the number of positive isolates, as identified by agglutination with the antiserum specific for E. amylovora. It should be noted that 14.1 per cent of the isolates were positive when tested with the antiserum.

The variability of the bacteria in susceptibility to the three phages made it possible to differentiate the strains of bacteria. It was not within the scope of this investigation to group the bacteria. The susceptibility to phage lysis is shown on Table 7, along with the source of isolation. If any one of the three phages produced a plaque, that particular culture was considered positive. The percentage of cultures found positive by phage typing was 16.2 per cent.

The two virulence tests compared favorably in the detection of the virulent strains (Table 8). The use of green pear fruit was an easier and more rapid method of differentiating the cultures. If either the green pear or the pear seedling showed the typical symptoms, that isolation was considered positive.

The virulence tests showed that 16.2 per cent of all cultures were virulent, as compared to 14.1 per cent positive with antiserum and 16.2 per cent positive with bacteriophage typing. It was found that of all E. amylovora cultures collected, 65.9 per cent were virulent when tested with a titer of 10^4 bacteria/ml.

Table 6. Cultures tested with *E. amylovora* antiserum

	Number of cultures	Number positive	Per cent positive
Mummy surface	9	1	11.1
Mummy interior	56	6	10.7
Stem surface	11	1	9.1
Stem interior			
Marginal	62	7	11.3
Distal	44	9	20.5
Soil	9	3	33.3
Total	191	27	14.1

Table 7. Testing of cultures for susceptibility to *E. amylovora* bacteriophage

	Number of isolations	Number which are phage positive			Total number positive cultures	Per cent positive cultures
		33	36	39		
Mummy surface	9	0	0	0	0	0
Mummy interior	56	6	1	6	6	10.7
Stem surface	11	3	0	3	3	27.3
Stem interior						
Marginal	62	9	7	10	11	17.7
Distal	44	8	5	9	9	20.5
Soil	9	2	1	2	2	22.2
Total	191				31	16.2

Location of Collections

It was supposed that certain trees were better hosts than others for overwintering; however, when apple and pear trees from which the collections were taken are compared to each other, there is very little difference between the Bartlett and resistant Anjou pear isolations (Table 9).

Characteristics of Isolates

Each of the cultures was tested according to agglutination, bacteriophage susceptibility and virulence. Table 10 shows the variability encountered with the different strains. However, since it is beyond the scope of this investigation, the strains were not grouped.

Table 8. Virulence of *E. amylovora* isolates taken from different sources

	Number isolates tested	Cultures causing ooze on pear fruit	Cultures causing disease symptoms on pear trees	Cultures causing symptoms on either	
				number	per cent
Mummy surface	9	1	1	1	11.1
Mummy interior	56	5	5	5	8.9
Stem surface	11	2	2	2	18.2
Stem interior					
Marginal	62	9	6	11	17.7
Distal	44	9	9	9	20.5
Soil	9	3	3	3	33.3
Total	191	29	26	31	16.2

Table 9. Comparison of tree types from which positive cultures were isolated

	Number of trees	Number of trees positive in any way	Per cent of trees positive
Apple	12	5	41.7
Pear	40	18	45.0
Bartlett	32	14	43.8
Anjou	8	4	50.0

Table 10. Reaction of cultures isolated from non-living sources, to serological, bacteriophage, and virulence tests. Only those isolates which were positive in at least one test were listed.

Culture number	Source	Date of collection	Shown to be positive by					
			Antiserum	Bacteriophage sensitivity			Inoculation of	
				Phage 33	Phage 36	Phage 39	Fruit	Tree
1B1a(3)	Stem-marginal	February 23	+	-	-	-	+	+
5A1b	Mummy-interior	February 23	+	-	-	-	+	+
6A1a(1)	Mummy-interior	February 23	+	-	-	-	+	+
6A1b(1)	Mummy-interior	February 23	+	-	-	-	+	+
10A2	Stem-distal	March 1	+	-	-	-	+	+
12B2	Stem-distal	March 1	+	+	+	+	+	+
17A	Mummy-interior	March 8	-	+	-	+	-	-
17B(1)	Stem-marginal	March 8	-	+	-	+	-	-
19A	Mummy-interior	March 8	-	+	-	+	-	-
19B(1)	Stem-marginal	March 8	+	+	+	+	+	+
19B(2)	Stem-distal	March 8	+	+	+	+	+	+
21A	Mummy-interior	March 8	-	+	-	+	-	-
24B(1)	Stem-marginal	March 16	-	-	-	-	+	-
24B(2)	Stem-distal	March 16	-	-	-	+	-	-
26B(2-2)	Stem-distal	March 22	-	+	-	-	+	+
26B(2-3)	Stem-distal	March 22	-	-	-	-	-	+
33B(2)	Stem-distal	March 29	-	+	+	+	-	-
34B(2)	Stem-distal	March 29	-	+	-	+	-	-
35B(2)	Stem-distal	March 29	-	+	-	+	-	-
35C(2)	Soil	March 29	+	-	-	-	+	+
35C(3)	Soil	March 29	+	-	-	-	+	+
36B2(1)	Stem-distal	March 29	+	+	+	+	-	-
36C	Soil	March 29	-	+	+	+	+	+
37B(1)	Stem-marginal	March 29	-	+	-	+	-	-
38B(1)	Stem-marginal	March 29	+	-	-	-	+	+
38B(2)	Stem-distal	March 29	+	+	+	+	+	+
38C	Soil	March 29	+	+	-	+	-	-
39A2	Mummy-interior	April 12	+	+	+	+	+	+
39B1	Stem-surface	April 12	-	+	-	+	+	+
41A2	Stem-marginal	April 12	-	-	-	-	+	-
42A2	Mummy-interior	April 12	+	-	-	-	-	-
43B2b	Stem-distal	April 29	+	-	-	-	+	+
44A1	Mummy-surface	April 29	-	+	-	+	+	+
45B2	Stem-marginal	April 29	-	-	-	+	-	-
47A(2)	Mummy-interior	May 12	+	+	-	+	+	+
47B(1)	Stem-surface	May 12	+	+	-	+	-	-
47B2(a)	Stem-marginal	May 12	+	+	+	+	+	+
47B2(b)	Stem-distal	May 12	+	-	-	-	+	+
48A1	Mummy-surface	May 15	+	-	-	-	+	+
48B2(b)	Stem-distal	May 15	+	-	-	-	+	+
50A2	Mummy-interior	June 11	+	-	-	-	-	-

DISCUSSION

The primary objective of this research was to determine if E. amylovora can overwinter outside of living plant tissue in Utah. The data leaves little doubt that many non-living areas are holdover inoculum sites, and potential sources for dissemination in the spring.

The soil has been overlooked as a major source of inoculum of fireblight bacteria. Ark (1932) found that the maximum length of time the bacteria could survive after being inoculated into soil was 54 days. He concluded that it could not overwinter in the soil. I found that soil was an ideal location to isolate E. amylovora in March and April. Spring rains may be accompanied with wind, which could blow minute soil particles contaminated with the bacteria to the young leaves and blossoms, possibly causing microscopic injuries and potential entrance route for the bacteria. I was able to isolate E. amylovora from fruit mummies, internal areas of dead stems, and the surfaces of twigs.

The presence of avirulent isolates could also be a means of overwintering. The conditions whereby these avirulent bacteria revert to virulent forms are not definitely known. This change may not occur naturally, but it has been induced by Goodman, Shaffer, and Baldwin (1962) in the laboratory. If it were possible for these bacteria to change from virulent to avirulent and vice-versa, this could be a reason for not experiencing fireblight during certain years. When the proper environmental conditions occur, these avirulent bacteria could change to the virulent form and thus cause fireblight.

Since the virulent strain of E. amylovora was readily isolated from many sources, it seems unnecessary to postulate this phenomenon of change.

The use of specific antiserum and bacteriophage typing made it possible to detect both virulent and avirulent forms of the isolates. The almost ubiquitous presence of the bacteria is indicated by being able to isolate E. amylovora from such diverse environments. The production of symptoms in pear seedlings and ooze in green pear fruit are reliable methods of testing virulence.

CONCLUSION

It was apparent, from this study, that E. amylovora is able to overwinter outside of living plant tissue in Utah.

All sites that were tested yielded positive isolates. The surface of stems and mummies seemed to be adequate sites for overwintering. Another good location for overwintering is within dead tissue such as fireblighted stems and mummies. The greatest percentage of E. amylovora was isolated from the soil. Further studies should reveal more information on the overwintering of E. amylovora in the soil.

Both virulent and avirulent isolates were found. The percentage of E. amylovora isolated increased as the weather warmed and spring approached.

None of the methods of testing the isolates was all inclusive; however, each method had its advantages. The antiserum and bacteriophage typing methods were capable of rapid identification of virulent and avirulent cultures. The testing of virulence with Bartlett pear seedlings and green pear fruit was reliable.

There appeared to be little difference between the collections made from apple or pear trees, thus showing that the host has relatively little bearing on the overwintering potential.

SUMMARY

The objective of this research was to determine where the fireblight bacteria could overwinter outside of living plant tissue. Isolations were made from non-living sources from February through June. It was evident that E. amylovora is capable of overwintering in both avirulent and virulent forms on the surface of tissues, inside of dead tissue and in the soil. There was a slight increase of positive isolates as spring approached and little difference was noted in apple and pear as preferred overwintering sites. The use of specific antiserum, bacteriophage typing, and pathogenicity tests as identification tools proved to be successful.

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