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Overwintering of Erwinia Amylovora Inside Living Host Tissue in Cache Valley, Utah

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OVERWINTERING OF *ERWINIA AMYLOVORA* INSIDE LIVING HOST TISSUE IN CACHE VALLEY, UTAH

by

G. Doyle Morrill

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G. Doyle Morrill
Garth Doyle Morrill

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Living Host Tissue in Cache Valley, Utah

by

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Utah State University, 1969

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Experiments were conducted to see if Erwinia amylovora (Burrill) Winslow et al. overwinters inside living host tissue in Utah. Bacteria, collected from apple, pear, mountain ash, chokecherry and Pyracantha, were used in the experiment. Isolates taken from buds and inner bark plated on nutrient yeast dextrose agar, were tested by serological and bacteriophage techniques. Those showing positive tests were then inoculated into Bartlett pear seedlings to test virulence.

Both virulent and avirulent isolates were isolated from each species of plant in the experiment. Pathogenic bacteria were isolated from diseased tissue near cankers, as well as from apparently healthy inner bark as far as six inches below the cankers. Pathogenic bacteria were also isolated from healthy appearing buds in the vicinity of cankers. Nearly 15 per cent of isolates from hosts other than apple and pear were pathogenic to Bartlett pear seedlings. A good deal of variation existed among the bacterial isolates.

Antisera developed from four isolates of Erwinia amylovora proved to be a good means of identification for the pathogen.

(47 pages)

INTRODUCTION

Fire blight, caused by Erwinia amylovora (Burrill) Winslow et al., is a serious bacterial disease of pears and apples in many fruit growing areas throughout the world. The growing of Bartlett pears in many humid areas of the United States is virtually impossible because of this disease. Fire blight is often severe in the arid areas of the western United States and was especially severe in Cache Valley, Utah,orchards in 1967.

Fire blight often develops rapidly in susceptible pear and apple varieties when temperature and moisture conditions are favorable. Bartlett and other susceptible pear trees may be killed, while infection in apple varieties is usually limited to blossoms and twigs. Infected blossoms, fruits and leaves turn brown or black and remain attached to the trees throughout the winter (Figures 1 and 2). Such trees have a scorched appearance giving rise to the name, fire blight. Cankers form in the bark of twigs and branches from which a bacterial exudate comes during wet weather (Figure 3). The bacteria are able to invade healthy tissue through wounds or natural openings such as stomates, pores, lenticels, etc.. However, they multiply rapidly and cause disease symptoms only in succulent tissues low in natural sugars. Multiplication of the bacteria takes place in the intercellular spaces and spread is by the dissolving of the middle lamella (5). Much of the disease occurs near blossom time, but green fruit and succulent growth is susceptible throughout the season.

Figure 1. Young pear severely infected with fire blight displaying typical winter symptoms. Note the dead leaves still clinging to the twigs.

Figure 2. Fire blighted branch of a mature pear tree with dead leaves and mummiefied fruits clinging to the twigs during winter.

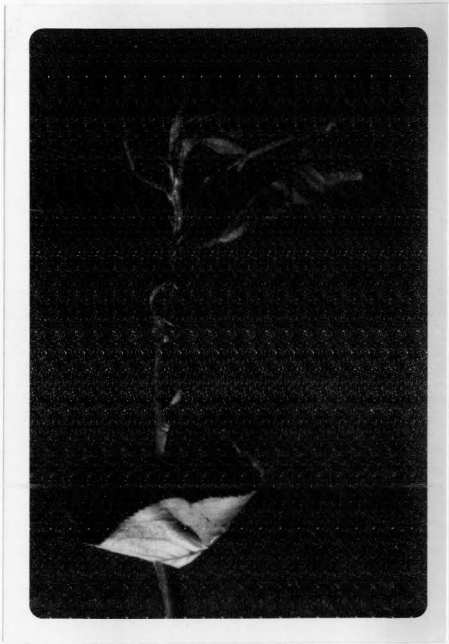

Figure 3. Twig from pear tree infected with fire blight showing dark shrunken bark and exudate.

Fire blight often flares spectacularly during warm, rainy weather in the spring and following severe hail or wind storms during the summer. The question of where the bacteria come from to cause so much disease at these times is not clearly understood. The bacteria are thought to overwinter in cankers and fruit mummies, but little information is available as to their overwintering in living tissues or other host plants. Therefore, I began this study to investigate the ability of Erwinia amylovora to overwinter in pear and apple tissues and in other hosts.

Objectives

The objectives of the study were to: (1) determine if Erwinia amylovora overwinters in living host tissue in Cache Valley, Utah, and if so, to determine sites of the overwintering bacteria on the apple and pear trees; (2) determine if Erwinia amylovora can be isolated from hosts other than apple and pear; (3) determine pathogenicity of isolates of Erwinia amylovora.

REVIEW OF LITERATURE

The disease was called fire blight by William Coxe as early as 1817 because of the scorched look it gave the trees (22). However, it was not until the late 1800's before the causative organism was identified as a bacterium by T. J. Burrill, Professor of Botany at the University of Illinois (28). The disease is of historical importance because it was the first plant disease which was shown to be caused by a bacterium. The bacterium has been known by several names:

Micrococcus amylovorus Burrill, 1882

Bacillus amylovorus (Burrill) Trevisan, 1889

Bacterium amylovorus (Burrill) Chester, 1897

Bacterium amylovorum (Burrill) Chester, 1901

Erwinia amylovora (Burrill), Winslow et al., 1920 (4).

Hosts

The pathogen has been isolated from nearly 80 species of the rose family. In addition to apple and pear, researchers have reported the disease on quince, flowering almond, hawthorn, firethorn, June berry, Flowering Quince, Spiraea (17, 18, 25, 26, 27), mountain ash, chokecherry, cotoneaster (17, 26, 27). Thomas (25) reported that few observations had been made of the disease among apricot, cherry, plum, and prune in the orchard, but that a degree of susceptibility approaching that of apple and pear had been reported on species of Pyracantha. Layne (14) found that living cowpea inoculated with *Erwinia amylovora* would produce

brownish-red slightly sunken lesions with a trace of water-soaking at the margins. Ooze never formed, but the bacterium could be isolated with ease from the discolored regions. Results of Layne's experiments showed that cultures of Erwinia amylovora could be maintained for over a two-month period on cowpea without any apparent reduction of pathogenicity to pear and apple.

Overwintering

Overwintering of the bacterium has been worked on by several authors. Thomas and Ark (26) stated that overwintering in sub-families other than the Pomoidae is unknown. Smith (22) stated that in tests made in 1919, 15 per cent of the bacteria survived after being frozen. Smith also stated that it is very unlikely that Erwinia amylovora overwinters outside of living host tissue. Rosen (20) felt that the pathogen could overwinter within the beehive, and was able to isolate the pathogen from beehive material and bees prior to development of blight in the spring. Thomas (24) was able to isolate virulent cultures of Erwinia amylovora from the surface of honey combs 35 days after inoculation. From this work he felt that it was possible, but improbable, for the bacteria to survive the winter in the beehive from the time of late blossoms in the autumn until the first blossoms in the spring. Goodman (7) isolated the organism from apple mummies still on the tree the following year and in later work (8) isolated the organism from apparently healthy apple buds. Overwintering studies by Pierstorff (18) showed that bacteria could be isolated after two years from bacterial ooze. Rosen (21) showed that when exudate was kept in an atmosphere approaching a relative

humidity of 0, the bacteria were still viable and infectious for about a year.

Dissemination, Entrance of the Pathogen, and Disease Spread

Dissemination of the bacterium has been a problem for many years. Stewart and Leonard (23) conducted experiments in which they showed that sucking insects, flies, and in fact all insects which visit host plants could spread the bacteria.

Bauske (3) in working with the dissemination of Erwinia amylovora by wind-blown water came up with some interesting findings. He stated that before 1900 observers had noted that fire blight spreads in the direction of prevailing winds. Also, workers had observed the downward passage of Erwinia amylovora to lower parts of the same tree or to interspersed branches of neighboring trees. He felt that bacteris suspended in meteoric water which fell or ran from infected blossoms, cankers, or twigs was the cause. Bauske used used simulated wind and rain and found that the fire blight pathogen could be transferred from infected to non-infected trees. It was also shown that wind barriers in the field sharply curtailed the spread of fire blight.

Entrance of the pathogen into the host plant has been worked on for many years. Lewis and Goodman (15) established that the fire blight pathogen could enter through natural openings in the upper surface of the Jonathan apple leaf and through lenticels of the stem. They also showed that foliar trichomes, hydathodes, stomates, and lenticels may serve as natural portals of infection, and wounding is not a prerequisite for infection. Bauske (3) showed that injury to pear foliage, caused by

wind, facilitated infection but that immature leaves were equally susceptible to infection whether wind damaged or not.

Rosen (19) found that the disease could be spread by spraying a suspension of the bacteria on tightly closed blossoms, which were placed in a moist chamber for 24 hours. Orton and Adams (16) in their work on collar blight showed that the disease spread from the cankers until the entire branch or trunk was girdled. Thomas and Ark (26) reported that the bacterium travels downward through the blossom spur or shoot, by way of the bark, forming an elliptical canker. This canker would then spread until the entire tree could be killed. Lewis and Goodman (15) reported that the bacterium spread through the tree via the phloem.

Pathogenicity

Pathogenicity of the organism varies from strain to strain. Ark (1) found differences in the size and form of colonies. He found two types, a rough and smooth. The rough form was found to revert to the smooth type when passed from 4 to 6 times through 2 per cent sucrose nutrient broth. He also found that the smooth form was more virulent than the rough form. Hildebrand (13) found that numerous differences occurred between various strains with the smallest bacteria being the most virulent. Farebee and Lockwood (6) found that a yellow nonpathogenic bacterium frequently was isolated with Erwinia amylovora. It was found that isolates of this bacterium inhibited growth of Erwinia amylovora on yeast extract agar due to an increase in acidity. This study was confirmed by Goodman (8). Goodman, Shaffer and Baldwin (11) were able to get the rough form to revert to the smooth form on exposure to aphid

extracts. Later, Goodman and Shaffer (10) found that growth in vitro revealed characteristic patterns for both the avirulent and virulent isolates; avirulent isolates grew more rapidly, reaching a maximum rate after 11 hours. The maximum rate of growth for the virulent isolates occurred at about 50 hours. It was found that the rough form was resistant to streptomycin at 1,000 ppm. where the smooth form was resistant at only the 6 ppm. level. Goodman (9) found that rapidly growing apple shoots inoculated with avirulent isolates of Erwinia amylovora were protected against infection from subsequent inoculation with a virulent strain of the pathogen.

MATERIALS AND METHODS

Three isolates of Erwinia amylovora (Burrill) Winslow, furnished through the courtesy of W. H. Shaffer, Jr., Department of Plant Pathology, University of Missouri, and one isolate obtained from the Midwest Culture Service served as test and control organisms. They were designated as Erwinia amylovora 33, 36, 39, and Erwinia amylovora MCS respectively.

Collections were made from a representative number of orchards in Cache Valley, Utah. Bartlett pear, Anjou pear, Wealthy apple and Jonathan apple collections were made during February, March and April. Pyracantha (Pyracantha coccinea Roem.) and chokecherry (Prunus virginiana L.) were collected during April, while mountain ash (Sorbus aucupuria L.) collections were made in February and April. Samples of blighted twigs were removed at least 12 inches below the margin of the canker (Figure 4). The twigs were placed in plastic bags, given a number, and taken to the laboratory.

Isolation of the bacteria was accomplished by three different methods. First, a small section of the twig which included a canker was removed and washed thoroughly in the vicinity of the canker with distilled water. The twig section was then placed in a solution of 10 per cent clorox and surface sterilized for 2 or 3 minutes. A flame sterilized scalpel was then used to cut through the bark in several areas below the canker. Flame sterilized forceps were used to peel back and remove a small piece of bark. The bark was placed on Nutrient Yeast Dextrose Agar (NYDA) and incubated at 30 centigrade for 50 hours. New NYDA plates

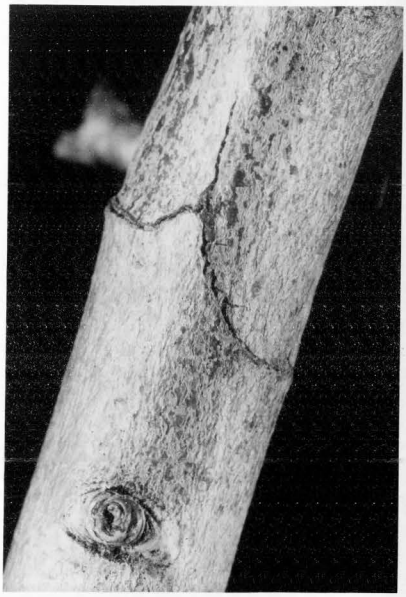

Figure 4. Canker on older pear branch with definite margin.

were then streaked with the isolated bacteria to isolate pure cultures. The pure culture isolates were then transferred to NYDA slants and stored at 10 C. for later testing.

Second, both lateral and terminal buds from living tissue were removed from the samples. The buds were surface sterilized in a 10 per cent clorox solution. The buds were ground in a Waring blender in 10 ml. of distilled water. NYDA plates were streaked with the ground buds both immediately after grinding and after a period of 50 hours. Some of the samples were streaked on selective media according to the formulae of Guthrie (12). This technique helped to remove some of the gram + bacteria from the sample. The isolates were then streaked on NYDA slants and stored as in method 1.

Third, one terminal or lateral bud was removed from near a canker of the sample. The bud was placed in a tube of Nutrient Yeast Dextrose Broth (NYDB) and incubated at 30 C. for 50 hours. NYDA plates were then streaked with the inoculated broth. Isolates were then isolated and stored in the same manner as above.

The objectives of the study were then tested by the following experiments.

Experiment 1: Identification by Use of Antisera

Antiserum from rabbits was developed from each of the four cultures of Erwinia amylovora. The bacteria were prepared for infection by suspending pure cultures of Erwinia amylovora 33, 36, 39, and MCS in physiological saline. The bacterial cells were killed by suspension in

a hot water bath at 80 C. for 30 minutes then stored in rubber-capped medicine vials at -20 C. except when being used to inject the rabbits. The rabbits were then injected according to the following schedule:

Day	Method of Injection	Amount
1	intraperitoneal	5 ml.
7	intraveinous (ear)	0.25 ml.
9	intraveinous (ear)	0.25 ml.
11	intraveinous (ear)	0.25 ml.
18	cardiac puncture	

After the injection period a titer check was run. Since the titer was high enough, twenty to fifty ml. of blood was removed by cardiac puncture on the eighteenth day. The serum was separated from the red blood cells by allowing the blood to clot. The serum was removed and any remaining red cells removed by centrifugation at 3100 X gravity for five minutes. The serum was stored in 5 ml. vials at -20 C.

Before testing the serum was diluated with physiological saline to the following levels:

Antiserum Number		
33	--	1/160
36	--	1/160
39	--	1/320
MSC	--	1/320

Tests were run on each isolate against the four antisera with agglutination considered a positive test. The results were then recorded. Controls were run for each serology test.

Experiment 2: Identification by Use of Bacteriophage

As a check to experiment one on identification the bacterial isolates were tested by bacteriophages specific for 33, 36, and 39. The technique of Baldwin and Goodman (2) was followed in this experiment. Bacteriophage was obtained from W. H. Shaffer, Jr., Department of Plant Pathology, University of Missouri. The bacteriophage was increased by inoculation of the bacteriophage into a culture of the respective bacteria and NYDB. The bacteria were allowed to multiply in the NYDB until the broth turned cloudy. Then an apparatus was set up to aerate the broth and the phage was inoculated into the broth. The phage was allowed to multiply until the NYDB turned clear. The phage was then stored under refrigeration for later use in testing.

Bacteria from each isolation were suspended in sterile distilled H_2O at approximately 10^7/ml. concentration. The suspension of bacteria was then placed into NYDA just before the agar hardened and swirled to mix properly. After the agar hardened, one drop of each of the three phages was placed in one fourth of the NYDA plate and the plate was incubated at 30 C. for approximately 50 hours. Clear plaques were considered positive tests. Controls were run in each case on the other fourth of the plate by using sterile NYDB.

Experiment 3: Identification by Use of Pathogenicity Tests

The pathogenicity of the isolates was tested by inoculation of bacterial suspensions into Bartlett pear seedlings. The pear seedlings

were planted individually in 1 gallon cans. The soil was fertilized with nitrogen, mixed with peat moss, and pasteurized using steam. They were then grown under greenhouse conditions until new growth was present. The inoculum was prepared by suspending an isolate of bacteria in sterilized distilled water at a concentration of 1×10^7 bacterial cells per ml. Each isolate was then tested by first inoculation into the petiole with a hypodermic needle. Second, by use of carborundum on the leaves with mechanical pressure of gauze pads with inoculum on upper surfaces. The inoculated area of the plant was then covered by a plastic bag to increase humidity. The trees were allowed to grow normally for about 3 weeks. Typical symptoms were considered a positive test. Controls were run in each case.

RESULTS

The results of the three experiments showed that Erwinia amylovora could be isolated from pear and apple, as well as Pyracantha, chokecherry and mountain ash during the winter months. The results also showed that there was a great deal of variation among the various isolates collected. However, it was found that in every method of collection, Erwinia amylovora could be isolated.

Experiment 1: Identification by Use of Antisera

Results of the serological tests showed variation depending on the method of isolation and the tree from which the isolate was obtained. There was also variation between bark and bud isolates. Of the eight Bartlett bud isolates from a selective medium, only two isolates gave a positive serological test to one each of the four antisera. Five Anjou bud isolates resulted in two isolates giving positive tests to antiserum 33 and 36 while one isolate gave a positive test to antiserum 39 (Table 1). On the other hand, Anjou bud isolates from buds in NYDB resulted in no positive serological tests, while three of eight Bartlett bud isolates gave positive tests to 33, 36, and 39 (Table 2).

Apple bud isolates from selective medium gave positive tests to all of the antisera (Table 1) and isolates from apple buds in NYDB gave positive tests to three of the four antisera (Table 2).

Table 1. Isolates from selective medium[a] showing positive tests

Buds	No. of isolates	MCS	Serological			Bacteriophage			Pathogenicity
			33	36	39	33	36	39	
Bartlett Pear	8	1	1	0	0	1	0	0	3
Anjou Pear	5	0	2	2	1	2	2	1	0
Apple	7	1	2	2	1	2	2	1	2

[a]Medium contained Nutrient Yeast Dextrose Agar plus .03 per cent crystal violet.

Table 2. Isolates from buds in Nutrient Yeast Dextrose Broth giving positive tests

Buds	No. of isolates	MCS	Serological			Bacteriophage			Pathogenicity
			33	36	39	33	36	39	
Bartlett Pear	8	0	3	3	3	1	1	1	0
Anjou Pear	3	0	0	0	0	1	0	0	0
Apple	11	0	4	3	2	3	4	2	3
Pyracantha	4	0	0	0	1	0	0	0	1
Chokecherry	2	0	1	0	0	1	0	0	1
Mountain Ash	3	0	0	0	0	0	0	0	0

The isolates from buds in NYDB of hosts other than apple and pear resulted in one Pyracantha isolate and one chokecherry isolate giving a positive reaction. No mountain ash isolates resulted in positive tests (Table 2). However, due to the small number of isolates tested, no definite comparative conclusions should be made in any of the experiments. The scope of this experiment was only to test the ability of the bacterium to overwinter in host tissue, not a complete ecology study. If more isolates from the hosts had been tested it is quite possible that different results would have been obtained.

Bark isolates showed higher percentages of positive serology tests than bud isolates for all hosts except mountain ash (Tables 3 and 4). Both bark and bud isolates from all hosts in the experiment gave positive serological tests to antisera specific for Erwinia amylovora. It was interesting to see the high percentage of bark isolates of Pyracantha and chokecherry that gave positive tests as compared to mountain ash which was quite low (Table 3). However, bud isolates from mountain ash resulted in 13.3 per cent positive serology tests while Pyracantha and chokecherry gave 9.4 and 10.0 per cent positive tests respectively (Table 4).

Both pear and apple bark isolates gave a higher percentage of positive tests than did the bud isolates of either host (Tables 3 and 4). However more bud isolates were tested.

The agglutination of the bacteria by the antiserum was quite easy to identify under the light microscope (Figure 5).

Table 3. Isolates from bark giving positive serological tests

Twigs	Month	No. of isolates	Per cent MCS	33	36	39	Total
Bartlett Pear	February	6	50.0	33.3	33.3	33.3	
	March	19	0.0	21.1	26.3	21.1	
	April	3	0.0	33.3	33.3	33.3	22.3
Anjou Pear	February	0	0.0	0.0	0.0	0.0	
	March	4	0.0	75.0	75.0	75.0	
	April	1	0.0	100	100	100	60.0
Apple	February	3	0.0	0.0	0.0	0.0	
	March	14	0.0	42.9	50.9	36.7	
	April	3	0.0	66.7	66.7	66.7	30.0
Pyracantha	April	6	0.0	33.3	33.3	50.0	29.2
Chokecherry	April	4	0.0	50.0	50.0	50.0	37.5
Mountain Ash	February	2	0.0	0.0	0.0	0.0	
	April	7	0.0	14.3	14.3	14.3	8.3

Table 4. Isolates from bud grinds which gave positive reactions to serological tests

Buds	Month	No. of isolates	Per cent MCS	33	36	39	Total
Bartlett Pear	February	13	15.4	23.1	7.7	15.4	
	March	27	0.0	33.3	23.8	19.0	
	April	9	0.0	11.1	22.2	33.3	16.9
Anjou Pear	February	1	0.0	0.0	0.0	0.0	
	March	10	0.0	37.5	25.0	12.5	
	April	2	0.0	33.3	33.3	33.3	20.5
Apple	February	7	0.0	0.0	0.0	14.3	
	March	26	5.9	23.5	17.6	11.8	
	April	9	0.0	14.3	14.3	42.9	12.9
Pyracantha	April	8	0.0	0.0	0.0	37.5	9.4
Chokecherry	April	5	0.0	20.0	0.0	20.0	10.0
Mountain Ash	February	3	0.0	33.3	33.3	33.3	
	April	12	8.3	8.3	8.3	16.6	13.3

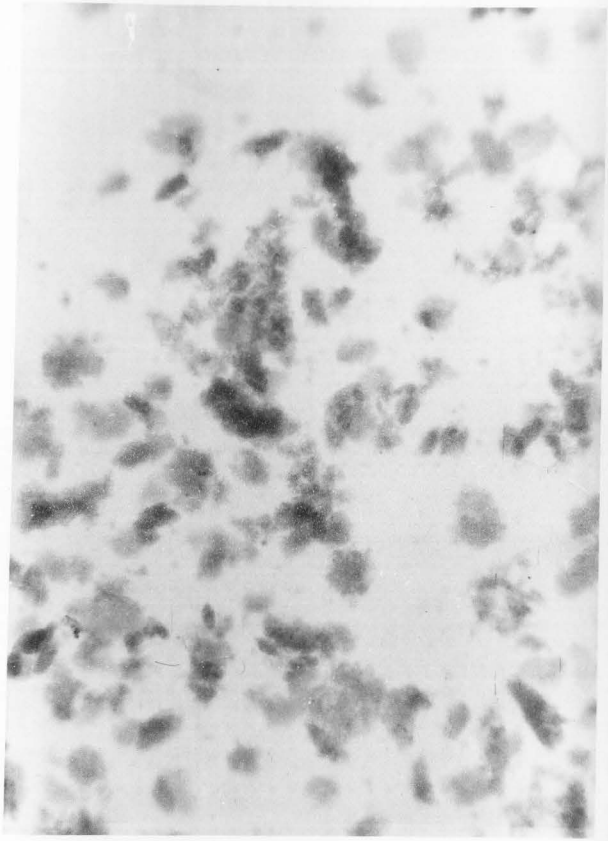

Figure 5. Agglutination of bacteria to antiserum as seen under light microscope.

Experiment 2: Identification by Use of Bacteriophage

Experiment Two showed that in every method of collection Erwinia amylovora was isolated. In all hosts except Pyracantha the bark isolates gave as high or higher percentages of positive bacteriophage tests than buds (Tables 5 and 6). Selective medium isolates from the buds of Bartlett pears, Anjou pear, and apple gave identical results in both the serology and bacteriophage tests (Table 1). However, isolates from NYDB gave varied results (Table 2). Apple bud isolates gave the same number of positive tests in both serology and bacteriophage tests, but only one of the Bartlett isolates resulted in positive bacteriophage test as compared to three isolates giving positive serology tests (Table 2). There were not enough isolates of Anjou pear, Pyracantha, chokecherry or mountain ash from buds in NYDB to make any comparisons. However, isolates from hosts other than apple and pear gave higher percentages of positive tests than they did in the serological tests (compare tables 3 through 6). The plaques were clear and quite easy to detect (Figure 6).

Experiment 3: Identification by Use of Pathogenicity

If the test for pathogenicity produced normal fire blight symptoms (scorching of leaves with rapid necrosis of leaves and twigs) it was considered positive (Figure 7). Of the isolates tested, those from Bartlett pear and apple trees gave the highest percentage of virulent isolates (Table 7). Anjou pear, Pyracantha and chokecherry isolates

Table 5. Isolates from bark giving positive bacteriophage tests

Twigs	Month	No. of isolates	Per cent 33	36	39	Total
Bartlett Pear	February	6	16.7	16.7	16.7	
	March	19	15.8	15.8	21.1	
	April	3	0.0	0.0	33.3	16.7
Anjou Pear	February	0	0.0	0.0	0.0	
	March	4	50.0	25.0	50.0	
	April	1	100	0.0	100	46.7
Apple	February	3	0.0	0.0	0.0	
	March	14	28.6	35.7	28.6	
	April	3	33.3	33.3	0.0	25.0
Pyracantha	April	6	16.7	33.3	33.3	27.8
Chokecherry	April	4	50.0	25.0	50.0	41.7
Mountain Ash	February	2	50.0	0.0	0.0	
	April	7	14.3	0.0	14.3	11.1

Table 6. Isolates from bud grinds which gave positive bacteriophage tests

Buds	Month	No. of isolates	Per cent 33	36	39	Total
Bartlett Pear	February	13	23.1	7.7	15.4	
	March	27	19.0	9.5	14.3	
	April	9	11.1	22.2	22.2	15.5
Anjou Pear	February	1	0.0	0.0	0.0	
	March	10	37.5	25.0	12.5	
	April	2	0.0	0.0	0.0	18.2
Apple	February	7	14.3	0.0	28.6	
	March	26	17.6	17.6	11.8	
	April	9	14.3	14.3	28.6	16.1
Pyracantha	April	8	50.0	25.0	12.5	29.1
Chokecherry	April	5	20.0	0.0	20.0	13.3
Mountain Ash	February	3	0.0	0.0	0.0	
	April	12	16.6	8.3	16.6	11.1

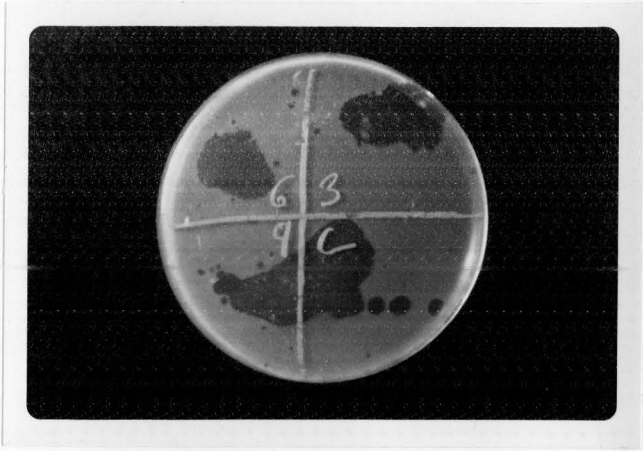

Figure 6. Plaques in culture of bacteria caused by lysis of bacterial cells by bacteriophage.

Figure 7. Positive test for pathogenicity of bacterial isolate on Bartlett pear seedlings. Plant at right is control plant.

Table 7. Isolates showing pathogenicity to Bartlett pear seedlings. Typical symptoms after 4 weeks were considered a positive test.

Host	Month	No. of isolates	Bud	No. of isolates	Bark	Total
Bartlett Pear	February	13	15.4%	6	50.0%	
	March	27	25.9%	19	21.1%	
	April	9	11.1%	3	33.3%	22.5%
Anjou Pear	February	1	0.0%	0	0.0%	
	March	10	10.0%	4	0.0%	
	April	2	0.0%	1	0.0%	5.6%
Apple	February	7	28.6%	3	0.0%	
	March	26	23.1%	14	28.6%	
	April	9	11.1%	3	33.3%	22.5%
Pyracantha	April	8	25.0%	6	0.0%	10.0%
Chokecherry	April	5	40.0%	4	0.0%	22.2%
Mountain Ash	February	3	33.3%	2	50.0%	
	April	12	8.3%	7	14.2%	16.6%

from bark were avirulent. However, only a few bark isolates were tested. All six trees tested had virulent bacteria isolated from buds.

Bud isolates showed higher percentages of pathogenicity than did the bark isolates in Pyracantha and chokecherry. Mountain ash showed the opposite with bark isolates being the higher. Results of apple and Bartlett pear isolates indicated that there was no appreciable difference between virulence of bud and bark isolates (Table 7).

Results showed that virulent isolates of Erwinia amylovora were collected from all six of the trees or shrubs in the experiment. Results also indicate that there was a good deal of variability among the isolates of Erwinia amylovora in the serological and bacteriophage tests (Table 8).

Table 8. Isolates showing a positive reaction to one or more of the three tests given

Host	Isolate No.	Serological				Bacterio-phage			Pathogenicity	
		MCS	33	36	39	33	36	39	Inoc.	Carb.
Bartlett Pear Bark	5 A	+	+	-	+	-	-	-	-	-
	17 B	+	+	+	+	-	-	-	+	+
	19 A	-	-	-	-	+	+	+	+	+
	22 A	+	-	+	-	-	-	-	+	+
	38 A	-	-	+	-	-	+	-	+	+
	38 B	-	-	-	-	+	-	-	-	-
	42 A	-	-	+	-	-	-	-	+	+
	60 A	-	+	+	+	-	-	+	-	-
	66 A	-	+	-	+	+	-	+	-	-
	72 B	-	+	+	+	+	+	+	+	+
	76 B	-	+	+	+	-	+	+	+	+
	84 A	-	+	+	+	-	-	+	+	+
Bartlett Pear Bud	1 A	-	-	-	-	+	-	-	-	-
	4 A	-	-	-	-	-	+	+	-	-
	11 A	-	+	+	+	-	-	-	-	-
	14 A	-	-	-	-	+	-	-	-	-
	14' A	+	+	-	-	-	-	-	-	-
	19' A	-	-	-	-	+	-	-	+	+
	33' A	+	-	-	-	-	-	-	+	+
	41 B	-	+	+	+	+	-	+	+	+
	51 A	-	+	+	-	-	-	-	+	+
	55 A	-	+	+	+	-	-	+	+	+
	55' A	-	+	-	-	-	-	-	+	+

Table 8. Continued.

Host	Isolate No.	Serological MCS	33	36	39	Bacteriophage 33	36	39	Pathogenicity Inoc.	Carb.
Bartlett Pear Bud	59 A	-	+	+	+	+	+	+	+	+
	63 A	-	+	-	+	+	-	-	+	+
	75 A	-	+	+	-	+	+	-	-	-
	81' A	-	-	-	+	-	-	-	+	+
	81' B	-	+	+	+	+	+	+	-	-
	83 A	-	-	+	+	-	+	+	-	-
	119 A	-	+	+	+	+	+	+	-	-
	119 B	-	+	+	+	-	-	-	-	-
	124 A	-	+	+	+	-	-	-	-	-
Anjou Pear Bark	44 A	-	+	+	+	-	+	+	-	-
	68 A	-	+	+	+	+	+	+	-	-
	78 A	-	+	+	+	-	-	-	-	-
	86 A	-	+	+	+	+	-	+	-	-
Anjou Pear Bud	31' B	-	-	-	-	-	-	+	-	-
	43' A	-	+	+	-	+	+	-	-	-
	67' A	-	+	+	+	+	+	-	-	-
	77' A	-	+	-	-	-	-	-	+	+
	117 A	-	-	-	-	+	-	-	-	-
Apple Bark	36 A	-	-	+	-	-	-	-	-	-
	36 B	-	-	+	-	-	+	-	+	+
	54 A	-	+	+	+	+	-	+	-	-
	58 B	-	+	+	+	-	+	-	+	+
	62 A	-	+	-	-	+	+	+	+	+

Table 8. Continued.

Host	Isolate No.	Serological				Bacteriophage			Pathogenicity	
		MCS	33	36	39	33	36	39	Inoc.	Carb.
Apple Bark	62 B	-	+	+	+	-	+	+	-	-
	70 B	-	+	+	+	+	+	+	+	+
	74 C	-	+	+	+	+	-	-	-	-
	80 B	-	+	+	+	-	+	-	-	-
	88 A	-	+	+	+	+	-	-	+	+
Apple Bud	7 B	-	-	-	-	+	-	+	+	+
	12' B	-	-	-	+	-	-	+	+	+
	35' A	+	+	+	+	+	+	+	+	+
	57' A	-	+	+	-	+	+	-	+	+
	69' A	-	+	+	+	+	+	+	-	-
	73 A	-	+	-	-	-	-	-	+	+
	79 B	-	+	+	+	-	+	-	+	+
	87' A	-	-	-	+	-	-	-	-	-
	103 A	-	-	-	-	-	-	+	+	+
	118 B	-	+	+	-	-	+	-	+	+
	120 B	-	+	-	+	+	+	-	+	+
	123 A	-	+	+	+	+	+	+	-	-
	123 B	-	+	+	-	+	+	-	-	-
Pyracantha Bark	201 C	-	+	+	+	+	+	+	-	-
	211 A	-	+	-	+	-	-	+	-	-
	211 B	-	-	+	+	-	+	-	-	-
Pyracantha Bud	202 A	-	-	-	+	+	+	-	-	-
	202 B	-	-	-	+	+	+	+	-	-

Table 8. Continued.

Host	Isolate No.		Serological MCS	33	36	39	Bacterio-phage 33	36	39	Pathogenicity Inoc.	Carb.
Pyracantha Bud	202'	A	-	-	-	-	+	-	-	+	+
	202'	B	-	-	-	-	+	-	-	-	-
	210	B	-	-	-	+	-	-	-	+	+
Chokecherry Bark	204	A	-	+	+	+	+	-	+	-	-
	204	B	-	+	+	+	+	-	+	+	+
Chokecherry Bud	203	B	-	+	-	-	+	-	-	+	+
	205	A	-	-	-	+	-	-	+	+	+
Mountain Ash Bark	18	A	-	-	-	-	+	-	-	+	+
	208	B	-	+	+	+	+	-	+	+	+
Mountain Ash Bud	9	B	-	+	+	+	-	-	-	+	+
	209	A	+	+	-	+	-	-	+	-	-
	204	A	-	-	+	+	+	+	+	+	+

DISCUSSION AND CONCLUSION

The main objective of this research was to determine if Erwinia amylovora overwintered in living tissue in Utah. The data left little doubt that holdover bacteria were present in both twigs below cankers and buds of host trees throughout the winter months. These bacteria could then be responsible for the new outbreaks of fire blight the following spring when conditions become right.

Work by Goodman and Shaffer published in 1962 (11) showed that both avirulent and virulent isolates of Erwinia amylovora could be isolated from the same tree. In later work they concluded that avirulent forms could be converted to virulent forms when passed through aphid extracts and that sugar concentration was a determining factor. The data of my experiments support the presence of both avirulent and virulent isolates of Erwinia amylovora in the same tree (Table 8). If Goodman's conclusions are correct, it could explain the rapid spread of fire blight by changing the bacteria from the avirulent to the virulent state when climatic conditions are ideal. This change is dependent upon the sugar concentration of the sap which is affected by climatic conditions such as rain fall, humidity, temperature and suculence of the tree.

For years, pathologists and orchardists in Utah have witnessed an immediate increase in fire blight following a hail storm, high winds, and wet weather. Bauske (3), in work with wind-borne rain, concluded that inoculum could be spread rapidly and effectively in this manner. His experiments indicated that injury to the trees facilitated the

spread of disease but that wounding was not a prerequisite to infection. Lewis and Goodman (15) showed that natural openings such as foliar trichomes, hydathodes, stomates, nectaries, and lenticels could serve as natural portals of entrance for the pathogen. Results and observations such as these indicate that holdover bacteria could cause epidemics when the right conditions prevail. My results, however, indicated that holdover bacteria are present not only in diseased but also in healthy tissue. Erwinia amylovora was present at nearly the same consistency in both apple and pear trees. The buds of both contained virulent as well as avirulent bacteria. Virulent bacteria were isolated as far as six inches below the cankers of apple and pear twigs. Virulent bacteria were isolated from all hosts.

The literature appears to be lacking in much information of inoculum overwintering in hosts other than apple and pear. I found that Erwinia amylovora could be isolated from Pyracantha, chokecherry, and mountain ash showing typical symptoms. Virulent isolates were found in buds as well as twigs. It is my opinion that large numbers of hosts other than apple and pear growing near orchards could be important sites for the overwintering of inoculum. Being aware of this could possibly be a help in control measures.

The use of antiserum was an effective tool in this experiment. The antiserum proved to be species specific but not strain specific. Results of the experiment indicate that there is a high degree of variability among the various strains of Erwinia amylovora.

Conclusions which can be drawn from the results of the study are: (1) Erwinia amylovora does overwinter in living host tissue in Cache Valley, Utah. Both virulent and avirulent forms can be isolated from

the same tissue. (2) Erwinia amylovora pathogenic to Bartlett pear seedlings can be isolated from Pyracantha, chokecherry, and mountain ash. (3) Virulent bacteria can be isolated from unexposed bark as far as six inches below the cankers on twigs of apple and pear trees. The bacteria can also be isolated from buds near the infected tissue of apple and pear trees. (4) Pathogenic isolates can be isolated from apple, pear, Pyracantha, chokecherry, and mountain ash in Cache Valley. Bartlett pear seedlings can serve as good test hosts. (5) Antiserum produced in rabbits is a good method for quick, easy identification of Erwinia amylovora. The antiserum is species specific, but not strain specific.

SUMMARY

The objectives of the study were to: (1) determine if Erwinia amylovora overwinters in living host tissue in Cache Valley, Utah, and if so, to determine sites of the overwintering bacteria on the apple and pear trees; (2) determine if Erwinia amylovora can be isolated from hosts other than apple and pear; (3) determine pathogenicity of isolates of Erwinia amylovora.

Erwinia amylovora was isolated from apple and pear trees throughout the winter months. Isolates were from a representative number of orchards throughout Cache Valley, Utah. Identification was serological by the use of antisera, developed from rabbits, which caused suspensions of Erwinia amylovora to agglutinate. The agglutinated bacteria could be seen under a light microscope at 450 X. As a check, the isolates were also bacteriophage tested. The phage was receivedfrom W. H. Shaffer, University of Missouri, and was specific for Erwinia amylovora. Positive tests were plaques of lysed bacteria on agar plates.

The pathogenicity of the isolates was tested by injection of the isolate into Bartlett pear seedlings growing under greenhouse conditions. This was done with a hypodermic syringe into the intercostal areas of leaves and in the apex of the stems. Inoculation was also done by dusting leaves with 600 mesh carborundum and the suspension of bacteria applied to the upper surface of the leaves with gauze pads by mechanical pressure.

Pathogenic bacteria were isolated from the living tissue up to six inches below the cankers and also from apparently healthy buds near the cankers. The isolate tissue from the twigs was phloem and bark parenchyma.

Finally, virulent Erwinia amylovora was isolated from mountain ash, chokecherry and Pyracantha in Cache Valley, Utah. Isolation and testing was by the same methods as those for apple and pear isolates. Nearly 15 per cent of isolates from hosts other than apple and pear were pathogenic to Bartlett pear seedlings.

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