The Role of the Stigma in Fire Blight Infections

S. V. Thomson

Associate professor, Department of Biology, Utah State University, Logan 84322.
Accepted for publication 15 November 1985 (submitted for electronic processing).

ABSTRACT

Thomson, S. V. 1986. The role of the stigma in fire blight infections. Phytopathology 76:476-482.

Erwinia amylovora occurs predominantly on the stigmas of epiphytically colonized flowers of *Pyrus communis*, *Malus sylvestris*, *Pyracantha* spp., *Crataegus* spp., and *Cotoneaster* spp. Rain facilitates the movement of bacteria from the stigmas to the hypanthia where infections generally occur. Bacteria survived better on the stigma than on the hypanthium and other flower parts. Small populations of bacteria declined when placed on the hypanthium, especially when the relative humidity was less than 20–30%. Bacteria survived at least 14 days on 80% of the pistil-inoculated flowers, whereas bacteria were reisolated from only 20% of the flowers inoculated on the hypanthium. Small populations of *E. amylovora* inoculated onto healthy stigmas multiplied to 10^5–10^6 per flower. Movement of these high populations of bacteria to the hypanthium resulted in infection.

The infection process of *Erwinia amylovora* (Burrill) Winslow et al has been studied extensively since 1892, when Waite (25) demonstrated the role of insects in fire blight epidemiology. Results of most studies suggest that widespread epidemics of fire blight result from rapid spread by rain and insects (7,14,16–18). The discovery (13) of large epiphytic populations of *E. amylovora* in apparently healthy flowers explains in part how severe outbreaks develop so suddenly. Epiphytic populations of *E. amylovora* from 10^5 to 10^7 bacteria per healthy flower are common on hosts of fire blight in the western United States (3,13,22,23). The presence of such high populations would suggest that the flowers are infected, but these infested flowers generally develop into normal fruit. Occasionally these epiphytically colonized blossoms become infected and a fire blight epidemic ensues. For example, numerous infections frequently follow rain and hail storms (7,13,24). Miller and Schroth (13) suggested that the infections developed because the injuries provided portals of infection for the entry of epiphytic bacteria.

Fire blight bacteria invade host flowers through natural openings or injuries. Hildebrand and MacDaniels (8) suggested that entry was through noncutinized stigmas and anthers, hydathodes on sepals, stomata on the style and sepals, and nectarthodes in the hypanthium. Injury allows entry into any wounded plant part.

Pierstorf (14) and Rosen (16) performed inoculation studies of pear and apple flowers and concluded that the most common site of infection was through the "nectariferous surface" of the floral cup (hypanthium) of pear flowers. Rosen (16) described nectarthodes as sites of infection on the hypanthium of pear flowers, but he also found that the stigmas of apple flowers served as infection sites. The difference in the susceptibility of pear and apple flowers was reportedly due to the shallow exposed tissue of the pear hypanthium compared to that of the hypanthium in apple, which is narrow and shielded by hairs.

Hildebrand (7) reported that inoculation of stigmas, anthers, and hypanthium of pear flowers with single cells of *E. amylovora* did not result in infection, whereas single-cell inoculations of the hypanthium of apple flowers resulted in infection. He was able to get infection via the stigmas of pear and apple flowers by using pollen contaminated with *E. amylovora* as inoculum and incubated the flowers at 20–42% RH. However, pear flowers inoculated only on the hypanthium and kept at 20–42% RH did not become infected. Infections were very high in inoculated apple or pear flowers kept at RH exceeding 58%, whether they had been inoculated on the hypanthium or the stigma.

Pear flowers have been reported to contain substances that were bactericidal to low inoculum levels of *E. amylovora*. Lelliot (12) observed a decline in populations of bacteria during the first 24 hr after inoculation on the hypanthium of pear flowers. Whereas, inoculum on the hypanthium of apple flowers started to multiply immediately. Similarly, Beer and Norelli (1) found a direct correlation between inoculum dose and symptom development on pear flowers. Inoculum levels of about 10^2 cells per blossom frequently did not develop symptoms, whereas flowers with populations of 10^7 bacteria were likely to be diseased 5 days later.

This study was made to determine where epiphytic bacteria are located on the host flower and how populations as high as 10^5–10^7 per flower can be present without causing infection. The study has been in progress for 10 yr and summarizes findings relative to the site of epiphytic colonization and the infection process under the arid growing conditions of California and Utah. Brief reports have been previously presented (20,21).

MATERIALS AND METHODS

Location of *E. amylovora* on epiphytically colonized flowers. Flowers of *Malus sylvestris* Mill., *Pyrus communis* L., *Pyracantha* sp. Roem., *Crataegus* sp. L. and *Cotoneaster* sp. Ehrh. were collected from orchards or plantings in Contra Costa, Lake, Mendocino, Sacramento, and Yolo counties in California, and Box Elder, Cache, and Utah counties in Utah. The plants had previously been shown to have epiphytic populations of *E. amylovora* on flowers by washing blossoms (22). Collection dates ranged from 1 April to 30 May in California and 15 May to 30 June in Utah. Flowers were aseptically collected by carefully placing a sterile disposable plastic tube (16 × 100 mm) over individual, open flowers and excising the pedicel by replacing the friction cap. Flowers in tubes were placed in an iced chest, transported to the laboratory, and processed within 2–24 hr. The number of flowers sampled ranged from 10 to 50 per location and were sampled through the years 1974 to 1983.

Flowers were removed from the tubes with sterile forceps and the five pistils from each flower were removed at the base with a second pair of sterile forceps. The appearance of the pistils was noted as either normal or necrotic. The pistils were inserted into a second tube containing 1 ml of sterile tap water (STW) and the remaining portion of the flower was washed in 10 ml of STW. Tubes were shaken on a vortex tube mixer and 0.1 ml of the wash water was spread on petri plates containing Miller-Schroth-sorbitol media (MSS) (22). These were incubated for 48–60 hr at 29 C, and the number of colonies were counted. To confirm the tentative identifications made on the selective media, representative colonies were tested periodically for reaction to antisera specific to *E.*

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked *"advertisement"* in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

amylovora and for ability to cause a hypersensitive reaction in tobacco leaves.

The individual pistils of 20 flowers were separated, washed, and aliquots of the wash water were plated as described above. Individual pistils were also placed on microscope slides and a drop of water was added while observing them at ×100 magnification.

Effect of rain on epiphytic populations. In two pear orchards in California and a mixed pear and apple orchard in Utah, the location of epiphytic populations of *E. amylovora* was monitored to determine the effect of precipitation on the location of epiphytic populations and symptom development. Sampling of individual flowers was performed as noted above before and after rain. Sample sizes ranged from 15 to 30 flowers per orchard and included the period from 1975 to 1983.

Inoculation of pistils and floral cups under controlled environments. The influence of the site of inoculation and incubation environment were studied in bouquets of pear flowers cut from an orchard in Lake County, CA. Bouquets incubated in the "dry" environment were placed in beakers of water in a lighted growth chamber at 21 C and relative humidity between 20 and 30%. The bouquets in the "humid" environment were also incubated in the light at 21 C, but the relative humidity was kept between 70 and 90%.

Inoculum of *E. amylovora* (Ea 27) was prepared from a 24-hr King's medium B slant (11). Flowers were inoculated by placing a 5-μl drop of bacterial suspension either in the floral cup at the base of the pistils or on the stigmatic ends of the five pistils. The droplet was calibrated to deliver 10^3 colony forming units (cfu). Inoculum levels greater than 10^3 cfu were not used because symptoms developed too rapidly to detect differences. The droplet placed on the stigmas in the "dry" environment usually remained in place until it dried. Flowers were dry during the remainder of the experiment. In the "humid" environment, the droplet intended for the stigmas occasionally ran down to the hypanthium and between the pistils. Condensation droplets usually formed on flower surfaces in the humid environment.

The populations in the flowers were checked immediately after inoculation and at 9, 21, 46, 67, and 117 hr. A sample of 10 flowers was taken at each time for each of the four conditions: hypanthium inoculated in humid and dry environments and stigma inoculated in humid and dry environments. Each flower was aseptically removed and dissected into two parts. The five pistils were placed in a tube and washed in 1 ml of STW. The remaining portion of the flower was also washed in 1 ml STW in a tube. Each tube was shaken for 15 sec on a vortex mixer, diluted by 10^{-2}, and 0.1 ml of the dilutions was spread on MSS with a bent glass rod. Controls consisted of flowers inoculated with STW and treated the same way except they were plated on MSS and King's medium B. Plates were incubated at 29 C and the colonies were counted. This experiment was repeated 7 May with bouquets of blossoms cut on 30 April and refrigerated to prevent premature opening.

Inoculation of pistil and floral cup outdoors. Flowers on a Bartlett pear tree in Berkeley, CA, were inoculated on 21 April 1977 with a 5-μl drop of Ea 49 calibrated to deliver 5.6×10^3 cfu on the stigmas or hypanthium. A sample of 20 flowers was taken 6 hr after innoculation and 1, 2, 4, 7, 12, and 14 days later. Washes of the pistils and hypanthia were plated on MSS media supplemented with streptomycin sulfate at 25 μg/ml.

Isolate Ea 49 was a naturally occurring, streptomycin-resistant strain of *E. amylovora* obtained from a pear orchard in Butte County, CA, in June 1976, and was used to expedite reisolation from the inoculated flowers. It was originally isolated on MSS media supplemented with 10 μg/ml streptomycin sulfate and subsequently found to be resistant to streptomycin at 100 μg/ml. It was characterized and found to conform to biochemical, serological, and pathological tests for *E. amylovora*.

Scanning electron microscopy. Pistils from pear and apple flowers suspected to be naturally colonized by *E. amylovora* were

TABLE 1. Incidence of *Erwinia amylovora* on floral parts of epiphytically colonized flowers

State and date	Location (county)	Host	Flowers		Population of *E. amylovora*	
			Sampled (no.)	Colonized (%)	Pistils[a] (cfu)	Flower[b] (cfu)
California						
1974						
12 Jun	Lake	Pear	10	100	1.1×10^6	7.1×10^3
26 Jun	Contra Costa	*Cotoneaster*	15	67	5.0×10^4	2.0×10^2
3 Jul	Contra Costa	*Pyracantha*	25	100	1.2×10^7	5.0×10
1975						
25 Apr	Solano	Pear	15	73	2.0×10^4	1.5×10^2
5 May	Yolo	Pear	28	89	2.4×10^4	4.2×10^2
7 May	Yolo	Pear	30	100	2.1×10^5	1.2×10^3
14 May	Yolo	*Crataegus*	15	53	3.6×10^5	2.1×10^3
16 May	Yolo	Apple	10	80	3.0×10^4	1.0×10
1976						
17 Apr	Yolo	Pear	50	100	5.0×10^7	2.0×10^2
28 Apr	Sacramento	Pear	15	100	6.7×10^5	1.0×10^2
5 May	Solano	Pear	23	65	2.3×10^5	1.7×10
1977						
24 May	Contra Costa	Pear	10	100	1.2×10^3	1.1×10
30 May	Mendocino	Pear	20	50	6.1×10^4	3.3×10^2
Utah						
1980						
21 May	Box Elder	Pear	10	70	5.2×10^4	1.2×10^2
21 May	Box Elder	Apple	10	100	3.1×10^5	2.1×10^2
21 May	Utah	Pear	10	60	1.1×10^4	0
21 May	Utah	Apple	10	90	2.6×10^4	7.2×10^2
1982						
1 Jun	Cache	Apple	20	55	5.5×10^7	8.1×10^4
1 Jun	Cache	Pear	20	60	1.2×10^5	4.4×10^4
1983						
2 Jun	Cache	Apple	25	92	8.1×10^5	2.3×10^2
2 Jun	Cache	Pear	25	100	1.4×10^5	6.2×10^3

[a] Represents the mean number of colony-forming units (cfu) from the groups of five pistils from the number of flowers sampled.
[b] Represents the mean number ot colony-forming units (cfu) from flowers without pistils.

divided into two groups. Two of the pistils from each flower were washed as noted above to determine the presence of epiphytic *E. amylovora*. If the two pistils were found to be colonized only with *E. amylovora*, then the other three pistils from the same flower were prepared for scanning electron microscopy.

Pistils were fixed in 2.0% osmium tetroxide in cacodylate buffer for 4 hr and rinsed twice in graduated ethanol. They were dehydrated in a series to 100% Freon. Specimens were critical-point dried in Freon and mounted on aluminum stubs. Gold was used to sputter coat the specimens. Microscopy was done on a Coates and Welter Model 50 scanning electron microscope.

RESULTS

Location of *E. amylovora* on epiphytically colonized flowers. Epiphytic populations of *E. amylovora* were found predominantly on the pistils of flowers (Table 1) in studies performed in California and Utah and on various hosts. The population of *E. amylovora* on the pistils was usually greater than the population on the remaining flower parts by a magnitude of one to six log units. In many cases, the population on flowers with pistils removed was so low it could have been attained by inadvertant contact of the pistils and other flower parts during transport and processing.

The physical appearance of the pistils was not an indication of the presence of *E. amylovora*. Many of the pistils were necrotic, especially in old flowers, but there was no relationship between the appearance of the pistils and the population of *E. amylovora* isolated. There was no water soaking nor any other evidence of infection. The incidence of disease in the respective orchards was always significantly less than the percentage of colonized flowers. In many cases, 90–100% of the flowers were colonized, but less than 1% of the flowers ever became diseased. Even flowers from orchards with populations of 10^7 bacteria per flower generally developed into apparently healthy fruit.

In studies in which individual pistils were washed separately, it was common to find that some of the pistils were free of bacteria. The number of colonized pistils ranged from 1 to 5 out of 5. Generally, when the population of bacteria was high (10^4 per flower) on the pistils there was a greater percentage of individual pistils colonized.

Bacterial streaming was apparent from epiphytically colonized pistils when viewed with the light microscope. The streaming originated from the stigmatic end and occurred within 15–30 sec after application of the water to the slide. Most of the bacteria appeared to be motile from the onset of streaming. Streaming continued for 5–15 min.

Effect of rain on epiphytic populations. Rain was instrumental in the redistribution of bacteria from pistils to other flower parts. (Table 2) A comparison of flowers before and after rain storms revealed that in most cases, the percentage of flowers with bacteria on floral parts, other than the pistil, increased dramatically. For example, prior to a 0.4-cm rain in a pear orchard in Yolo County, CA, only 20% of the flowers (pistils removed) were colonized with an average population of 2.1×10^2 per flower. However, 3 hr after the storm there was an increase to 75% of the flowers (without pistils) colonized with an average population of 1.3×10^4. Redistribution was similar in pear, apple, pyracantha, and hawthorne in California and Utah.

Inoculation of pistil and hypanthium under controlled environments. Bacteria survived on the pistils of almost all of the pistil-inoculated flowers whether incubated in the humid or dry environments (Table 3). Recovery from the hypanthium of pistil inoculated flowers 117 hr after inoculation was less frequent than pistil recovery but still averaged over 60% in the humid environment and about 32% in the dry environment (Table 3). At 117 hr after inoculation on the pistil, 100% of the pistils still had viable *E. amylovora*.

The percent recovery from the hypanthium-inoculated flowers

TABLE 2. Effect of rain on the redistribution of *Erwinia amylovora* on flowers

	Flower parts colonized (%)		Populations[a]	
	Pistils	Flowers[b]	Pistils	Flowers[c]
California				
Yolo County, 5 May 75				
(0.5 cm rain, 30 pear flowers)				
Before rain	100	17	7.4×10^4	4.2×10^2
Rain + 2 hr	90	53	5.1×10^4	2.2×10^3
Rain + 2 days	100	60	4.0×10^4	6.5×10^3
Yolo County, 14 May 76				
(0.4 cm rain, 15 pear flowers)				
Before rain	46	20	3.6×10^4	2.1×10^2
Rain + 3 hr	75	75	4.4×10^4	1.3×10^4
Sacramento County 28 Apr 77				
(1.27 cm rain, 15 pear flowers)				
Before rain	80	33	6.7×10^3	1.0×10
Rain + 5 hr	75	69	1.5×10^2	2.6×10^2
Utah				
Cache County, 27 May 80				
(0.2 cm rain, 20 pear flowers)				
Before rain	100	25	2.7×10^4	1.2×10^2
Rain + 1 hr	100	88	3.1×10^4	2.4×10^3
Cache County, 27 May				
(0.2 cm rain, 20 apple flowers)				
Before rain	90	35	3.2×10^5	7.1×10^2
Rain + 1 hr	85	75	2.1×10^5	5.4×10^3
Cache County, 2 Jun 83				
(0.76 cm rain, 25 *Pyracantha* flowers)				
Before rain	80	28	2.5×10^4	2.0×10
Rain + 1 hr	88	84	1.7×10^4	2.2×10^3
Cache County, 2 Jun 83				
Before rain	60	20	7.7×10^3	1.2×10
Rain + 1 hr	68	48	2.3×10^3	6.7×10^2

[a] Mean population of *E. amylovora* per flower part.
[b] Percent of flowers, with pistils removed and colonized with *E. amylovora*.
[c] Mean population of *E. amylovora* on flowers with pistils removed.

was considerably less than that of pistil-inoculated flowers and averaged less than 15% on flowers under humid or dry incubation conditions (Table 4). At 117 hr, the recovery did not exceed 20% of the inoculated flowers on either pistil or hypanthium.

The reisolation of *E. amylovora* from flower parts previously inoculated on the pistil was approximately one log unit less than the 10^3 cfu used in the inoculation (Figs. 1 and 2). The populations continued to decline on flower parts inoculated on the hypanthium up to the 8-hr reisolation in both the high and low humidity environments (Figs. 1 and 2). However, the population on the pistil-inoculated flowers in the humid environment remained the same or showed a slight increase at 8 hr and continued to increase to 10^6–10^7 per flower part (Fig. 1). The population on flower parts inoculated on the hypanthium and incubated in the humid environment remained less than the original inoculum until the 117-hr sample was taken. At that time, reisolation from the pistils was almost as high as that from the pistil-inoculated flowers (Fig. 1).

E. amylovora multiplied on the pistils of pistil-inoculated flowers in the dry environment about the same as on those inoculated in the humid environment. However, multiplication on the hypanthia of pistil-inoculated flowers was considerably less. Reisolations from the hypanthium-inoculated flowers was generally very low or nil (Fig. 2).

Although bacteria survived on flowers and multipied to populations between 10^5–10^7 per flower by 117 hr, only a small percentage of the flowers developed fire blight symptoms. At the conclusion of the study, most of the flowers had been used in the reisolation process. However, 73 flowers were kept for symptom observation. In the dry environment, only 8% of the pistil-inoculated flowers and none of the hypanthium-inoculated flowers became diseased while in the moist environment, 31 and 13% of the pistil- and hypanthium-inoculated flowers became diseased, respectively. The experiment repeated on 7 May yielded similar results.

TABLE 3. Recovery of *Erwinia amylovora* from parts of pear flowers inoculated on the pistils

Postinoculation time (hr)	Recovery[a] (%)			
	Humid incubation[b]		Dry incubation[c]	
	Pistil	Hypanthium	Pistil	Hypanthium
0	100	60	100	60
8	100	50	60	0
21	90	60	70	10
46	100	70	100	40
67	100	70	100	30
117	100	60	90	50
Mean	98.3	61.7	86.7	31.7

[a]Percent of 10 flowers from which *E. amylovora* was reisolated at the indicated postinoculation times.
[b]Humidity maintained between 70 and 90%.
[c]Humidity maintained between 20 and 30%.

TABLE 4. Recovery of *Erwinia amylovora* from parts of pear flowers inoculated on the hypanthium

Postinoculation time (hr)	Recovery[a] (%)			
	Humid incubation[b]		Dry incubation[c]	
	Pistil	Hypanthium	Pistil	Hypanthium
0	20	40	20	40
8	10	10	0	0
21	10	10	0	20
46	0	20	10	10
67	0	0	0	0
117	20	10	0	20
Mean	10	15	5	15

[a]Percent of 10 flowers from which *E. amylovora* was reisolated at the indicated postinoculation times.
[b]Humidity maintained between 70 and 90%.
[c]Humidity maintained between 20 and 30%.

Inoculation of pistil and floral cup outdoors. Temperatures throughout the study were atypically cool with maximum temperatures ranging from 15 to 20 C. Continuous, light rains occurred on the 3rd and 4th day after inoculation. The precipitation for the 2-day period was 7.6 mm. A second, similar rainy period occurred 9, 10, and 12 days after inoculation. Precipitation during this period was 8.2 mm.

Bacteria survived for up to 14 days on the pistils of 80% or more of the flowers inoculated on the pistils. In contrast, recovery from flower parts inoculated on the hypanthium declined to 20% or less within 2 days after inoculation. Rain was responsible for moving bacteria from the pistils of pistil-inoculated flowers to the hypanthium. For example, only 20% of the hypanthia were

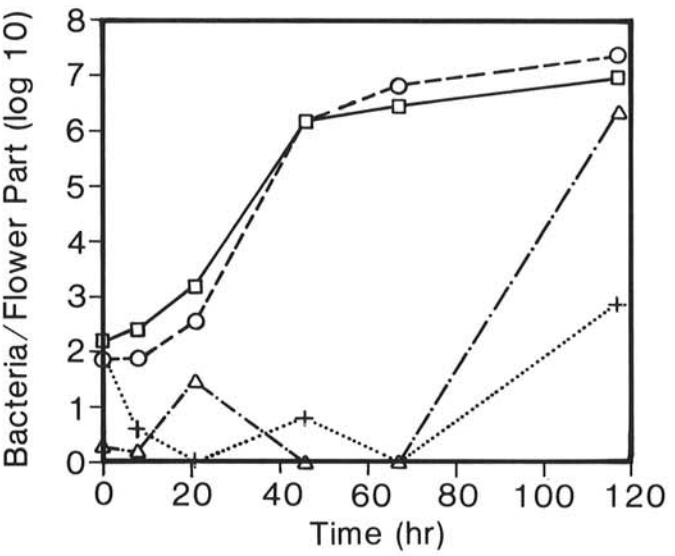

Fig. 1. Population of *Erwinia amylovora* reisolated from the pistils or hypanthium (minus pistils) of pear flowers incubated at 70–90% relative humidity for various lengths of time after inoculation of the pistils or hypanthium with *E. amylovora*. Pistil inoculation-pistil recovery □——□, pistil inoculation-hypanthium recovery o-----o, hypanthium inoculation-pistil recovery Δ–·–·Δ, hypanthium inoculation-hypanthium recovery +·····+.

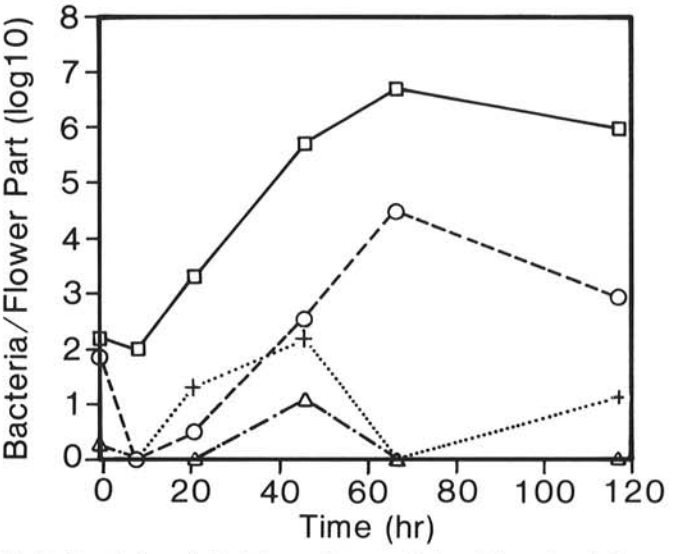

Fig. 2. Population of *Erwinia amylovora* reisolated from the pistils or hypanthium (minus pistils) of pear flowers incubated at 20–30% relative humidity at various lengths of time after inoculating the pistils or hypanthium with *E. amylovora*. Pistil inoculation-pistil recovery □——□, pistil inoculation-hypanthium recovery o-----o, hypanthium inoculation-pistil recovery Δ–·–·Δ, hypanthium inoculation-hypanthium recovery +·····+.

colonized on day 2 prior to the rain, but 100% were colonized after rain on days 2 and 4 (Table 5). There was a consistent increase in the percentage of colonized hypanthia following rain regardless of where inoculation occurred.

The decline in populations following inoculations on the hypanthium was similar to that noted in the inoculations made in the controlled environments. Populations recovered from the the hypanthium were about 800 cfu per flower immediately after inoculations on the hypanthium, but within 6 hr, they declined to 80 cfu per flower (Fig. 3). No bacteria were isolated from 20 flowers (pistils removed) previously inoculated on the hypanthium when sampled at 1 day. In contrast, populations of bacteria recovered from the pistils of pistil-inoculated flowers showed a slight decline from 5.6×10^3 down to 3×10^3 at 6 hr after inoculation but increased to 10^5 and 10^6 per flower at 2 and 4 days after inoculation respectively. At 14 days after inoculation, there were still populations of over 10^6 cfu on the pistils of pistil-inoculated flowers (Fig. 3).

The populations reisolated from all flowers on the 7th day after inoculation ranged from 10^4 to 5×10^6. However, symptoms were not apparent until 15 days after inoculation. Counts made 21 days after inoculation revealed that 62% (175/283) of the flowers inoculated on the pistil showed fire blight symptoms while only 18% (47/261) of the flowers inoculated on the hypanthium were infected.

TABLE 5. Percentage of flower parts colonized with *Erwinia amylovora* after inoculation of the pistils or hypanthium of Bartlett pear flowers outdoors at Berkeley, CA

Postinoculation time (hr)	Recovery[a] (%)			
	Pistil inoculated		Hypanthium inoculated	
	Pistil	Hypanthium	Pistil	Hypanthium
0.25	100	20	20	20
1	100	38	60	0
2	100	20	19	0
3 rain[b]				
4 rain	94	100	19	44
7	94	81	25	19
9 rain				
10 rain				
12 rain	88	88	75	75
14	80	70	0	10
Mean	93.7	59.6	31.1	24.0

[a] Percent of 20 flowers from which *E. amylovora* was reisolated at the indicated postinoculation times.

[b] Light rains on days 3 and 4 totaled 7.6 mm and on days 9, 10, and 12 totaled 8.2 mm.

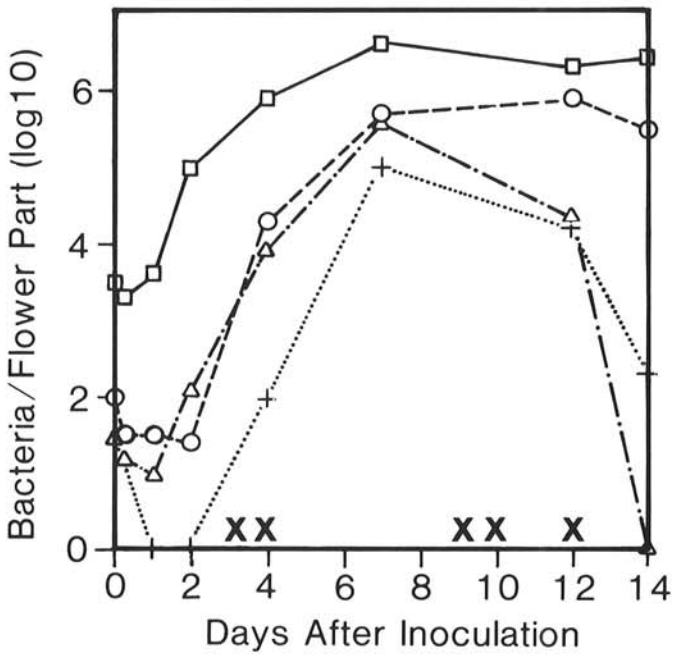

Fig. 3. Population of *Erwinia amylovora* recovered from the pistils or hypanthium (minus pistils) of pear flowers inoculated on the pistil or hypanthium of a Bartlett pear tree in Berkeley, CA. Pistil inoculation-pistil recovery □——□, pistil inoculation-hypanthium recovery o-----o, hypanthium inoculation-pistil recovery Δ-·-·Δ, hypanthium inoculation-hypanthium recovery +·····+, and rain, ×.

Scanning electron microscopy. Scanning electron microscopy revealed that bacteria were primarily present on the stigma (Fig. 4A to D). The style was usually free of bacteria except for a few cells close to the stigma. Bacteria were seen on the entire surface of the convoluted areas of the stigma. A mucilaginous material appeared to be present on the surface of most stigmas. Bacteria appeared to be undergoing binary fission based on the appearance of dividing cells (Fig.4B). There was no evidence of a pathogenic response. There was no apparent difference in the appearance or location of bacteria on pear or apple flowers (Fig. 4C and D).

DISCUSSION

The epiphytic populations of *E. amylovora* were located primarily on the stigmatic areas of pistils of every fire blight host surveyed including pear, apple, pyracantha, cotoneaster, and hawthorne. They were present at populations of 10^3 to 10^7 on pistils without causing symptoms and only very seldom did disease result from the infestations. This is in contrast to the results of Beer and Norelli (1) who found that infections were likely when epiphytic flower populations reached 10^6–10^7 cfu. However, their studies were performed with bacteria atomized on every flower part and under higher relative humidities than those experienced in this study.

Under the arid conditions in the west, where daytime humidities are between 20 and 60% and usually in the low range, the bacteria multiply on the stigmatic surfaces, frequently attaining populations of 10^5 to 10^7. The remaining portions of the flower are usually free of bacteria or have only a very small population. Colonization may be limited by the availability of moisture. The stigmatic surfaces provide a moist surface, whereas the hypanthium is usually dry under low relative humidities (7). A hydrophilic low-molecular-weight compound which augments moisture uptake has been identified and termed a "stigmatic hydration factor." This acts as a hygroscopic agent to maintain the relative humidity in excess of 98% on the stigma which is necessary for pollen germination (5). Amino acids, glycoproteins, and some carbohydrates are also present on stigmatic surfaces. These conditions appear ideal for the growth of bacteria. In addition to *E. amylovora*, populations of fluorescent pseudomonads were common inhabitants of the stigmas.

The growth of bacteria on the stigmas allows for the development of high populations which are transferred to other flower parts during a rain storm or with heavy dew. The deposition of greater populations of bacteria on the hypanthium is more likely to result in infection (7,8,14,16,17) than low populations because the latter generally decline rapidly due to natural defense mechanisms (6,12,15). This transfer of high populations of bacteria at one time explains how widespread disease epidemics develop following a rain storm. A heavy dew may also provide a means for movement of bacteria and could explain why infections occur without rain. A concentration of sugars in excess of approximately 30% in the nectar of pear and apple flowers was proposed as the mechanism restricting development of fire blight. Rain was thought to act as a diluent allowing growth of bacteria and resulting in infection (9,10). Sugar concentration was not investigated in this study, but these findings show that the bacteria are not present in nectar prior to rain and that physical movement by rain, rather than dilution of nectar, is the phenomenon that initiates infection after a rain storm.

Although it is difficult to prove conclusively, it appears that most infections of pear flowers result when high populations of bacteria reach the hypanthium. The inoculation of stigmas in this study eventually resulted in significant populations on other flower parts,

but only when flowers were incubated under high relative humidities or when rain occurred during the course of the experiment. The highest percentage of infections occurred when inoculations were made on the stigmas. However, the infection of these flowers probably occurred when the hypanthium of stigma-inoculated flowers became colonized with high populations of the pathogen transported there by rain or a meniscus of water between the pistils.

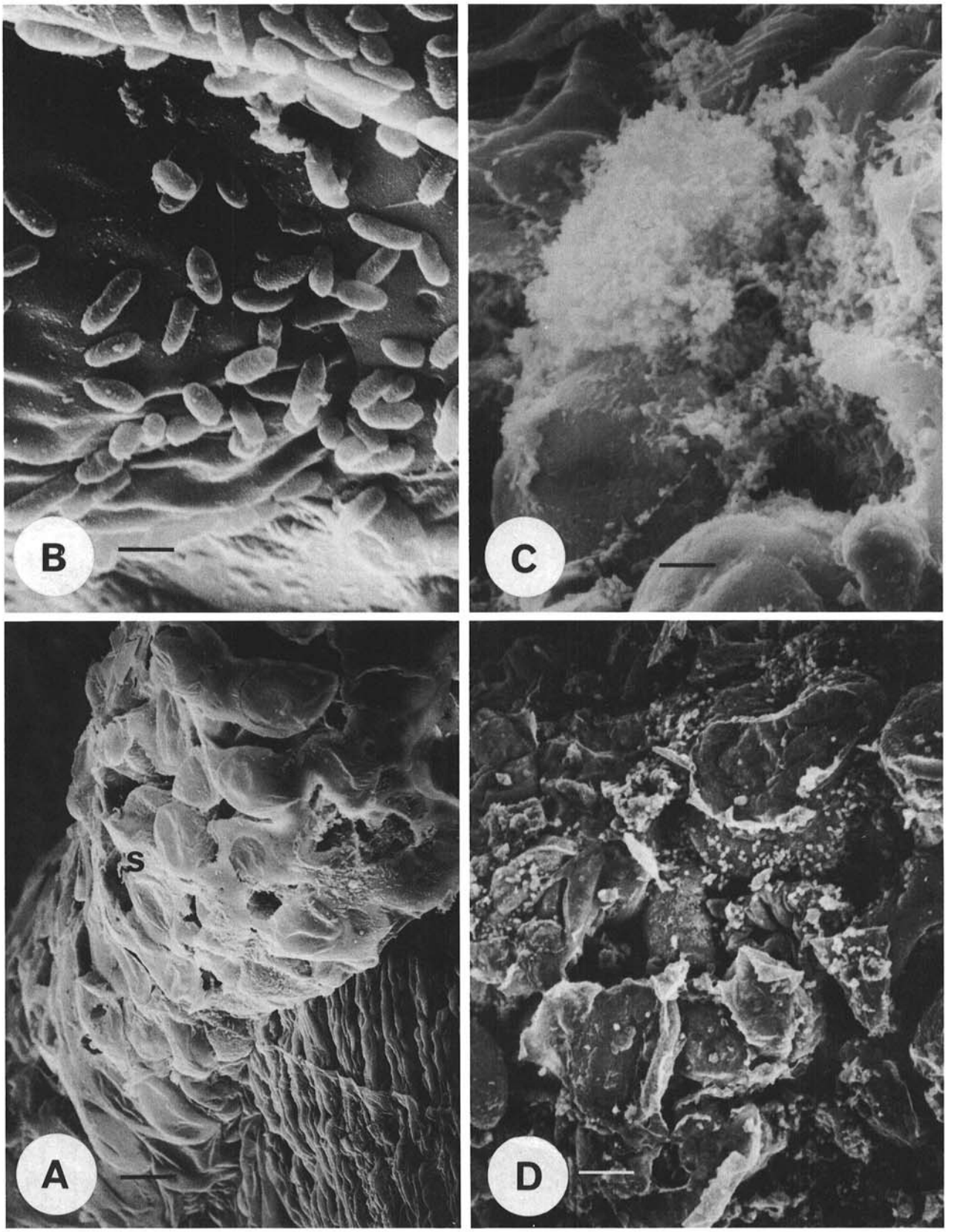

Fig. 4. Scanning electron micrographs of *Erwinia amylovora* on the stigmas of epiphytically colonized flowers. **A,** Stigma(s) of pear flower pistil with bacterial cells primarily present on the stigmatic surface. Bar approximately 10 μm. **B,** Close-up of bacterial cells on stigmatic surface of pear flower pistil. Bar approximately 1.0 μm. **C,** Mass of bacterial cells on stigmatic surface of pistil of apple flower. Bar approximately 20 μm. **D,** Bacterial cells on stigmatic surface of apple flower pistil. Bar approximately 20 μm.

The phenomenon of the growth of bacteria on the stigma in arid climates may explain why epiphytic populations are present in the west and not in New York and Michigan and other areas with similar humid climates (2,4,19). The frequent rains and high relative humidity present in the northeastern U.S. probably results in rapid growth of bacteria on the hypanthium where infections occur before a detectable epiphytic population develops. Infections are therefore more frequent and occur before they can be predicted by using epiphytic population measurements as a basis for forecasting when to apply bactericides (23).

There are several reasons why insect inoculation of the hypanthium of pear flowers is not likely to result in infection. The number of cells deposited during an insect visit may not be enough to cause immediate infection. The presence of hydroquinone in pear tissues may be involved in a defense mechanism preventing infection of flowers by low numbers of bacteria (6,15). The movement of insects while feeding on nectar and pollen result in frequent physical contact of the thorax with the stigmatic surfaces, whereas the hypanthium is generally limited to occasional contacts by only the probosis of nectar-feeding insects.

LITERATURE CITED

1. Beer, S. V., and Norelli, J.L. 1975. Factors affecting fire blight infection and *Erwinia amylovora* populations in pome fruit blossoms. (Abstr.) Proc. Am. Phytopathol. Soc. 2:95.
2. Beer, S. V., and Opgenorth, D. C. 1976. *Erwinia amylovora* on fire blight canker surfaces and blossoms in relation to disease occurrence. Phytopathology 66:317-322.
3. Covey, R. P. 1975. Fire blight control strategies—A look in the future. Wash. State Hortic. Assoc. Proc. 71:64-65.
4. Dueck, J., and Morand, J. B. 1975. Seasonal changes in the epiphytic population of *Erwinia amylovora* on apple. Can. J. Plant Sci. 55:1007-1012.
5. Ferrari, T. E., Lee, S. S., and Wallace, D. H. 1981. Biochemistry and physiology of recognition in pollen-stigma interactions. Phytopathology 71:752-755.
6. Hildebrand, D. C. 1970. Fire blight resistance in *Pyrus*: Hydroquinone formation as related to antibiotic activity. Can J. Bot. 48:177-181.
7. Hildebrand, E. M. 1937. Infectivity of the fire blight organism. Phytopathology 27:850-852.
8. Hildebrand, E. M., and MacDaniels, L. H. 1935. Modes of entry of *Erwinia amylovora* into the flowers of the principal pome fruits. (Abstr) Phytopathology 35:20.
9. Ivanoff, S. S., and Keitt, G. W. 1941. Relations of nectar concentration to growth of *Erwinia amylovora* and fire blight infection of apple and pear blossoms. J. Agric. Res. 62:733-743.
10. Keitt, G. W., and Ivanoff, S. S. 1941. Transmission of fire blight by bees and its relation to nectar concentration of apple and pear blossoms. J. Agric. Res. 62:745-753.
11. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
12. Lelliot, R. A. 1979. Effect of temperature on populations of *Erwinia amylovora* in host blossoms. (Abstr.) Page 527 in: Proc. 4th Int. Conf. Plant Pathogenic Bact., Angers, France.
13. Miller, T. D., and Schroth, M. N. 1972. Monitoring the epiphytic population of *Erwinia amylovora* on pear using a selective medium. Phytopathology 62:1175-1182.
14. Pierstorff, A. L. 1931. Studies on the fire blight organism, *Bacillus amylovorus*. N. Y. (Cornell) Agric. Exp. Stn. Mem. 136. 53 pp.
15. Powell, C. C. Jr., and Hildebrand, D. C. 1970. Fire blight resistance in *Pyrus*: Involvement of arbutin oxidation. Phytopathology 60:337-340.
16. Rosen, H. R. 1935. The mode of penetration of pear and apple blossoms by the fire blight pathogen. Science 81:26.
17. Rosen H. R. 1936. Mode of penetration and progressive invasion of fire blight bacteria into apple and pear blossoms. Arkansas Agric. Exp. Stn. Bull. 331. 68 pp.
18. Stewart, V. B., and Leonard, M. D. 1915. The role of sucking insects in the dissemination of fire blight bacteria. Phytopathology 5:117-123.
19. Sutton, T., and Jones, A. L. 1975. Monitoring *Erwinia amylovora* populations on apple in relation to disease incidence. Phytopathology 55:1009-1012.
20. Thomson, S. V. 1979. Stigmatic surfaces of pear pistils as a source of inoculum for *Erwinia amylovora*. (Abstr.) Page 816 in: Proc. 4th Int. Conf. Plant Pathgenic Bact., Angers, France.
21. Thomson, S. V., and Schroth, M. N. 1976. The stigmatic surfaces of pistils as the site of epiphytic colonization of pear flowers by *Erwinia amylovora*. Proc. Am. Phytopathol. Soc 3:257 (Abstr.).
22. Thomson, S. V., Schroth, M. N., Moller, W. J., and Reil, W. O. 1975. Occurrence of fire blight of pear in relation to weather and epiphytic populations of *Erwinia amylovora*. Phytopathology 65:353-358.
23. Thomson, S. V., Schroth, M. N., Moller, W. J., and Reil, W. O. 1982. A forecasting model for fire blight of pear. Plant Dis 66:576-579.
24. Van Der Zwet, T., and Keil, H. L. 1972. Importance of pear-tissue injury to infection by *Erwinia amylovora* and control with streptomycin. Can. J. Microbiol 18:893-900.
25. Waite, M. B. 1902. The relation of bees to the orchard. Cal. Cult. 18:390-391.