Erwinia amylovora on Fire Blight Canker Surfaces and Blossoms in Relation to Disease Occurrence

S. V. Beer and D. C. Opgenorth

Assistant Professor and former Experimentalist, respectively, Department of Plant Pathology, Cornell University, Ithaca, N. Y. 14853.

Present address of junior author: Department of Plant Pathology, University of Wisconsin, Madison 53706.

We acknowledge the technical assistance of J. B. Bruno, J. F. W. Eve, Jr., and L. S. Negi, the cooperation of the operators of the monitored commercial orchards, and that of the personnel and J. P. Tette, manager of the New York Apple Pest Management Project.

Accepted for publication 28 August 1975.

ABSTRACT

BEER, S. V., and D. C. OPGENORTH. 1976. Erwinia amylovora on fire blight canker surfaces and blossoms in relation to disease occurrence. Phytopathology 66: 317-322.

One pear orchard and four apple orchards in Wayne County, New York State, were monitored for the presence of *Erwinia amylovora* and the occurrence of blossom blight. The pathogen was isolated from holdover canker surfaces in all five orchards by a canker-swabbing technique in which two selective media were utilized. It was detected in blossoms of three of the five orchards 1-3 weeks before blossom infection appeared. The pathogen was not confirmed in blossoms from these orchards in sufficient time to indicate implementation of effective spray applications. Although blossom blight developed in all monitored orchards, more developed in the three orchards in which the pathogen had been detected previously in blossoms. *Erwinia amylovora* was detected sporadically throughout the season on cankers that had been produced the previous year. Some cankers apparently were active for most of the spring, others were only temporarily active, and *E. amylovora* was not isolated from the surface of others. Initiation of canker activity appeared to be positively correlated with warm (> 17 C), moist conditions at least 1 day prior to sampling. These studies indicated that holdover cankers may provide inoculum prior to, or without visible evidence of, renewed canker activity, and that *E. amylovora* may be detected in pome fruit orchards in New York before the development of blossom infection.

Additional key words: epidemiology.

Fire blight, caused by *Erwinia amylovora* (Burrill) Winslow et al., was the first plant disease to be attributed to a bacterial agent (1). In the northeastern United States, fire blight is devastating in some years, but of little or no importance in others (13). Therefore, fruit growers often are lax in implementing recommendations (4) for its control. Recently, the disease has assumed increased importance on apple in North America because of the shift to younger trees of highly susceptible cultivars grown on fire blight-suspectible size-controlling rootstocks (2, 8).

The blossom blight phase of the disease usually occurs prior to blight of leaves or vegetative shoots. It is important because it reduces yields (S. V. Beer, *unpublished*), may cause severe tree damage, and provides inoculum for other tree-damaging phases of the disease (9, 17). Absence of blossom blight may be explained by a lack of sufficient inoculum (10), unfavorable environmental conditions prior to bloom (15) or during and after bloom (13), nonsusceptibility of blossoms (19), or a combination of these factors.

The development of selective differential media (7, 12) has facilitated the detection of the pathogen in and around orchards. Studies on pear in California (12, 19) have indicated that large populations of *E. amylovora* may be found in blossoms and on young fruit and leaves prior to the appearance of blossom blight. Commercial enterprises are now using this methodology to monitor epiphytic populations of the bacterium in pear orchards (B. Zoller, *personal communication*). The data are used to improve the timing of spray applications to control fire blight in California (19).

The purposes of these studies were to determine whether *E. amylovora* could be detected in pome fruit orchards in the northeastern USA prior to the appearance of blossom blight, and thus to explore the possibility of developing spray recommendations that might be more cost-effective than those now in common use in New York (4). We also wished to determine whether the methodology of Miller and Schroth (12) or suitable modifications of it would be useful under New York State conditions.

MATERIALS AND METHODS

Orchards sampled.—Commercially maintained orchards (one pear and four apple) located in Wayne County, New York, and with a history of fire blight problems were selected for study. A summary of tree descriptions and of the extent of fire blight in the five orchards is given in Table 1.

Groups of 40 contiguous trees, located generally in the center of each orchard, were selected for monitoring. Temperature, humidity, and rainfall data were collected

TABLE 1. Orchards in Wayne County, New York, monitored for fire blight in 1974

Orchard	Cultivar[a]	Rootstock	Orchard age (years)	1973 Cankers[b] (Mean no. per tree)	Blossom infections[d] (Mean no. per tree)	Blossom infections[d] (Maximum no. per tree)
Apple						
1	Idared	M.7	8	<5	2.0	17
2	Twenty Ounce	Seedling	11	25	5.9	75
3	Idared	M.7	7	5	0.2	4
4	Monroe, Rome Beauty	Seedling	19	(25)[c]	0.8	20
Pear						
5	Bartlett, D'Anjou	Quince	12	5	0.1	2

with recording hygrothermographs and simple rain gauges placed in the center of each plot. An automatic recording rain gauge was located between Orchard 2 and Orchard 4. The 40 trees in each orchard were examined for fire blight cankers that remained from infection in 1973 at the start of our studies.

Monitoring procedures.—The orchards were visited about twice weekly starting at the 12.7-mm (half-inch) green growth stage (6) until mid-July. During each visit, estimates of flower bud development were made, weather stations were serviced, and cankers from the previous season's infections were examined. Careful surveys for new infections on blossom clusters and vegetative shoots were made. Because the trees were small, most blossom clusters were clearly visible from a distance of less than 1.5 meters. When infections were noted, the type (blossom or vegetative shoot) and location of each was recorded.

Five to 10 specific cankers formed in 1973 that had penetrated 1-year or older tissues were selected for monitoring in most orchards. In Orchard 4, 25 cankers were monitored. Cankers were examined for evidence of renewed pathological activity by visual inspection for surface ooze or watersoaking. Occasionally slight cuts were made near the margin in noncankered tissues to determine whether bark and cortex tissues were discolored and/or watersoaked. Surface populations of *E. amylovora* were sampled by swabbing intact canker margins with sterile cotton-tipped applicators (Swube®, Falcon Plastics, Oxnard, CA) previously dipped in sterile distilled water. The applicators were maintained in previously sterilized tubes in an ice chest between collection and processing.

Starting at the pink stage (6), 200 blossoms or young fruits (five from each tree) in each orchard (except Orchard 4) were collected in a random manner. Before picking, hands were dipped in 70% (v/v) ethanol and allowed to air-dry. The 200-blossom sample from each orchard was placed in a new polyethylene bag, and held in an ice chest until processed.

Assay of orchard samples.—Canker swabbings and blossom samples were usually processed the day of collection and in all cases within 24 hours after collection. Canker swabs were plated on one general, and two specific, bacteriological media. Plates were swabbed directly with the moist applicators or with washings from the applicators. Swabs were washed by adding 4-ml of sterile distilled water to each tube containing an applicator and shaking for 0.5 hour. Aliquots (0.1 ml) of wash water (or 1:100 dilutions in sterile distilled water) were plated in triplicate on prepared plates of each of the three media discussed below. The sample was spread on the agar surface with an L-shaped glass rod with the aid of a turntable (Fisher Scientific, Rochester, NY). Plates were then incubated inverted at 27 ± 1 C for 48-72 hours.

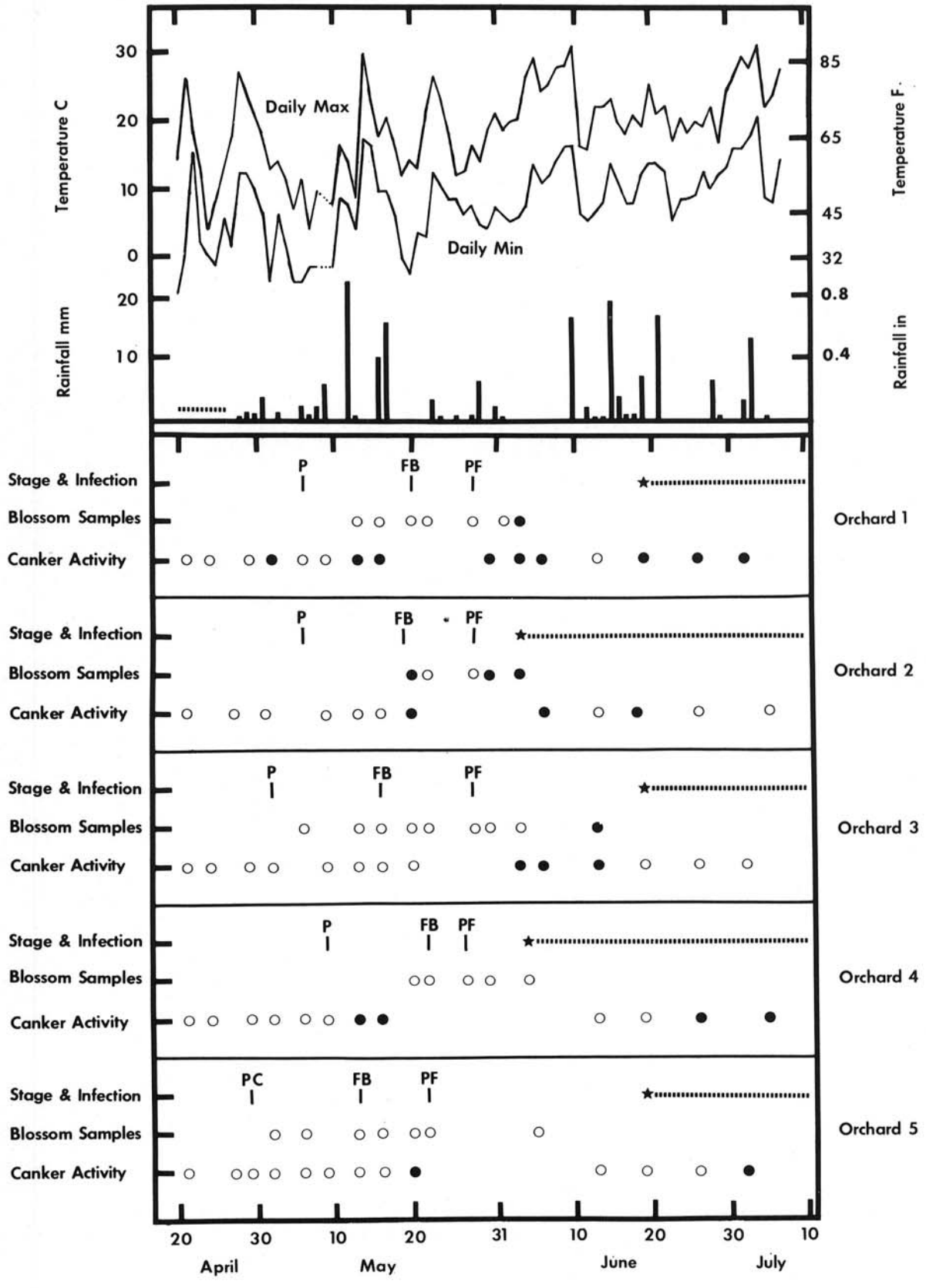
Fig. 1. Monitoring of fire blight cankers, pome-fruit blossoms, and young fruits for *Erwinia amylovora* and the appearance of blossom infection as related to growth stage, ambient temperature, and rainfall. Four apple orchards (Orchards 1-4) and one pear orchard (Orchard 5) were located in Wayne County, New York, within 33 km of each other. Temperature data are from Orchard 5. Daily rainfall data (starting 26 April) are from a site located between Orchard 2 and Orchard 4. Canker activity was determined by swabbing intact canker margin surfaces with cotton-tipped applicators, and plating washings of the applicators on three bacteriological media. Blossoms and young fruits were collected, washed with sterile water, and the washings were plated. The pathogenicity of resulting colonies was tested by inoculating *Cotoneaster pannosa* shoots or *Pyrus communis* fruits. Open circles indicate that *E. amylovora* was not isolated from blossoms, or from any canker sampled on the indicated date. Closed circles indicate that either blossom samples, or at least one canker, yielded *E. amylovora*. Blossom cluster infection was first observed on the dates marked by stars, and was present thereafter (dashed line). Growth stages are abbreviated as follows: P = Pink, PC = Popcorn, FB = Full Bloom, PF = Petal-Fall. *E. amylovora* was isolated from all orchards before blossom blight was noted.

[a]Orchards 1-4 had *Malus sylvestris* cultivars; Orchard 5 had *Pyrus communis* cultivars.

[b]All except Orchard 2 were pruned before April, 1974, by the orchard operators in an attempt to remove all fire blight cankers. The approximate mean number of cankers per tree remaining in 40-tree blocks in April, 1974, is indicated.

[c]An attempt was made to remove all cankers from this block except for 25 on one tree.

[d]Cumulative number of blossom cluster infections observed prior to 3 July 1974 in the monitored 40-tree block in each orchard.


Temperature C
30
20
10
0
Daily Max
Daily Min
85
65
45
32
Temperature F.
Rainfall mm
20
10
0.8
0.4
Rainfall in
Stage & Infection
Blossom Samples
Canker Activity
P
FB
PF
Orchard 1
Orchard 2
Orchard 3
Orchard 4
PC
Orchard 5
20
30
10
20
31
10
20
30
10
April
May
June
July

Blossom samples from each orchard were suspended in 200 or 300 ml of sterile distilled water in 300- or 500-ml Erlenmeyer flasks, respectively. The flasks were shaken vigorously for 0.5 hour on a wrist-action shaker at 20-25 C. The blossom wash water was plated in the same manner as applicator wash water.

Miller and Schroth's medium with sorbitol as the carbon source (12) (M-S) was used as modified (19) by the addition of cobaltous chloride (167 mg/liter). On M-S medium, after incubation for 48-72 hours at 27 C, colonies of *E. amylovora* are smooth, slightly domed with entire translucent margins, and generally are creamy-orange with brighter orange centers. A sucrose crystal-violet medium (C-G) (7) was also used on which colonies of *E. amylovora* usually develop characteristic surface craters after incubation for 48-72 hours at 27 C. Nutrient agar (N-A) (Difco Laboratories, Detroit, MI) was used as a nonselective control medium. Media (22-ml amounts in 100 × 15 mm polystyrene petri dishes) used in monitoring was not more than 2 weeks old and generally was less than 1 week old.

Colonies that grew on selective media were tentatively identified as *E. amylovora* on the basis of colony appearance (morphology, color, and size) relative to that of an authentic isolate of *E. amylovora* (18). Two or three sample colonies that had the characteristic appearance of *E. amylovora* on one specific medium were transferred to the other specific medium and incubated at 27 ± 1 C for 48-72 hours. Colonies that appeared similar to the authentic isolate of *E. amylovora* on both media were tested for pathogenicity by inoculating shoots of *Cotoneaster pannosa* or immature *Pyrus communis* 'Bartlett' fruits by the procedures of Seemüller and Beer (18). A colony was considered to be *E. amylovora* only if it gave the expected reaction on both specific media, and also a positive pathogenicity test. Bacteria isolated from cankers and blossoms were treated similarly.

RESULTS

Fire blight blossom infection developed in all the monitored orchards during the 1974 season. The pathogen was detected on canker surfaces in all five orchards, and in blossoms in three orchards, prior to the observation of blossom infection. A summary of the monitoring results in each orchard is presented in Fig. 1. The daily maximum and minimum ambient temperatures measured in centrally located Orchard 5 are presented. Daily rainfall data from a site midway between Orchard 2 and Orchard 4 also are presented. Weather conditions were similar in all orchards.

Orchard 1.—*Erwinia amylovora* was isolated from the surface of one of five swabbed cankers on 2 May, and from two other cankers during bloom. Canker margin surfaces were slightly reddened, but no ooze was apparent at the time of swabbing. One canker that became active on 16 May continued to yield the pathogen throughout the study. Ooze was first noted on its surface 2 weeks later. *Erwinia amylovora* was not recovered from blossoms until after petal-fall. During bloom, up to 1,500 unidentified bacteria per blossom grew on nutrient agar. Up to 600 colonies per blossom grew on the M-S and C-G media, but samples of these were not pathogenic. Many of the colonies that developed on both specific media from the petal-fall blossom sample were similar in appearance, and resembled sample colonies that were pathogenic.

On 19 June, six and 12 blossom cluster infections were first noted on the two trees that had active cankers during bloom. Adjacent trees had nine, 13, zero, zero, and one blossom cluster infections. Other trees in the 40-tree block had a mean of six-tenths infected cluster per tree.

Orchard 2.—*Erwinia amylovora* was first isolated from the surface of one canker and from the blossom samples collected during full bloom. No ooze was apparent on the canker surface, and it did not yield the pathogen again. Blossom cluster infection was first observed 1 week after petal-fall on the tree with the canker that was active earlier. Swabbings of a canker (not previously swabbed) on another infected tree on 5 June contained the pathogen. Following several days of warm rains, *E. amylovora* was isolated on 19 June from three of seven swabbed cankers. These three had reddened margins and appeared active as evidenced by red-brown discoloration of extra-cambial tissues, but ooze was not present on the canker surfaces. Another canker (not swabbed previously) was oozing and the pathogen was isolated from its surface. The four active cankers did not yield the pathogen subsequently.

The full-bloom blossom sample yielded about 2,500 unidentified colonies per blossom on nutrient agar, and 1,300 colonies per blossom on both M-S and C-G media. Some of the colonies that grew on M-S and C-G media were *E. amylovora*. Two subsequent blossom samples also contained the pathogen.

Fire blight had been particularly severe in Orchard 2 in 1973 (Table 1). Many of the previous years' lesions had penetrated 3-year-old wood. More fire blight eventually developed in Orchard 2 than in the other orchards studied. Several trees had 40-50 blossom cluster infections and many vegetative shoot infections.

Orchard 3.—*Erwinia amylovora* was isolated from one oozing canker after petal-fall on 3 June, and again on the two subsequent sampling dates. *Erwinia amylovora* was found in the 13 June blossom and small fruit samples. Two blossom cluster infections, first observed on 19 June, were on the same tree and within 30 cm of the swabbed canker that had yielded *E. amylovora* 16 days earlier. One canker on a tree just outside the 40-tree monitored block had visible surface ooze during full bloom on 20 May.

Orchard 4.—The monitoring design in this orchard differed from the others. Before growth started, we attempted to remove all cankers from the 40-tree block, except for those on one tree. This was done in an attempt to pinpoint the potential source of fire blight inoculum to one tree. Twenty-five cankers from 1973 were selected and six to eight of these were swabbed on each visit to the orchard. One canker yielded *E. amylovora* twice during bloom; some cankers were inactive until late June. None of the cankers that yielded *E. amylovora* had obvious ooze, but some pathological activity was evident on several when tissues near the canker margins were cut. Fifty-blossom samples from the cankered tree, and 200-blossom samples from the eight nearest trees were collected. *Erwinia amylovora* was not confirmed in any of those samples. As many as 2,500 unidentified colonies per blossom grew on N-A from the cankered tree blossom samples, and more than 2×10^4 colonies per blossom grew on N-A from the blossom samples of surrounding trees.

The cankered tree had no blossom infection on 13 June, while several of the adjacent trees did. Removal of a larger proportion of the blossoms from the cankered tree during sampling may account for these observations.

Orchard 5.—This was the only pear orchard studied. *Erwinia amylovora* was recovered from the surface of only one canker on only one occasion (20 May during bloom). Many unidentified bacteria grew on N-A and the selective media from most blossom collections. One blossom cluster and one vegetative shoot infection were observed on 19 June, more than 1 month after full bloom. This orchard was sprayed with streptomycin twice during bloom on 11 May and 14 May. These sprays likely prevented more severe blossom blight in this orchard (Table 1).

DISCUSSION

Erwinia amylovora was isolated from the surfaces of fire blight cankers in one pear and four apple orchards, often before any external symptoms or signs of pathological activity were apparent. Many of the cankers from which the pathogen was recovered initially by swabbing later oozed or had typical water-soaking and red to brown discoloration of the extra-cambial tissues. *Erwinia amylovora* was not recovered from canker surfaces before bloom except in one instance, and it was not isolated from blossom samples taken during early bloom stages. The pathogen was recovered from later blossom samples before blossom blight was observed in three of the apple orchards.

Neither of the two selective media used to monitor *E. amylovora* was ideal. Under New York orchard conditions, platings of blossom and canker washings usually resulted in many colony types. When *E. amylovora* was isolated, it was rarely isolated exclusively. Many colonies that grew on M-S medium had colony characteristics resembling those described by Miller and Schroth (12) for *E. amylovora,* but many of these were not pathogenic and did not form craters when transferred to C-G medium. In processing many samples on the C-G media, it was difficult to examine all colonies, but many had no craters. In some cases, fast-growing bacteria overgrew other colonies, and prevented a determination of cratering. However, each isolate that gave the expected reaction on both M-S and C-G media was pathogenic. *Erwinia amylovora* might not have been detected in some blossom or canker samples because the pathogenicity of only a small proportion of the colonies that grew on the two selective media was tested. Thus, the data presented in Fig. 1 are probably biased in favor of negative indications of the pathogen.

The canker-swabbing technique used in these studies is of value for determining the activity of "holdover cankers" [sensu Brooks (5)] prior to visual evidence of activity. It is also useful for identifying those cankers that may produce significant amounts of inoculum but never produce visually detectable ooze. Brooks (5) suggested, "In some cases exudation (of ooze) takes place even before the disease is macroscopically apparent in the tissues." Through canker swabbing, we have demonstrated the merit of his suggestion. Our work also may explain Rosen's (16) frustration (and that of other earlier workers cited by Rosen) caused by failure to identify sources of initial inoculum in orchards where, despite careful observations, oozing cankers were not found before blossom blight developed.

Brooks (5) noted that exudation of ooze by new infections and by holdover cankers was favored by relatively warm temperatures (18.3 − 29.5 C) and by relative humidities of 80% or more. Environmental and/or other factors that effect the initiation of growth of the pathogen in holdover cankers are not understood. In our studies, the pathogen was isolated from the surface of some cankers early in the season, but much later in others. Some cankers apparently were active for a relatively short time; others were active for most of the season. Analysis of our weather data suggested that initiation of canker activity is preceded by relatively warm temperatures (greater than 17 C) and rainfall. Few cankers were active immediately following very cool temperatures (<5 C) or frost. Inactivity of cankers might account for Powell's (15) observations that severe blossom blight does not occur unless at least 30 degree-days [temperature base = 18.3 C (65 F)] are accumulated between the last frost and early bloom.

Parker (14) concluded that the initial dissemination of the fire blight pathogen from its source (holdover cankers) to the primary infection court (usually a blossom) is accomplished by crawling and flying insects and by rain. In the three orchards where *E. amylovora* was detected in blossom samples, there was rainfall (and an opportunity for dissemination) in the time interval between detection of the bacteria on canker surfaces and in blossoms.

Large populations of *E. amylovora* [relative to those found in California (12, 19)] were rarely detected in blossom samples prior to the development of blossom infection. The absence of large populations of the pathogen and the development of low levels of blossom blight are probably related. The frequent absence of the pathogen from our bulk blossom samples might be caused by small populations in individual blossoms, or a small proportion of infested blossoms in the orchards. The sensitivity of the techniques used may have been insufficient to detect small populations of *E. amylovora* in the presence of large numbers of other bacteria.

Our data suggest that the amount of fire blight that occurred in the monitored orchards in 1974 was limited by the amount of inoculum in blossoms. The weather conditions during and just after bloom (Fig. 1) apparently were favorable and satisfied Mills' (13) criteria for the development of infection. Some blossom infection was observed in all orchards. In addition, in inoculation experiments carried out in the same area and at the same time (3, and S. V. Beer, *unpublished*), the amount of blossom cluster infection produced was correlated with the concentration of *E. amylovora* applied to apple blossoms during bloom.

Erwinia amylovora was not detected in blossoms in sufficient time to prevent infection by the immediate application of bacteriotoxic compounds. Under our conditions, by the time the pathogen was confirmed in blossoms, the disease could not have been controlled with available pesticides (11). Blossom monitoring appears to be of more value in California because there the primary and rattail bloom periods are long. If *E. amylovora* is detected in blossoms and sprays are indicated, the sprays

apparently protect blossoms which open subsequent to sampling; not those that were open at the time of sampling. Under New York conditions, bloom periods are generally short (less than 2 weeks) and significant numbers of rattail blooms are rare. However, epiphytic *E. amylovora* in blossoms must be considered also as a source of inoculum for later vegetative shoot infection.

The studies reported here are continuing. Similar studies in other geographical areas are needed. It is hoped that through a more thorough understanding of the factors affecting canker activity and dissemination of inoculum, it may be possible to predict, in advance of bloom, the presence of *E. amylovora* in pome fruit orchards and the probability for the development of severe epiphytotics.

LITERATURE CITED

1. BAKER, K. F. 1971. Fire blight of pome fruits: the genesis of the concept that bacteria can be pathogenic to plants. Hilgardia 40:603-633.
2. BEER, S. V., and H. S. ALDWINCKLE. 1974. Fire blight in New York State. N. Y. Food Life Sci. Quart. 7(1):16-19.
3. BEER, S. V., and J. F. W. EVE, JR. 1975. Apple–fire blight. Page 10 *in* C. W. Averre, ed. Fungicide and nematicide tests–results of 1974, Vol. 30. Am. Phytopathol. Soc., St. Paul, MN. 209 p.
4. BRANN, J. L. JR., P. A. ARNESON, and G. H. OBERLY. 1975. 1975 Treefruit production recommendations. N. Y. State Coll. of Agric. and Life Sci. 48 p.
5. BROOKS, A. N. 1926. Studies of the epidemiology and control of fireblight of apple. Phytopathology 16:665-696.
6. CHAPMAN, P. J. 1966. Standard names for key apple bud stages. Proc. N. Y. State Hortic. Soc. 111:146-149.
7. CROSSE, J. E., and R. N. GOODMAN. 1973. A selective medium for and a definitive colony characteristic of Erwinia amylovora. Phytopathology 63:1425-1426.
8. CUMMINS, J. N., and H. S. ALDWINCKLE. 1973. Fire blight susceptibility of some apple rootstock clones. Hortic. Sci. 8:176-178.
9. EDEN-GREEN, S. J., and E. BILLING. 1974. Fireblight. Rev. Plant Pathol. 53:353-365.
10. HILDEBRAND, E. M. 1937. Infectivity of the fire-blight organism. Phytopathology 27:850-852.
11. LUEPSCHEN, N. S., K. G. PARKER, and W. D. MILLS. 1961. Five-year study of fire blight blossom infection and its control in New York. N. Y. Agric. Exp. Stn. Bull. 963:1-19.
12. MILLER, T. D., and M. N. SCHROTH. 1972. Monitoring the epiphytic population of Erwinia amylovora on pear with a selective medium. Phytopathology 62:1175-1182.
13. MILLS, W. D. 1955. Fire blight development on apple in western New York. Plant Dis. Rep. 39:206-207.
14. PARKER, K. G. 1936. Fire blight: overwintering, dissemination, and control of the pathogene. N. Y. (Cornell) Agric. Exp. Stn. Memoir 193:1-42.
15. POWELL, D. 1963. Prebloom freezing as a factor in the occurrence of the blossom blight phase of fire blight of apple. Trans. Ill. State Hortic. Soc. 97:144-148.
16. ROSEN, H. R. 1929. The life history of the fire blight pathogen, Bacillus amylovorus, as related to the means of overwintering and dissemination. Ark. Agric. Exp. Stn. Bull. 244:1-96.
17. SCHROTH, M. N., S. V. THOMSON, D. C. HILDEBRAND, and W. J. MOLLER. 1974. Epidemiology and control of fire blight. Annu. Rev. Phytopathol. 12:389-412.
18. SEEMÜLLER, E. A., and S. V. BEER. 1976. Absence of cell wall polysaccharide degradation by Erwinia amylovora. Phytopathology 66: (In press).
19. THOMSON, S. V., M. N. SCHROTH, W. J. MOLLER, and W. O. REIL. 1975. Occurrence of fire blight of pears in relation to weather and epiphytic populations of Erwinia amylovora. Phytopathology 65:353-358.