Monitoring the Epiphytic Population of Erwinia amylovora on Pear with a Selective Medium

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ABSTRACT

A selective differential medium devised for monitoring epiphytic populations of *Erwinia amylovora* inhibited growth of most other microorganisms. Although all *Erwinia* sp. that were tested grew on the medium, each species was identified by its distinctive colony morphology and color. The few pseudomonads and other bacteria that grew on the medium were blue or green in contrast to the red-to-orange hues characteristic of *Erwinia* sp. Generation times and the percentage of *E. amylovora* cells producing colonies were similar for the selective medium and standard media. *E. amylovora* existed as an epiphyte in pear flowers and other plant parts during spring. The bacterium was not detected in leaf or flower buds during winter and early spring months. However, it was detected in flowers and on the surface of cankers (no visible ooze) prior to evidence of flower infection. Epiphytic populations of *E. amylovora* varied among orchards and trees, and were related to disease severity. Every flower assayed during an epiphytotic was infested with bacteria at populations commonly ranging from 10^4 to 10^6 cells/flower. However, relatively few flowers became infected (100 flower infections/tree). In one orchard, 7% of the aborted flowers had a surface population of *E. amylovora*. Neither the population of *E. amylovora* nor disease incidence was affected by the occurrence of saprophytic bacteria in flowers. Four to 33% of the flowers were colonized only by *E. amylovora*. The insects, *Pegamya* sp. and *Minettia* sp., carried surface populations of fire blight bacteria ranging from 10^1 to 10^5 cells/insect.

The first naturally occurring streptomycin-resistant strain of *E. amylovora* was found in a severely diseased orchard. Concentrations of streptomycin up to 200 μg/ml did not affect growth of this strain.

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Fireblight disease of pome fruit is one of the oldest and most intensively studied plant diseases (1). Many details of the infection process, life cycle, and epidemiology have been clearly elucidated by the keen observations and adroit experiments of early workers. Examples of this critical work are contained in reports on overwintering in cankers and twigs (16, 22); dissemination by insects (8, 10, 12, 13, 14, 15, 16, 19, 20, 23) and water (10, 22); ingress through natural openings in flowers, shoots, and leaves (13, 22); and infection pathways (16, 18).

Despite this body of definitive information, it seems incongruous that little agreement exists concerning factors that promote rapid spread of the bacterium and contribute to epiphytotics. Some workers champion the role of water (6, 10, 22), whereas others emphasize the activity of insects (12, 17, 19, 21). Since none of these factors, or combinations thereof, satisfactorily explains the occurrence of a sudden epiphytotic after a hail storm or frost, or occasionally during periods of dry warm weather, presumably some other aspect of the pathogen's life cycle is significant but has escaped consideration. Accordingly, we examined the possibility that existence of the bacterium as an epiphyte might account for phenomena in disease development that remain unexplained. The determination of whether or not *E. amylovora* occurred as an epiphyte required the monitoring of the bacterial population on plant parts and the development of the necessary methodology.

This paper reports on the development and use of a selective differential medium and its use in locating and quantitating epiphytic populations of *E. amylovora* on pear parts. An abstract of this work has been published (11).

Preparation of the medium.—The selective medium was prepared by adding the following compounds, in the order listed, per liter of distilled water: mannitol, 10.0 g; nicotinic acid, 0.5 g; L-asparagine, 3.0 g; K_2HPO_4, 2.0 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; sodium taurocholate (Difco), 2.5 g; Tergitol anionic 7 (sodium heptadecyl sulfate, Union Carbide), 0.1 ml; nitrilotriacetic acid (NTA), 10 ml of 2% aqueous solution (NTA is first neutralized with ca. 0.73 g of KOH/g NTA); bromothymol blue, H_2O-soluble (Matheson Colman & Bell), 9 ml of a 0.5% aqueous solution; neutral red, H_2O-soluble (Matheson Colman & Bell), 2.5 ml of a 0.5% solution; agar, 20.0 g. The medium was adjusted with 1 N NaOH (ca. 5 ml) to pH 7.2 to 7.3. The preparation was autoclaved at 121 C for 15 min. The pH of the medium after autoclaving should be ca. 7.4. Fifty mg Actidione (cyclohexamide, Nutritional Biochemical Corp.), and 1.75 ml of a 1.0% solution thallium nitrate (K & K Rare Chemicals) were added to the autoclaved medium.

Growth of E. amylovora on the selective medium.—The effectiveness of most selective media is limited by slow bacterial growth, low percentage detection of the total population, or by toxicity of the ingredients. These characteristics were examined

TABLE 1. Generation times and plating efficiency of *Erwinia amylovora* when grown on selective and standard media

Strain	Generation time (min)		Plating efficiency[b]	
	Selective	Nutrient[a]	Nutrient[a]	PDP[c]
FB 1	127	98	61	106
FB 9	110	88		
FB 13	135	125	108	98
FB 14	120	115		
FB 15	105	105	204	102
FB 17	133	115	96	
FB 19	160	100		
FB 22	138	108	94	91
FB 23	125	100		
FB 24	100	80		
FB 25	130	128	259	107
FB 37			109	134
Mean	125	105	133	106

[a]Nutrient broth was supplemented with 0.5% glucose.

[b]The figures are expressed as the percentage number of colonies growing on standard media in comparison to that on selective medium. An equal number of cells was plated on each medium.

[c]PDP = Potato-dextrose peptone.

by comparison of the *Erwinia* selective medium with standard media. Generation times of *E. amylovora* strains were obtained in a broth preparation of the selective medium and in nutrient broth plus 0.5% glucose (NBG). Bromothymol blue and neutral red which affect colony color were omitted from the selective medium to enable turbidity readings. Twelve strains of *E. amylovora* were grown for 18 hr on potato-dextrose peptone (PDP) slants. Five ml of a bacterial suspension with a turbidity reading of 100 on a Klett-Summerson colorimeter (green filter) were added to 250-ml side-arm flasks containing 50 ml of either NBG or selective medium broth. The flasks were incubated at 28 C for 12 hr on a rotary shaker (175 rpm), and readings were made every hour. To determine possible toxicity of the selective medium, the percentage of cells that produced colonies on solid media was determined by plating a known concentration of cells on selective medium, nutrient-glucose (NG), and PDP agar media. Aqueous suspensions were made of 18-hr cultures (10^3 to 3×10^3 cells/ml) and 0.1 cc aliquots pipetted onto the various media and distributed with an L-shaped glass rod. The bacteria was incubated at 28 C for 4 days.

The selective medium was nearly as good a growth medium as NBG or NG. The generation time of 12 *E. amylovora* strains averaged 125 min in the selective broth and 105 min in NBG (Table 1). The percentage of cells that produced colonies on the selective medium compared favorably to that on NG and PDP media (Table 1). However, recovery percentages varied considerably depending on which medium and strain was used for the comparison.

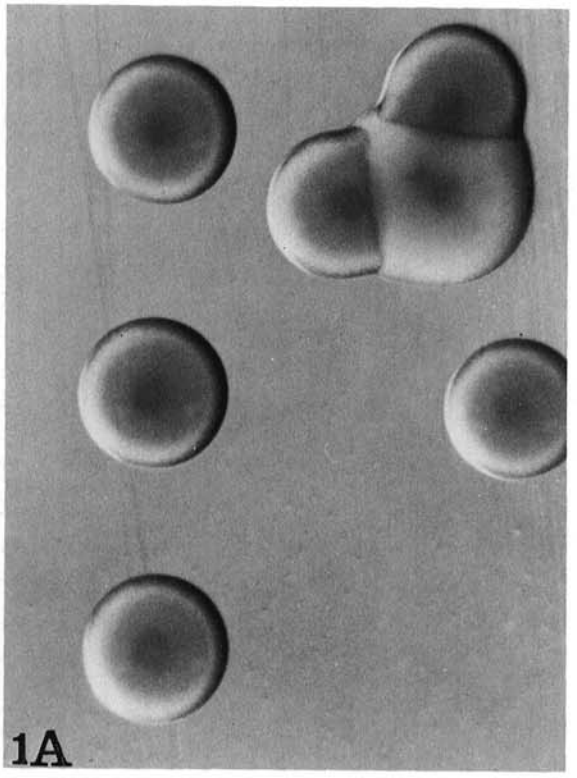
Selectivity of the medium.—Two hundred forty-nine bacterial strains representing plant pathogens and saprophytes from five genera and 80 species were evaluated for their capacity to grow on the selective medium. Inhibition was rated by comparison of growth on selective and standard media. Cultures were streaked on the media and incubated at 28 C for 96 hr; and readings were made at 24-hr intervals. Growth was arbitrarily rated from 1 to 10, with 10 comparable to growth on standard media. A rating of 3 or less represented pinpoint colonies or a thin film of growth.

Erwinia spp. obtained a growth rating of 10 in 24-36 hr. Only 17 *Pseudomonas* strains representing 12 species produced growth comparable to that of *Erwinia* spp. However, most isolates required 36-96 hr to attain a rating of 5-10. The results within a single species were not always consistent; one strain of *P. cichorii, P. coronafaciens, P. lachrymans, P. marginalis,* and *P. primulae* differed from other strains by exhibiting growth (data table available upon request).

Differentiation.—Reddish-orange colored colonies were indicative of the *Erwinia* genus on the selective medium. Colonies of pseudomonads and other bacteria which occasionally appeared were green to blue in color. Scrutiny with a 10X dissecting microscope revealed the distinctive colony morphology peculiar to each species within the *Erwinia* genus. Several factors influenced identification. The medium surface must be dry to restrict colonies from spreading. Proper spatial relations were essential, since typical colony characters were not revealed when colonies overlapped or if the population was dense. Identification of colonies with a dissecting microscope was effected by illumination from below with a flat mirror surface and transmitted parallel light. Greatest differentiation was accomplished when the periphery of the illuminated area was focused on the colony. *E. amylovora* colonies were smooth, with dark orange centers, and had entire translucent margins (Fig. 1-A). The width of the margin varied slightly among strains, but all tested strains produced a distinctive translucent margin. This margin distinguished *E. amylovora* from *E. herbicola,* which was otherwise similar. Other *Erwinia* spp. were easily discerned from *E. amylovora* by their peculiar colony characteristics. For example, *E. atroseptica* and *E. carotovora* colonies were rough, with diffuse orange centers and serrated edges (Fig. 1-B).

Methods for monitoring epiphytic populations of E. amylovora.—A Bartlett pear (*Pyrus communis* L.)

Fig. 1-2 1-A) Distinctive colony morphology of *Erwinia amylovora* and 1-B) *E. atroseptica* on selective medium. The varying hues of red and orange further distinguish among *Erwinia* species. 2-A) Typical populations of *E. amylovora* when washings from apparently healthy pear flowers were plated on selective medium; and 2-B) potato-dextrose peptone agar. Fast-growing saprophytes and *Bacillus* species often inhibit growth of *E. amylovora* on standard media.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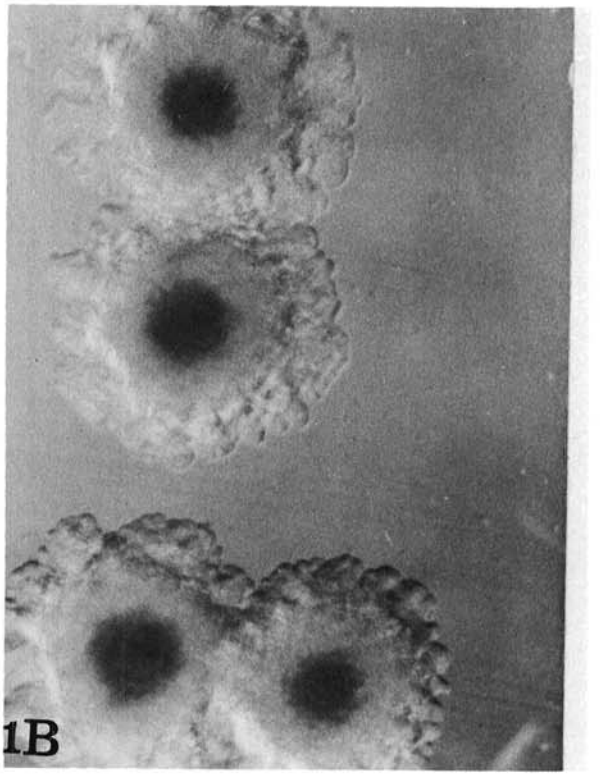
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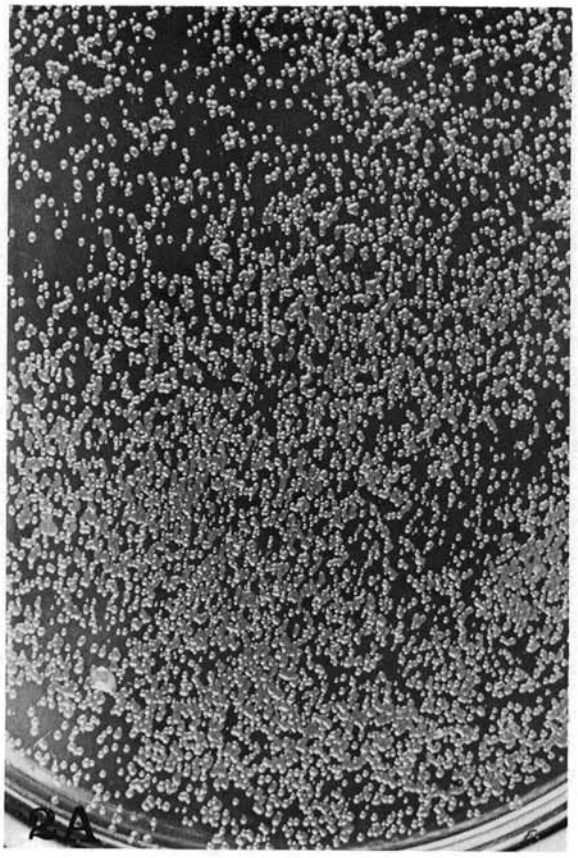
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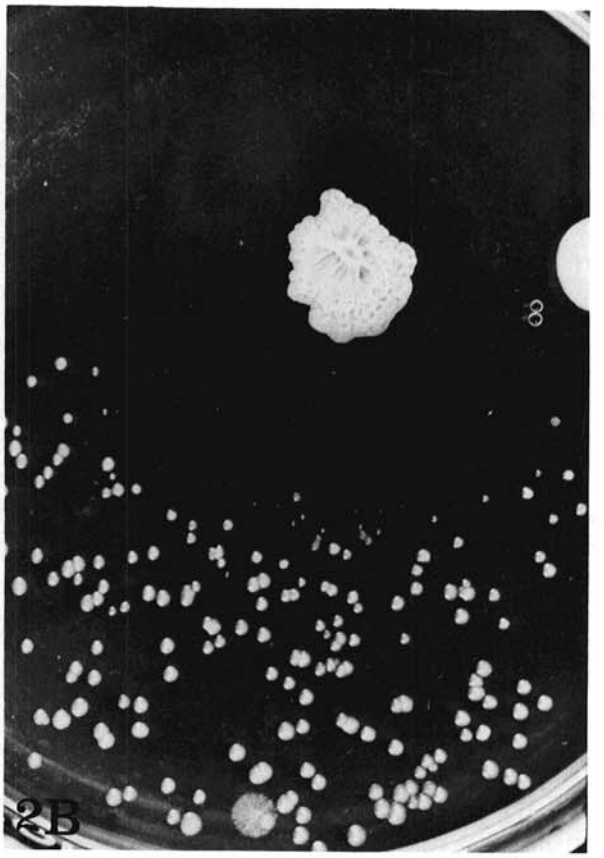
2B

orchard (Walnut Creek, Calif.) was monitored in 1971 for epiphytic populations of *E. amylovora* on plant parts. One-half the orchard had been abandoned for 1 year, and had not received fireblight control measures for 2 years. During 1970, the orchard sustained an average of 50 fireblight strikes/tree. We examined the occurrence of *E. amylovora* as an epiphyte by making weekly isolations from buds (dissected), flowers, fruits, and blighted wood infected in the previous year. Over 1,000 isolations were made from two sets of six trees during a 10-week period. Buds, flowers, and small fruits were individually washed with 5 ml of sterile distilled water in 50-ml covered beakers. The beakers were vibrated for 15 sec with a vortex mixer. To determine whether or not *E. amylovora* might be present on the exterior of cankers, we washed 3- to 5-cm sections of blighted twigs, 5- to 8-mm in diam, for 60 sec after coating the cut ends with paraffin to prevent any outward movement of bacterium from the interior. Black aborted blossoms also were examined for *E. amylovora*, since their occurrence is a common phenomenon with pears, and there has been conjecture whether the pathogen might account for some of the blossom drop. Aliquots (0.1 ml) from each wash sample, as described above, were distributed evenly on selective and PDP media (two replications) with an L-shaped rod. The media were kept for 3 to 4 days at 28 C, and the numbers and description of the bacteria growing on both media were recorded.

A Bartlett pear orchard in Butte County was examined in 1971 for epiphytic populations of the fireblight bacterium during an epiphytotic. Pear parts were sampled for *E. amylovora* from a random selection of trees as previously described, except that only one dish of each medium was used. Insects also were examined for presence of *E. amylovora*. The trees were uniformly infected, averaging 100 flower and 25 shoot infections/tree.

A Bartlett pear orchard in Sacramento was examined in 1970 for the presence of *E. amylovora* as an epiphyte. The procedures differed from those described above because techniques were in the process of refinement. Buds, leaves, fruits, and flowers were washed in 10 ml of sterile distilled water in a 50-ml beaker and vibrated, and 1-ml aliquots of the washings were distributed over the agar surface on each of two plates of the selective medium and of PDP. The excess liquid was drained from the dishes, the agar surface was allowed to dry for 20 min at 40 C, and the dishes were placed in an incubator.

Cultures representative of each colony type that developed on the selective medium during the investigations were tested for pathogenicity by inoculation of young excised pear fruits, slices of young pear fruit, or succulent pyracantha shoots.

Occurrence of E. amylovora as an epiphyte.—*E. amylovora* was present as an epiphyte on various parts of the pear tree during certain times of the year. Data from the Sacramento and Butte County orchards (Table 2) indicated that most flowers were infested during an epiphytotic. Flowers in the Butte County orchard were 100% infested, with 6% exhibiting a surface population exceeding 10^6 cells. *E. amylovora* was also present on 92% of the young fruit, 76% of the leaves, and 26% of the young shoots and axillary buds. Figure 2-A and B are representative of the epiphytic population of *E. amylovora* when washings from flowers were plated on selective and PDP media, respectively. Although 100% of the flowers were infested, relatively few became infected, as indicated by the number of diseased flowers (100/tree) and the setting of a crop. Although *E. amylovora* was not monitored into the summer in the Butte County orchard (temperatures over 100 F are common), a one-time monitoring of the Sacramento orchard in summer did not reveal the presence of the bacterium on leaves, leaf buds, or rat-tail bloom (common euphemism for blossoms developing after normal bloom).

E. amylovora was detected in the Walnut Creek orchard in healthy flowers 4 weeks before symptoms were evident. The monitoring of six trees (3 March - 26 April, Table 2) further showed that blight bacteria may be present as an epiphyte without subsequently causing disease; none of the six trees became diseased. However, disease incidence was higher for trees with a still higher epiphytic population of the bacterium, as shown with another six trees monitored from 3-10 May.

E. amylovora bacteria were not detected early in the year (Walnut Creek and Sacramento orchards) in flower or leaf buds (Table 2). However, they were detected on the surface on cankers of several small limbs in Walnut Creek orchards prior to and during flowering. There was no evidence of ooze on these cankers.

Seven per cent of the black aborted flowers assayed in Walnut Creek orchard revealed the presence of *E. amylovora*. Some flowers had a surface population exceeding 10^4 cells.

The predominant insects (identified by E. I. Schlinger) during late bloom in Butte County were *Pegamya* sp., *Minettia* sp., and syrphid flies. Fifty-six per cent of the insects carried fireblight bacteria ranging from ca. 10^1 to 10^5 cells/insect. Figure 3 shows a trail of fireblight colonies from a captured *Pegamya* sp. that walked over the selective medium.

Data provided by plating the washings from pear plant parts on the selective and PDP media did not indicate a positive relationship between the occurrence of saprophytic bacteria and disease incidence (Table 3). Whereas several bacteria on PDP produced zones in which *E. amylovora* was inhibited, there was no evidence that these bacteria affected the population of *E. amylovora* in flowers (Table 4). In general, the population of saprophytic bacteria was low, ranging from 10^2 to 10^3 cells/flower, whereas *E. amylovora* averaged about 25×10^4 cells/flower (Butte County orchard). Many flowers contained only *E. amylovora*. The occurrence of saprophytic bacteria, however, appeared to affect the population count of *E. amylovora* on PDP; counts of *E. amylovora* were 10% greater from platings on selective media than on PDP. *E. herbicola*-like bacteria were found commonly in the Sacramento orchard in late

TABLE 2. Epiphytic populations of *Erwinia amylovora* on *Pyrus communis* L. Bartlett pear plant parts from three orchards at different localities

Monitoring date (month/day)	Number of pear parts or insects tested[a]	Number of pear parts infested and numbers of cells/pear part							
		0	$1\text{-}10^2$	$10^2\text{-}10^3$	$10^3\text{-}10^4$	$10^4\text{-}10^5$	$10^5\text{-}5 \times 10^5$	$5 \times 10^5\text{-}10^6$	$>10^6$
Sacramento orchard (1970)[b]									
2/7	100 leaf buds	100	0	0	0	0	0	0	0
3/31	100 leaf buds	100	0	0	0	0	0	0	0
4/3	100 flower buds	100	0	0	0	0	0	0	0
4/27–Disease incidence: ca. 100 blighted flowers/tree.									
5/1	30 flowers	20	0	10	0	0	0	0	0
	19 fruits (<6 mm in diam)	9	2	1	3	1	0	2	1
	24 leaves	21	2	0	0	0	0	1	0
5/15	35 flowers	5	0	0	0	0	0	10	20
	64 fruits (<8 mm in diam)	50	0	6	3	1	2	2	0
	64 leaves	62	1	1	0	0	0	0	0
7/16	115 rattail flowers	115	0	0	0	0	0	0	0
	92 fruits	92	0	0	0	0	0	0	0
	94 leaves	94	0	0	0	0	0	0	0
	94 leaf buds	94	0	0	0	0	0	0	0
Walnut Creek orchard (1971)[c]									
3/3	60 swelling flower buds	60	0	0	0	0	0	0	0
	30 small limbs with cankers	30	0	0	0	0	0	0	0
3/11	60 opening flower buds	60	0	0	0	0	0	0	0
3/16	60 opening flower buds	60	0	0	0	0	0	0	0
	12 small limbs with cankers	11	0	0	1	0	0	0	0
3/23	12 small limbs with cankers	11	0	0	0	1	0	0	0
	60 opening flower buds	60	0	0	0	0	0	0	0
3/29	60 flowers (50% bloom stage)	60	0	0	0	0	0	0	0
4/5	120 flowers (90% bloom)	119	0	1	0	0	0	0	0
4/12	120 flowers (full bloom)	119	0	0	1	0	0	0	0
4/19	120 flowers (fruit forming)	119	1	0	0	0	0	0	0
4/26	120 fruits (>5 mm in diam)	120	0	0	0	0	0	0	0
	36 aborted flowers	36	0	0	0	0	0	0	0
4/30–Blight first observed: an average of one blighted flower/10 trees.									
5/3	120 late and rat-tail blooms	112	4	3	0	1	0	0	0
	36 aborted flowers	29	0	1	0	3	1	1	1
5/10	120 rat-tail blooms	116	2	0	2	0	0	0	0
	36 aborted flowers	35	0	1	0	0	0	0	0
5/10–Disease incidence: Blight was not detected on the six trees from which pear parts were monitored 3/3-4/26; a total of 20 blighted blossoms was found on six trees monitored 5/3-5/10.									
Butte County orchard (1971)[b]									
5/4–Disease incidence: ca. 100 blighted flowers/tree.									
5/4	145 flowers (full bloom stage)	0	0	8	11	40	51	25	10
	72 young fruits (>4 mm in diam)	6	0	27	17	15	0	0	7
	54 leaves	13	0	12	7	16	3	2	1
	43 shoot tips (terinal 15 cm)	33	0	7	1	2	0	0	0
	43 axillary leaf buds	32	0	6	4	1	0	0	0
	23 insects	8	0	13	1	1	0	0	0

[a]All plant parts except cankered limbs and aborted flowers were healthy in appearance.
[b]Plant parts were selected at random throughout the orchard.
[c]Plant parts were selected from the same six trees between 3/3 and 4/26, and a different set of six trees between 5/3 and 5/10.

summer (1970), but were not detected during 1971 in either Walnut Creek or Butte County orchards.

The authenticity of suspected *E. amylovora* colonies that developed on selective media from various experiments was examined by testing the pathogenicity. All 60 of the tested isolates were pathogenic.

Streptomycin resistance.—That repeated streptomycin applications did not affect disease severity in the Butte County orchard suggested the possibility that the fireblight strain was resistant to streptomycin. Accordingly, 50 isolates from the orchard were tested for resistance by plating cells on NG with streptomycin sulfate (744 μg/mg). All isolates were resistant to streptomycin. When equal numbers of cells were tested on NG with and without streptomycin, colony numbers or growth were not significantly affected at streptomycin concentrations up to 200 μg/ml.

DISCUSSION.—The selective medium was an

Fig. 3. Population of *Erwinia amylovora* from an infested *Pegamya* sp. captured in pear orchard. The insect (arrow) was placed in the petri dish, and distributed the bacteria by walking over the medium.

effective tool for monitoring the population of a bacterium in its ecological niche, since it was highly selective and of differential value; and detected a high percentage of propagules. Although growth of California strains on the selective medium compared favorably with that on standard media, the inherent variability of the species is such that no medium appears best for all strains. The medium does not prevent growth of other *Erwinia* spp.; however, their general absence from pear foliage during these tests, coupled with the distinctive characteristics of each *Erwinia* sp., enabled rapid identification of *E. amylovora*.

The capacity of *E. amylovora* to live as an epiphyte is an important aspect in the disease cycle, and explains many curious phenomena reported by early investigators. For example, this property accounts for the uniform presence of inoculum requisite for the occurrence of a sudden epiphytotic such as after a hail storm, where up to 95% of the blossoms may be diseased. It also explains a common situation where an orchard may have no obvious disease, no holdover cankers, and warm dry weather prevailing during blossoming; yet suddenly an epiphytotic occurs. The capacity of *E. amylovora* to live as an epiphyte on healthy pear parts further discounts the necessity for inoculation to occur at some precise time when the plant is most susceptible. Thus, once the bacterium encounters a favorable niche, such as a flower, its ability to perpetuate itself as an epiphyte greatly increases the opportunity for infection, since this insures that inoculum is present should environmental conditions occur which favor infection.

E. amylovora was termed an epiphyte since it was cultured from the plant surface sensu Leben (9). However, since R. A. Lelliott (*personal communication*) found that it readily multiplied on the surface of flowers, and we confirmed that it effects this with only occasional development of disease (*unpublished data*), *E. amylovora* may be considered a pathogen that has a resident phase in its disease cycle.

The finding that 100% of the flowers in the Butte County orchard were infested with *E. amylovora* with only relatively few flowers becoming diseased (100 flower strikes/tree) indicates there is much to learn about conditions favoring infection in nature. Although low nectar concentrations may be important in diseases development (8, 20), this orchard sustained enough rain to reduce nectar to a low concentration. We suspect, moreover, that many physiological factors are involved in susceptibility, and that as a general rule only a few infested flowers become

TABLE 3. The absence of a relationship between the occurrence of saprophytic resident bacteria and *Erwinia amylovora* on Bartlett pear plant parts and disease incidence (Butte County orchard)

	Flower		Fruit[b]		
Location	No. samples	% with saprophytic bacteria[a]	No. samples	% with saprophytic bacteria	Disease severity
Sacramento	114	96	172	96	ca. 100 blighted flowers/tree
Walnut Creek	665	64	260	63	ca. 1 blighted flower/tree
Butte County	145	67	72	93	ca. 100 blighted flowers/tree

[a]Average population of saprophytic bacteria/infested flower and fruit was 550 cells.
[b]Fruits were ca. 6 cm in diam.

diseased. However, data suggest that blossom drop may in part be caused by small localized infections.

The literature abounds with contestations concerning the roles of meteoric water, overwintering cankers, insects, wind, and environment in contributing to epiphytotics. Theories on the role of water as a major factor contributing to epiphytotics lack corroboration in California, since frequent epiphytotics occur during extensive periods of dry weather and conversely may be absent during wet weather. Other workers (12, 17, 21) also have questioned the role of water on similar grounds. We consider insects to be a dominant factor in disseminating *E. amylovora* among flowers, trees, and orchards. It seems pointless to add to the numerous reports that insects disseminate fireblight bacteria, as any animal that contacts the bacteria is capable of disseminating them. The insect appears to be a most efficient inoculating agent, as it carries large amounts of inocula directly to the infection court. This has particular importance since inoculum potential may be critical in development of the fireblight disease. Hildebrand (7) reported that single cells were unable to infect pear flowers unless flowers were maintained in a high humidity. R. A. Lelliott (*personal communication*) found that multiplication in the flowers was dependent on the presence of a large number of cells. Ercolani (4) reported similar results with *Pseudomonas syringe*. One might conjecture from these fndings that, in nature, inoculation may often be effected by a mass or large number of cells and not by "low dosages" which some workers logically contend should be used when conducting pathogenicity tests in greenhouse and field studies to duplicate natural processes. Plant bacteriologists have long been puzzled by the common necessity to use seemingly high amounts of inoculum (10^3-10^4 cells/ml) to achieve infection with many bacterial plant pathogens. Perhaps the low dosages are not typical of natural inoculation and infection processes in nature.

The first step in the epiphytic stage in the life cycle of *E. amylovora* presumably involves its dissemination from an overwintering site to a flower or some other suitable niche. Our observations and data reaffirm the role of determinant and indeterminate cankers on twigs and limbs as the primary reservoir of bacteria. Some workers (16) have questioned the role of cankers as a reservoir for bacteria, since they were either unable to find them in an orchard prior to manifestation of an epiphytotic or could not find ooze about cankers prior to infection. However, it seems unlikely that small indeterminate cankers would be found. There also may be a preoccupation with the necessity of finding ooze, as *E. amylovora* was detected on the exterior of cankers and blossoms where ooze was not evident.

Although there are suggestions that *E. amylovora* may overwinter in healthy buds (3), the evidence is not conclusive. Our data suggest that epiphytic activity of *E. amylovora* is sporadic, with populations increasing rapidly during favorable conditions, and terminating during hot summer weather and cold winters. Even in orchards with severely diseased trees, fireblight bacteria were not detected in buds or flowers during the hot summer months, or in dormant leaf and flower buds during winter months. Although Baldwin & Goodman (2) reported the presence of *E. amylovora* in apple buds as detected by phage typing, the bacteria were avirulent, which casts doubt as to their identity as *E. amylovora*.

TABLE 4. The absence of a relationship between the occurrence of saprophytic bacteria and populations of *Erwinia amylovora* in pear flowers (Butte County orchard)

No. flower samples	Saprophytic bacteria[a]	Population of *E. amylovora*/ flower
22	Isolate 14 (*Bacillus* sp.)	214,295 ± 73,500
28	Isolate 28	176,400 ± 62,245
35	Isolate 32	383,425 ± 81,795
13	Mixture of saprophytes	244,415 ± 76,260
47	No saprophytes detected	287,950 ± 58,150

[a]Population of saprophytes ranged from 10^2 to 10^3 cells/flower. No attempt was made to identify the saprophytes. Isolates 14, 28, and 32 were the only saprophytes detected in the flower samples, whereas flowers designated as having a mixture of saprophytes contained two to four different colony types of about equal proportions.

The absence of a detectible positive relationship between the occurrence of saprophytic bacteria and populations of *E. amylovora* or with disease incidence casts some doubt on the role or use of saprophytic bacteria in suppressing flower infection. Although the introduction of large numbers of a saprophytic bacterium in flowers may inhibit *E. amylovora* (5), the small numbers of naturally occurring saprophytic bacteria suggest that the flower is not a particularly favorable habitat for bacteria. Furthermore, bacteria which produced zones of inhibition of PDP may not have the proper substrate in the flowers to elaborate inhibitors. The most significant role of these antagonistic and competitive bacteria may well be in inhibiting growth of *E. amylovora* on standard media, thereby contributing to erroneous conclusions as to the population of the pathogen in nature.

The finding for the first time of a streptomycin-resistant *E. amylovora* strain occurring naturally in the field seems remarkable in view of the many years of streptomycin applications. Perhaps resistant strains have been present but were not detected because of the sporadic nature of disease. This might account for occasional complaints by growers in the past that streptomycin was not effective.

The use of the selective medium to monitor the population of *E. amylovora* should provide an effective tool in studying the effects of the environment on disease development, or in studying the effects of bactericides and antagonists on epiphytic populations. It also provides a mechanism to forecast whether or not it may be necessary to apply protective materials. For example, the low population of bacteria detected in the Walnut Creek orchards cor-

related with low blight incidence, even though one-half of this orchard had no protective materials applied.

NOTE: While this paper was in press we found that the substitution of sorbitol, 10.0 g, for mannitol in the selective medium restricted growth of *E. herbicola* like bacteria which otherwise are easily confused with *E. amylovora*.

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