

# Role of Wind-Driven Rain, Aerosols, and Contaminated Budwood in Incidence and Spatial Pattern of Fire Blight in an Apple Nursery

P. S. McMANUS and A. L. JONES, Department of Botany and Plant Pathology and the Pesticide Research Center, Michigan State University, East Lansing 48824-1312

## ABSTRACT

McManus, P. S., and Jones, A. L. 1994. Role of wind-driven rain, aerosols, and contaminated budwood in incidence and spatial pattern of fire blight in an apple nursery. *Plant. Dis.* 78:1059-1066.

The role of wind-driven rain, rain-generated aerosols, and *Erwinia amylovora* contaminated budwood in the epidemiology of fire blight in a Michigan apple (*Malus × domestica*) nursery was investigated in 1992 and 1993 and in a simulated nursery planting at Michigan State University (MSU) in 1993. A degree-day model, MARYBLYT, was used to assess whether fire blight outbreaks were associated with storms containing wind-driven rain. Most outbreaks, especially those occurring early in the season, were associated with previous storms. Spatial lag autocorrelation analysis indicated that when fire blight was initially detected in the nursery each season, significant autocorrelations among spatial lags generally were within-row or formed tight clusters. Following storms containing wind-driven rain, autocorrelation matrices showed significant values across rows, often at high-order, noncontiguous spatial lags. Ordinary runs analysis indicated strong within-row aggregation of fire blight in the MSU nursery throughout the season, whereas significant across-row aggregations were apparent only later in the season following storms containing wind-driven rain. The possibility that *E. amylovora* becomes suspended in aerosols during rain was investigated by collecting air samples using a six-stage microbial impaction air sampler. All air samples collected during rain contained *E. amylovora*, whereas samples collected during dry periods contained very few or no colony-forming units of the pathogen. Using traditional plating techniques, *E. amylovora* was not detected in 115 10-bud samples collected in 1992 from a budwood orchard. In 1993, *E. amylovora* was detected in 6 and 16% of washings from budsticks and leaves by plating and a polymerase chain reaction method, respectively. We conclude that wind-driven rain was the most important factor involved in spreading *E. amylovora* in the nursery.

Fire blight, caused by *Erwinia amylovora* (Burrill) Winslow et al. is the most destructive bacterial disease affecting apple (*Malus × domestica* Borkh.), pear (*Pyrus communis* L.), and rosaceous ornamentals. *E. amylovora* is difficult to control and nearly impossible to eradicate, because once established in its host, low populations persist and overwinter in symptomless tissue (5,7,24,34,38,45). Despite intense efforts to prevent its introduction, *E. amylovora* is often present in pome tree nurseries (3,42,48). Nursery trees are at high risk for fire blight since regular fertilization and irrigation promote succulent growth, which is especially vulnerable to the pathogen (3,8,12,27,42,48). Contaminated propagation wood and nursery stock are potential means of long-distance dispersal of *E. amylovora* (2,9,38,42,46). Maintaining uncontaminated nursery stock is crucial in preventing the introduction of *E. amylovora* into areas where blight is not known, and in limiting the introduction of new genotypes of the pathogen to areas where blight is already established.

Few studies have attempted to determine how *E. amylovora* enters nurseries or how it is disseminated after introduction. Pear trees in a nursery in New York succumbed to blight after *E. amylovora* was apparently introduced on budwood and disseminated by means of contaminated budding knives (42). Internally infested scionwood was implicated as the source of *E. amylovora* when three of 600 pear rootstock seedlings became infected after being grafted with surface-sterilized, symptomless buds (47). Pruning tools have also been reported to transmit the blight organism (42, 44,48). Bauske (3) reported that the incidence of fire blight in pear nurseries in Iowa was directly related to the degree of exposure of trees to prevailing southerly winds, and spread of blight was reduced by the use of windbreaks. Moreover, winds of 3–6 m/sec moved waterborne *E. amylovora* at least 1 m, the distance between nursery rows (3,4). While the pattern of blighted trees in the nursery was affected by the weather, comprehensive weather data were not recorded. Thus, a correlation between wind-driven rain and disease incidence was not established. Also, no attempt was made to identify the original sources of *E. amylovora*.

Spatial lag autocorrelation analysis has been used to test whether the value of a variable at one location in a field

plot is dependent on values of the variable at neighboring locations (10,11,16, 17,19,20,36). Spatial pattern analyses, including lag autocorrelation and ordinary runs, have been used to relate incidence and aggregation of bacterial diseases in citrus nurseries in Florida and Argentina to biological and environmental factors, including cultural practices (16,18,19,21). In the citrus nursery studies, factors affecting spatial patterns of disease included host susceptibility, aggressiveness of the pathogen strain, defoliation and regrowth of the host, introduction of the pathogen on contaminated rootstock, bactericide application, mechanical spread during routine maintenance operations, and wind-driven rain. Within-row aggregation of disease was generally attributed to cultural and mechanical factors, whereas across-row aggregation of disease was related to wind-driven rain.

The purpose of this study was to identify factors involved in the introduction and spread of fire blight at an apple nursery by analyzing spatial patterns of disease and relating these patterns to weather phenomena and cultural practices. In addition, entry of *E. amylovora* into the nursery on contaminated budwood was investigated.

## MATERIALS AND METHODS

In 1992, three plots were established at an apple nursery in Michigan. Plots were 30 m long and consisted of 10, 11, or 13 rows running north-south. Trees were spaced about 25 cm apart within rows, with 1.5 m between rows. Thus, a plot of 10, 11, or 13 rows contained approximately 1,200, 1,320, or 1,560 trees, respectively. Trees were fire blight susceptible cultivars grafted to M.26 rootstock, also susceptible to fire blight. In 1993, four plots were established in a nursery block 1.6 km from the 1992 site. Plots were 30 m long and consisted of 15 rows running east-west, and spacing of trees and rows was the same as for the 1992 plots. Each plot contained approximately 1,800 trees. Trees were fire blight susceptible cultivars on M.9 or Mark rootstocks, both susceptible to fire blight. In all nursery plots, the scion cultivar was uniform within rows but varied among rows within a plot. The trees were trickle-irrigated, and fertilizer and pesticides, including copper bactericides and streptomycin, were applied as needed to maintain vigorous growth.

This research was supported in part by the Michigan Agricultural Experiment Station.

Accepted for publication 15 August 1994.

Plots were monitored for fire blight at least once per week from June through August of each year. On each census date, the position of each blighted tree was mapped within the plot and then the tree was removed from the nursery.

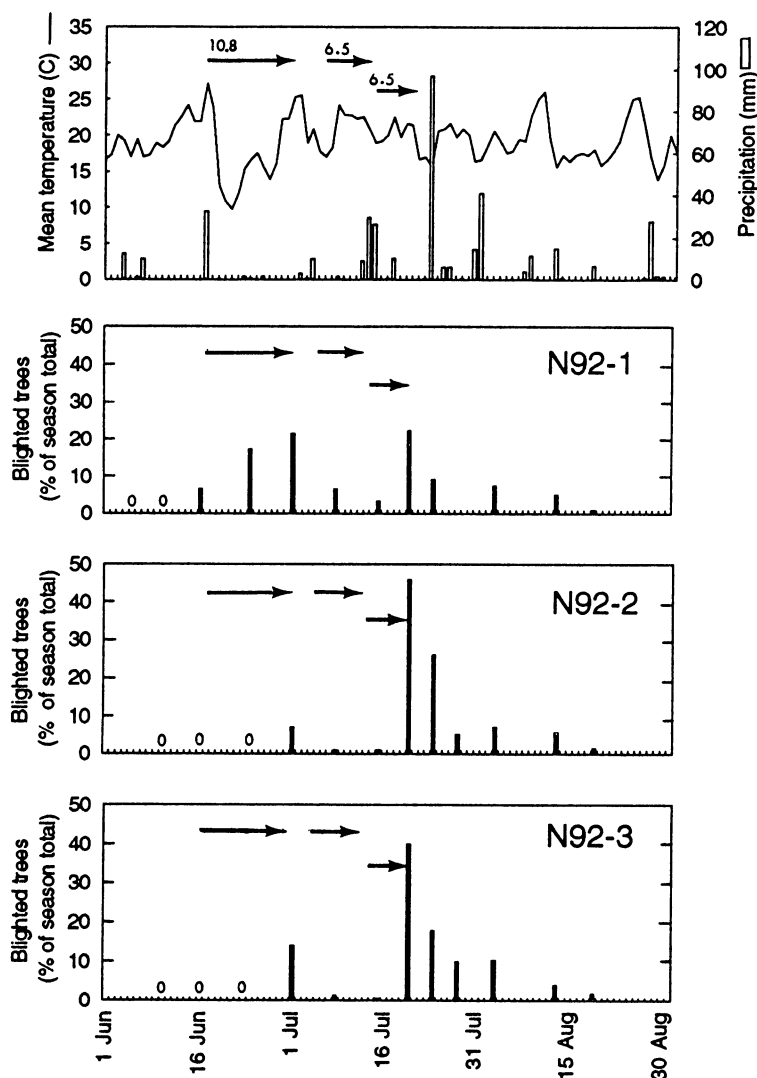
A simulated nursery planting of four plots, each consisting of five rows of 22 1-yr-old apple trees, was established at the Botany and Plant Pathology Farm of Michigan State University (MSU) at East Lansing on 3 June 1992. Trees were planted 30 cm apart within rows, with 1.5 m between rows, which ran north-south. Trees were fire blight susceptible cultivars on M.26 rootstock. In 1993, trees were cut back to 5–7 cm above the graft union, and the scion was trained to a single shoot. The trees were trickle-irrigated, and fertilizer and pesticides (no bactericides) were applied as needed until late August. A nalidixic acid resistant mutant of *E. amylovora* was selected from plates of King's B medium (25)

supplemented with nalidixic acid at 100  $\mu\text{g/ml}$  inoculated with 0.1 ml of a suspension of *E. amylovora* containing approximately  $10^9$  cfu/ml. Two trees in the center of each plot were inoculated with the nalidixic acid resistant strain on 15 June 1993 by wounding succulent apical tissue with a dissecting needle and smearing bacteria into the wounds. After symptoms developed on inoculated trees, the plots were monitored two to three times per week for fire blight symptoms, and the locations of diseased trees were recorded. Bacterial ooze or water-soaked tissue was streaked onto CCT, the differential medium of Ishimaru and Klos (23), supplemented with nalidixic acid at 25  $\mu\text{g/ml}$  and cycloheximide at 100  $\mu\text{g/ml}$  (CCTnc) to verify that the marked strain was present in blighted trees.

**Relation of meteorological events to fire blight incidence.** In 1992, temperature and precipitation at the nursery were

measured with a wet/dry bulb thermometer and tipping bucket rain gauge, respectively; and data were recorded with an RSS 411 apple scab predictor (Reuter-Stokes, Inc., Cleveland, OH). Wind speed was measured with a three-cup totalizing anemometer and was averaged hourly with a CR10 micrologger (Campbell Scientific, Inc., Logan, UT) at a station 15 km from the nursery. In 1993, temperature, precipitation, wind speed, and wind direction were monitored at the nursery every minute with a thermistor probe, tipping bucket rain gauge, three-cup totalizing anemometer, and wind vane, respectively, and averaged hourly with a 21 $\times$  micrologger (Campbell Scientific, Inc.). Temperature and precipitation at MSU were measured with a thermistor probe and a tipping bucket rain gauge, respectively; and data were recorded with an EnviroCaster (Neogen Corp., Lansing, MI). Wind speed and direction data were obtained from a National Weather Service station located 12 km from the MSU plots. The MARYBLYT computer program (28, 40, 41) was used to link major outbreaks of fire blight with weather events that occurred approximately 57 cumulative degree days  $>12.7$  C prior to the outbreaks. This is the number of degree days from inoculation required for the appearance of symptoms (41). For both 1992 and 1993, predictions were initiated when rain fell during an hour when the mean wind speed was  $\geq 6.5$  m/sec. In 1993, the fastest 1-min mean wind speed for each day, as well as hourly mean wind speeds, were recorded. Therefore, predictions were also initiated when rain fell during an hour in which a 1-min mean wind speed was  $\geq 7.7$  m/sec and hourly mean wind speed was  $\geq 4.0$  m/sec. Our goal was not to compare disease incidence among plots, but rather to evaluate disease incidence on each assessment date and then, using MARYBLYT, determine if major outbreaks were correlated with previous storms containing wind-driven rain. Thus, disease incidence was standardized by defining it as the number of new infections in a plot expressed as a percentage of the seasonal total number of blighted trees in that plot.

**Spatial pattern analyses.** After data were collected, nursery plots were partitioned into 1.5-m<sup>2</sup> quadrats, each containing approximately six trees within one row. The number of blighted trees in each quadrat was determined. Disease assessment dates were grouped according to the observed clumping of fire blight outbreaks. Spatial lag autocorrelation analysis was performed using the LCOR2 computer program (20) to assess autocorrelation among counts of diseased trees within each quadrat. Autocorrelation matrices were generated in which each quadrat count was compared to counts in all proximal quadrats. Autocorrelation matrices and their associated



**Fig. 1.** Relation of weather to the incidence of fire blight at an apple nursery in Michigan in 1992. Arrows in each graph originate on dates when storms occurred and span the period required to accumulate 57 degree days  $>12.7$  C according to the MARYBLYT model (41). Numbers above arrows in the top graph are mean hourly wind speeds (m/sec) recorded during the storms. Blighted trees in plots N92-1, N92-2, and N92-3 are new fire blight infections detected on each assessment date. A zero indicates that no blighted trees were detected. Disease was not assessed on other dates.

two-dimensional proximity patterns were interpreted as described previously (11,16,17,19,20,36). Because the MSU plots were relatively small, quadrat-based analysis was not appropriate. However, trees were arranged in a lattice of rows and across-row columns, and the status (i.e., diseased or healthy) of every tree was known, permitting the use of ordinary runs (30) to analyze the spatial pattern of disease. Disease assessment dates were grouped as described above. In each plot, within-row runs were calculated by treating the five rows of trees as a single continuous row by counting runs up one row and down the next (10,31). Similarly, across-row runs were calculated by treating the 22 columns of trees as a single column. A continuity correction (0.5) was added to the observed number of runs prior to calculating Z-statistics (15). A nonrandom distribution of diseased trees was indicated if the probability of the Z value was  $\leq 0.050$ .

**Air sampling.** An Andersen six-stage microbial impaction sampler (1) (Andersen 2000 Inc., Atlanta, GA) was used in 1993 to sample air in an MSU apple orchard with fire blight and, later in the season, in the simulated nursery plantings at MSU. Glass petri dishes designed for use with the air sampler were filled with 27 ml of CCT supplemented with cycloheximide (CCTc) at 100  $\mu\text{g}/\text{ml}$  for sampling in the orchard, or CCTnc for sampling in the plots. The sampler was supported in a horizontal position and placed among foliage with conspicuous bacterial ooze. A battery-powered vacuum pump drew air through the sampler for 20 min at a flow rate of 0.028  $\text{m}^3/\text{min}$ . Eleven samples were collected during rain, and 10 samples were collected during dry weather; although in some cases, leaves were wet from dew or previous rain. During rain, the sampler was shielded from above with an umbrella to minimize splashing near the sampler orifice. Counts for each stage were summed to determine total counts per air sample and expressed as cfu per cubic meter.

**Screening of budwood for *E. amylovora*.** Budwood from various cultivars of apple in the main budwood orchard for the nursery was collected during late July through August of 1992 and 1993. Although the trees were symptomless at the time of collection, fire blight infections had been pruned from trees in this orchard during June and July of both years. In 1992, 10-bud samples from each of 115 trees were ground in 5 ml of 0.01 M potassium phosphate buffer (PPB), pH 7, enriched with 5 ml of CCT prepared without agar, and shaken for 12–16 hr at room temperature. Aliquots (100  $\mu\text{l}$ ) and  $10^{-1}$  and  $10^{-2}$  dilutions of each sample were spread onto plates of CCTc. In 1993, 106 approximately 50-cm budsticks were defoliated and cut into several

pieces. Leaves and stems were shaken in 100 ml of PPB for 1–2 hr. Samples were filtered (Whatman No. 1) to remove debris, concentrated by centrifugation for 15 min at 6,000 rpm, and resuspended in 210  $\mu\text{l}$  of water. Aliquots (100  $\mu\text{l}$ ) and  $10^{-1}$  and  $10^{-2}$  dilutions of the suspension were spread onto plates of CCTc. Aliquots (10  $\mu\text{l}$ ) of the concentrated sample were subjected to the polymerase chain reaction (PCR) to detect *E. amylovora* (6). Primers were 17-mer oligonucleotides from the borders of a 0.9-kb *Pst*I fragment of a plasmid common to all strains of *E. amylovora* (6,14), and were synthesized at the Macromolecular Facility Laboratory at MSU with an automatic 380B DNA Synthesizer (Applied Biosystems, Foster City, CA). PCR was carried out in a total volume of 50  $\mu\text{l}$  containing (final concentrations) 25 pmol of each primer; 2 U of Taq DNA polymerase (Gibco, BRL, Gaithersburg, MD); 0.2 mM each dATP, dCTP, dGTP, and dTTP (Promega Corp., Madison, WI); 20 mM Tris-HCl, pH 8.4; 50 mM KCl; 1.5 mM  $\text{MgCl}_2$ ; 10 mM 2-mercaptoethanol; 8  $\mu\text{g}$  of bovine serum albumin; 5% dimethyl sulfoxide; and 1% Tween 20. Sterile water (10  $\mu\text{l}$ ) and 10  $\mu\text{l}$  of a suspension containing *E. amylovora* at approximately  $10^4$  cfu/ml were run as negative and positive controls, respectively. A dilution series of *E. amylovora* and a series put through the centrifugation, filtering, and resuspension steps were run to determine the sensitivity of the assay. Samples were overlaid with a drop of light mineral oil, and PCR was performed in a Coy TempCycler (Coy Corp., Grass Lake, MI). Denaturation

was at 93 C (in first cycle for 2 min and in subsequent cycles for 1 min), annealing was at 52 C for 2 min, and polymerization was at 72 C for 2 min. After 37 cycles, the PCR products were separated on 1.0% agarose gels, stained with ethidium bromide, and photographed on a UV transilluminator. The presence of a 0.9-kb DNA fragment was indicative of *E. amylovora* (6).

**Pathogen identification.** Representative colonies characteristic of *E. amylovora* on CCTc or CCTnc were tested with an *E. amylovora* specific DNA probe (14,33). Pathogenicity of putative strains of *E. amylovora* was tested by inoculating immature pear fruit.

## RESULTS

**Relation of meteorological events to fire blight incidence.** Disease sufficient for analysis developed at the nursery in three plots (N92-1, N92-2, and N92-3) in 1992, and in one plot (N93-3) in 1993. Since diseased trees were removed immediately after the detection of fire blight, data for each assessment date represented only new infections and not cumulative counts of blighted trees. At MSU, disease sufficient for analysis developed in two plots (MSU2 and MSU3).

Most major outbreaks of fire blight in the nursery plots were associated with wind-driven rain; however, some outbreaks did not appear to be storm related. Conversely, wind-driven rain did not always result in major outbreaks (Figs. 1–3, Table 1). In 1992, three severe storms occurred at the nursery during mid-June through mid-July, and fire

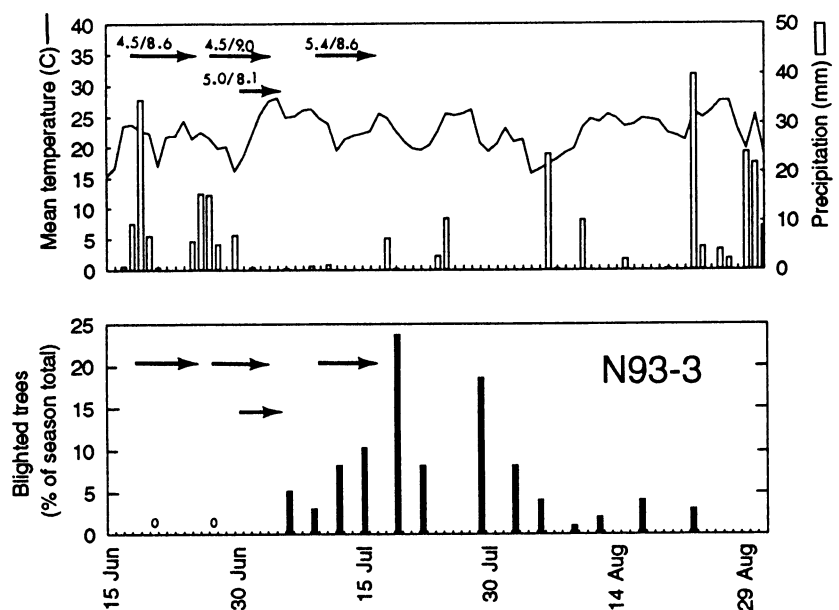


Fig. 2. Relation of weather to the incidence of fire blight at an apple nursery in Michigan in 1993. Arrows in both graphs originate on dates when storms occurred and span the period required to accumulate 57 degree days  $>12.7$  C according to the MARYBLYT model (41). Numbers above arrows in the top graph are mean hourly wind speeds followed by maximum 1-min mean wind speeds (m/sec) recorded during the storms. Blighted trees in plot N93-3 are new fire blight infections detected on each assessment date. A zero indicates that no blighted trees were detected. Disease was not assessed on other dates.

blight symptoms were expected on 1 July, 12 July, and 20 July (Fig. 1, Table 1). On 1 July, following the storm on 17 June, the incidences of blighted trees in plots N92-1, N92-2, and N92-3 were 22, 8, and 14%, respectively, based on seasonal totals of 121, 197, and 155 blighted trees, respectively. On the combined dates of 20 and 24 July, following the storm on 13 July, the incidences of new infections were 32, 73, and 57% in the three respective plots. However, little blight was found in any of the plots on 15 July following the storm on 4 July.

In 1993, fire blight outbreaks were expected at the nursery following storms on 18 June, 27 June, 30 June, and 9 July (Fig. 2, Table 1). No disease was observed following the 18 June storm. Blight was first observed in plot N93-3 on 6 July, following the storms on 27 and 30 June. Twenty-four percent of the seasonal total of 97 blighted trees was recorded on 19 July, following the storm on 9 July. Although 19% of the seasonal total of blighted trees was recorded on 29 July, no storm could be associated with this outbreak (Fig. 2).

At MSU, symptoms on focus trees were detected on 19 June. Fire blight outbreaks were expected on 29 June, 16

July, 13 August, and 29 August (Fig. 3, Table 1). Symptoms on nonfocus trees in plots MSU2 and MSU3 were first noted on 30 June, following the 19 June storm. Later in the season, outbreaks of fire blight were not clearly associated with storms, and storms did not necessarily result in disease outbreaks. In plot MSU2, the incidence of blighted trees, based on a seasonal total of 51 blighted trees, was relatively constant through mid-July and increased during late August, with 22% of the seasonal total recorded on 29 August. A similar trend was detected in plot MSU3, with 22% of the seasonal total of 55 diseased trees recorded on 24 August.

**Spatial pattern analyses.** No consistent pattern of significantly autocorrelated spatial lags was noted early in the season, with two plots (N92-1 and N92-2) showing strong within-row patterns, while the other two plots (N92-3 and N93-3) showed tightly clustered patterns (Fig. 4). On later dates, proximity patterns showed a few significant across-row autocorrelations, often noncontiguous and at high-order spatial lags. Examination of the autocorrelation matrices corresponding to diagonal directions revealed significant high-order autocor-

relations by mid-July in all plots (not shown). Significant negative autocorrelations were not detected. For plot N92-1, there was extensive significant autocorrelation among spatial lags within field rows (north-south) and no autocorrelation across rows (east-west) when data from early assessment dates were combined. Throughout July, within-row autocorrelations decreased, and across-row autocorrelations were detected. Later in the season, matrices for the diagonal directions indicated significant autocorrelations among high-order, noncontiguous spatial lags at an oblique (southwest-northeast) angle across rows. For plot N92-2, extensive within-row (north-south) autocorrelations and first-order across-row (east-west) autocorrelations among spatial lags were detected on 1 July. On later dates, matrices for diagonal directions indicated significant autocorrelations among high-order, noncontiguous spatial lags at an oblique (southwest-northeast) angle across rows. For plot N92-3, a large, tight cluster of significantly autocorrelated spatial lags noted on 1 July disintegrated by mid-July despite extensive disease in the plot. However, matrices for both diagonal directions indicated several significant autocorrelations at high-order spatial lags. Late-season infections resulted in first-order autocorrelation in a diagonal direction, second-order autocorrelation across rows (east-west), and at the ninth spatial lag within rows (north-south). For plot N93-3, first-order autocorrelations were initially significant within (east-west) and across (north-south) rows. The cluster of quadrats with significant autocorrelations expanded during July. However, later in the season, the pattern of new infections resulted in significant autocorrelations at high-order, discontinuous spatial lags. Matrices for the diagonal directions indicated significant autocorrelations among high-order, discontinuous spatial lags at an oblique (southwest-northeast) angle.

Ordinary runs analysis of data from plots MSU2 and MSU3 showed significant within-row (north-south) aggregation throughout the season (Table 2). There was no evidence of across-row aggregation until late July through early August. The Z-values calculated from across-row runs were much less negative than Z-values calculated from within-row runs.

**Air sampling.** All 11 air samples collected during rain contained *E. amylovora*; the mean was 103 cfu/m<sup>3</sup> with a standard deviation of 106. Three of 10 air samples collected during dry weather contained *E. amylovora*; the mean was 2.4 cfu/m<sup>3</sup> with a standard deviation of 1.0. Eighty-two percent of aerosols containing *E. amylovora* were deposited on stages 1-3 and were therefore >2.1 μm in diameter, while 18% of aerosols

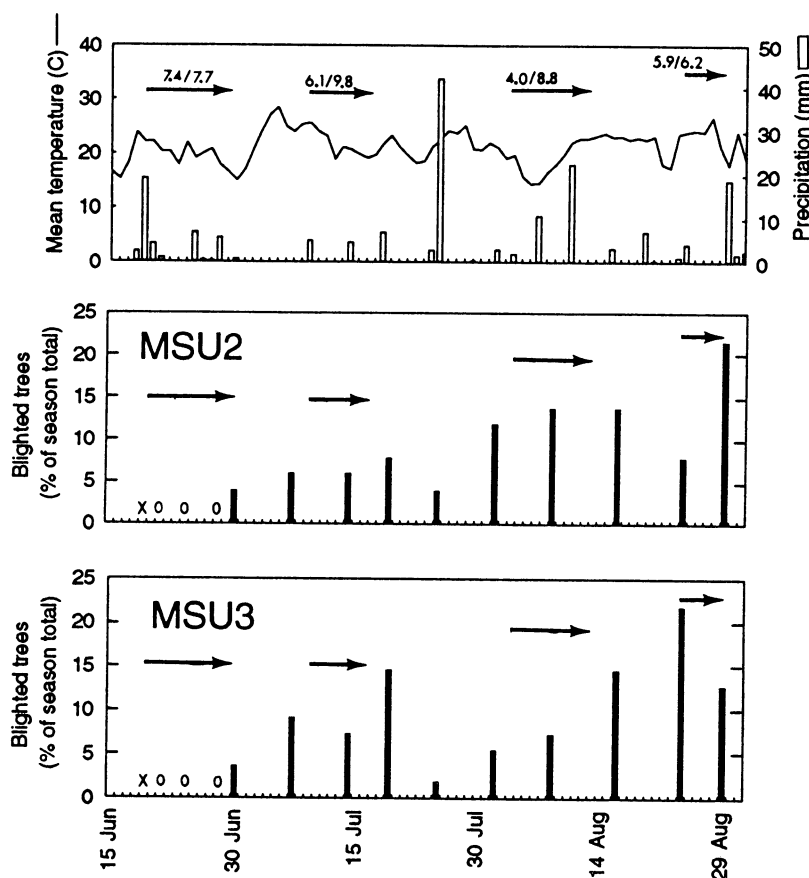


Fig. 3. Relation of weather to incidence of fire blight in simulated nursery plots at Michigan State University in 1993. Arrows in each graph originate on dates when storms occurred and span the period required to accumulate 57 degree days >12.7 C according to the MARYBLTY model (41). Numbers above arrows in the top graph are mean hourly wind speeds followed by maximum 1-min mean wind speeds (m/sec) recorded during the storms. The date on which focus trees first showed symptoms is indicated by X. Blighted trees in plots MSU2 and MSU3 are new fire blight infections detected on each assessment date. A zero indicates that no blighted trees were detected. Disease was not assessed on other dates.

containing *E. amylovora* were deposited on stages 4–6 and were therefore <2.1  $\mu\text{m}$  in diameter. Representative colonies were identified as *E. amylovora*.

**Screening of budwood for *E. amylovora*.** *E. amylovora* was not detected in any of 115 10-bud samples collected in 1992. In 1993, *E. amylovora* was detected in 6% of 106 samples using traditional plating and identification methods, and in 16% of the samples using PCR. All samples testing positive on plates also tested positive by PCR. Bacterial populations of the plate-positive samples ranged from  $2 \times 10^4$  to  $2 \times 10^5$  cfu/ml, corresponding to  $2 \times 10^2$  to  $2 \times 10^3$  cfu per PCR reaction, assuming a plating efficiency of 100%. The lower detection limit of the PCR assay was  $1 \times 10^2$  cfu/ml when *E. amylovora* was added directly to the reaction buffer and  $5 \times 10^2$  cfu/ml when bacteria were put through the manipulations that field samples underwent.

## DISCUSSION

Wind-driven rain was the most important factor involved in spreading *E. amylovora* after the initial detection of disease in the nursery. Severe outbreaks of fire blight could often be traced back

to a storm containing wind-driven rain. When blight was initially detected in the nursery each season, significant autocorrelations among spatial lags generally were within-row or formed tight clusters (Fig. 4). However, after severe weather, autocorrelation matrices for directions parallel with and at diagonals to the plots often showed significant values at high-order, noncontiguous spatial lags. For example, proximity patterns for plots N92-1 and N92-2 indicated high-order, across-row spatial lag autocorrelations aligned not only with the east–west axis (Fig. 4), but also with the southwest–northeast axis following summer storms. Such patterns are consistent with strong southwesterly winds, which are capable of blowing inoculum several meters and are typical in summer storms in Michigan.

The role of weather in the incidence and spatial pattern of fire blight at the nursery was more easily discerned early in the summer when disease was first detected and following the first major storm of the season. By late July and throughout August, interpretation of data was complicated by the formation of secondary foci. In our study, secondary foci did not expand because diseased

trees were continually removed by nursery personnel. This is in contrast to studies of *Xanthomonas campestris* pv. *citri* in citrus nurseries, where extensive secondary foci coalesced by later assessment dates (19,21). It was suggested that spatial lag distances between primary and secondary foci might provide an estimate of the distance that inoculum is splash-disseminated (19). Secondary foci increased the amount of bacteria-laden ooze available to initiate new infections and might be associated with an increase in epiphytic *E. amylovora* (13,35,43,45). As ooze became more abundant, dispersal of the pathogen to healthy trees probably occurred even in the absence of severe storms. It was also possible for disease to occur throughout a plot with no significant autocorrelations among spatial lags (Fig. 4, N92-3). This might be expected if disease were randomly dispersed among quadrats.

The MSU plots were established to evaluate the development and spread of fire blight when diseased trees were not removed. A buildup of bacterial ooze and epiphytic *E. amylovora* was probably the reason that new infections steadily increased as a percentage of the seasonal total in the MSU plots. Within-row

**Table 1.** Weather data in relation to dates of predicted and observed outbreaks of fire blight in nursery trees<sup>a</sup>

Location Year	Date of storm	Rain (mm) <sup>b</sup>	Wind speed (m/sec) <sup>c</sup> and direction <sup>d</sup>	Date of symptoms		Plot:incidence (%) <sup>f</sup>
				Predicted	Observed <sup>e</sup>	
Nursery 1992	17 June	32.5	10.8 na	1 July	1 July <sup>g</sup>	N92-1: 22 N92-2: 8 N92-3: 14
	4 July	9.7	6.5 na	12 July	15 July	N92-1: 3 N92-2: 1 N92-3: 1
	13 July	29.5	6.5 na	20 July	20 and 24 July	N92-1: 32 N92-2: 73 N92-3: 57
	18 June	9.4	4.5; 8.6 SW	25 June	28 June	N93-3: 0
1993	27 June	15.2	4.5; 9.0 SW	4 July	6 July <sup>h</sup>	N93-3: 5
1993	30 June	7.1	5.0; 8.1 SE	5 July	6 July <sup>h</sup>	N93-3: 5
1993	9 July	0.8	5.4; 8.6	16 July	19 July	N93-3: 24
Michigan State University 1993	19 June	19.3	7.4; 7.7 SW	29 June	30 June <sup>i</sup>	MSU2: 4 MSU3: 4
	9 July	5.1	6.1; 9.8 SW	16 July	19 July	MSU2: 8 MSU3: 15
	3 Aug.	2.0	4.0; 8.8 SW	13 Aug.	16 Aug.	MSU2: 14 MSU3: 15
	24 Aug.	4.1	5.9; 6.2 S	29 Aug.	29 Aug.	MSU2: 22 MSU3: 13

<sup>a</sup>In 1992 and 1993, predictions were initiated on dates when rain fell during an hour when mean wind speeds were  $\geq 6.5$  m/sec; in 1993, predictions were also initiated on dates when rain fell during an hour in which a 1-min mean wind speed was  $\geq 7.7$  m/sec and hourly mean wind speed was  $\geq 4.0$  m/sec. Predicted dates for fire blight outbreaks were approximately 57 cumulative degree days  $> 12.7$  C following each storm (41).

<sup>b</sup>Total rainfall on date of storm.

<sup>c</sup>Maximum mean hourly wind speed (m/sec) recorded during rain; fastest 1-min mean wind speed recorded during rain.

<sup>d</sup>S = south; SW = southwest; SE = southeast; na = not available.

<sup>e</sup>Disease assessment dates within 4 days following the date that symptoms were predicted.

<sup>f</sup>The number of new infections expressed as a percentage of the seasonal total number of blighted trees.

<sup>g</sup>Date on which fire blight was first detected in plots N92-2 and N92-3.

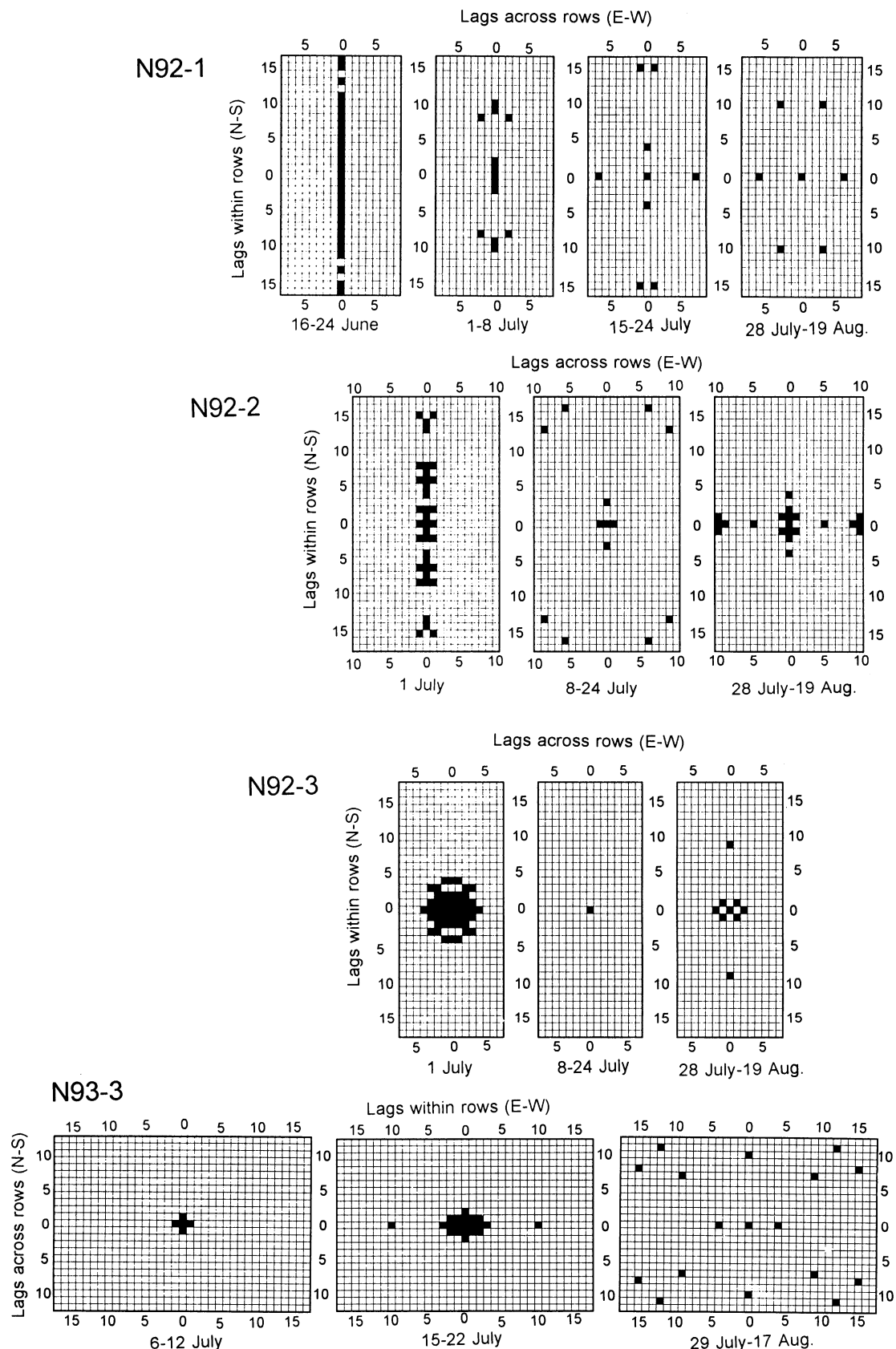
<sup>h</sup>Date on which fire blight was first detected in plot N93-3.

<sup>i</sup>Date on which fire blight was first detected on nonfocus trees in plots MSU2 and MSU3.

aggregation of disease was noted throughout the season (Table 2). The distance between trees within rows was much less than that across rows, allowing

more frequent contact and transfer of inoculum among trees in the same row. Cross-row aggregation of disease was significant only later in the season,

possibly because of a storm on 9 July. Although total precipitation from the storm was not great (5.1 mm), it fell in a short period of time (approximately



**Fig. 4.** Proximity patterns of significant spatial lag autocorrelations of fire blight in an apple nursery in Michigan. Black squares denote significant positive autocorrelations ( $P \leq 0.050$ ) at the indicated spatial lag positions. For plots N92-1, N92-2, and N92-3, lags north-south (N-S) are within field rows; for plot N93-3, lags east-west (E-W) are within field rows. Plots N92-1, N92-2, N92-3, and N93-3 contained 11, 13, 10, and 15 field rows, respectively.

20 min) and was accompanied by strong gusts of wind from the southwest. Alternatively, across-row aggregation may have been due to the simultaneous spread of disease within adjacent rows.

Differences in the degree of susceptibility among trees within a plot were probably minor and had little or no consequence in our spatial analyses. In 1992, disease pressure was high due to abundant inoculum and favorable environmental conditions, and cultivars believed to be moderately resistant became blighted. Thus, differences in susceptibility among the highly susceptible trees in the plots were likely masked. Furthermore, when blight spread across rows, there was no evidence that trees in particular rows consistently escaped the disease.

The importance of rain in the release and dispersal of *E. amylovora* was further supported by the air sampling data. Air samples collected during rain always contained *E. amylovora*, whereas samples collected during dry periods contained few or no cfu of the pathogen, consistent with studies in which aerosolization of plant-associated bacteria was enhanced by rain (26,37), but in contrast to reports of greater aerosolization during warm, sunny periods (29,32). Whether a pathogen is present as a leaf epiphyte or is embedded in a matrix of bacterial ooze probably affects its potential to be aerosolized under various environmental conditions. We shielded the sampler with an umbrella to minimize the entry of splash droplets, which would create artificially high colony counts on stage 1 of the sampler. However, this may have also reduced the number of bacterial-laden aerosol particles near the sampler orifice, since *E. amylovora*-containing aerosol particles are probably generated when raindrops hit bacterial ooze.

Southey and Harper (39) reported that *E. amylovora* remained viable up to 2 hr in aerosol particles exposed to the open air. Aerosols containing soft rot *Erwinia* spp. remained suspended for at least 1 hr (22). Thus, wind might result in the dispersal of viable *E. amylovora* even after rain has stopped. We assumed a cfu represented at least one bacterium. However, since cells of *E. amylovora* are approximately  $0.7 \times 1.0 \mu\text{m}$  (48), and most colonies arose from aerosol particles  $>2.1 \mu\text{m}$  in diameter, it would be possible for a single particle to carry more than one cell. Under favorable environmental conditions, bacteria would multiply rapidly if aerosols landed on highly susceptible wind-damaged shoot tips (12,27). We observed that infection was frequently associated with wind-damaged leaves. Thus, aerosols could be an epidemiologically significant source of *E. amylovora* even if the number of cells per particle were low.

Contaminated budwood has been implicated as a means by which *E.*

*amylovora* enters pome tree nurseries (2,9, 42,47,48). Populations of *E. amylovora* are apparently very low in buds from symptomless shoots of apple and pear, since numerous attempts to recover the pathogen from such tissue have yielded few positive results (7,9,35,45). Although not a model epiphyte (35), *E. amylovora* has commonly been isolated from the surfaces of apparently healthy shoots of apple and pear, but only after symptoms were observed in the orchard (35,43,45). Although we failed to isolate *E. amylovora* from buds alone, we detected the pathogen in samples of budsticks and leaves by plating and PCR. Dueck and Morand (13) reported that in Ontario, Canada, populations of *E. amylovora* on apple leaves were highest during mid-July through August. If conditions were similar in Michigan, then high populations of *E. amylovora* would coincide with the collecting and grafting of buds. In the budwood orchard, bundles of budsticks with leaves are wrapped in wet cheesecloth and stored for up to 3 days before buds are grafted onto rootstocks. Thus, *E. amylovora* might have entered the nursery externally on budwood and invaded the rootstocks after grafting with contaminated, rather than infected, buds.

Several lines of circumstantial evidence support the premise that budwood was a source of *E. amylovora* in the nursery. Rootstocks in plots N92-1 and N92-2 were budded in 1991, and there was strong within-row aggregation of disease on the earliest dates that blight was detected during the 1992 season. In plot N92-1, a few rootstocks within one row had active cankers by mid-May. At the other nursery location, we noted that several rootstocks that had been budded with the same cultivar exhibited fire blight cankers at the graft union 2-4 wk after budding in 1992. Infected trees were not randomly dispersed down the row but appeared to be aggregated, indicating that they might have been budded from the same budstick or bundle of budsticks. Removal of infected trees in the autumn of 1992 eliminated potential sources of inoculum in the spring of 1993 and may

have contributed to the lower incidence of fire blight in 1993.

Due to the large number of buds required by nurseries and the limitations in detection of *E. amylovora* in buds, it would be impractical to screen all budwood prior to grafting. Furthermore, budwood sources are finite, and it may be impossible to maintain a mature budwood orchard completely free of the pathogen, especially in apple-growing regions. Thus, the introduction of *E. amylovora* on budwood into nursery plantings may be inevitable. However, our study has demonstrated the urgency of preventing the overwintering of inoculum in rootstocks, because once *E. amylovora* is established in the nursery, it is readily disseminated by wind-driven rain and becomes aerosol-borne during rain with or without wind.

#### ACKNOWLEDGMENTS

We thank Tim R. Gottwald for providing LCOR2 software, and Gail R. Ehret and M. Beatrix Jones for assistance in the field.

#### LITERATURE CITED

- Andersen, A. A. 1958. New sampler for the collection, sizing, and enumeration of viable airborne particles. *J. Bacteriol.* 76:471-484.
- Baldwin, C. H., Jr., and Goodman, R. N. 1963. Prevalence of *Erwinia amylovora* in apple buds as detected by phage typing. *Phytopathology* 53:1299-1303.
- Bauske, R. J. 1967. Dissemination of waterborne *Erwinia amylovora* by wind in nursery plantings. *Am. Soc. Hortic. Sci. Proc.* 91:795-801.
- Bauske, R. J. 1971. Wind dissemination of waterborne *Erwinia amylovora* from *Pyrus* to *Pyracantha* and *Cotoneaster*. *Phytopathology* 61:741-742.
- Beer, S. V., and Oppenorth, D. C. 1976. *Erwinia amylovora* on fire blight canker surfaces and blossoms in relation to disease occurrence. *Phytopathology* 66:317-322.
- Bereswill, S., Pahl, A., Bellemann, P., Zeller, W., and Geider, K. 1992. Sensitive and species-specific detection of *Erwinia amylovora* by PCR-analysis. *Appl. Environ. Microbiol.* 58:3522-3526.
- Bonn, W. G. 1979. Fire blight bacteria in symptomless dormant apple and pear buds. *Can. J. Plant Pathol.* 1:61-62.
- Brooks, A. N. 1926. Studies of the epidemiology and control of fireblight of apple. *Phytopathology* 16:665-696.
- Calzolari, A., Peddes, P., Mazzucchi, U., Mori, P., and Garzena, C. 1982. Occurrence of *Erwinia amylovora* in buds of asymptomatic apple plants in commerce. *Phytopathol. Z.* 103:156-162.
- Campbell, C. L., and Madden, L. V. 1990. Intro-

**Table 2.** Ordinary runs analysis of fire blight on apple trees in simulated nursery plots at Michigan State University (MSU)

Plot	Disease assessment dates	Z-values <sup>a</sup> (P) <sup>b</sup>	
		Within row (north-south)	Across row (east-west)
MSU2			
	25 June-14 July	-6.80 (<0.001)	0.03 (0.382)
	16 July-8 Aug.	-5.07 (<0.001)	-1.93 (0.027)
	11 Aug. 29 Aug.	-5.49 (<0.001)	-1.06 (0.145)
MSU3			
	25 June 14 July	-5.81 (<0.001)	-0.23 (0.409)
	16 July-8 Aug.	-4.33 (<0.001)	-1.65 (0.050)
	11 Aug. 29 Aug.	-6.30 (<0.001)	-1.64 (0.051)

<sup>a</sup>Standardized variable; large negative values indicate a nonrandom, aggregated distribution of diseases.

<sup>b</sup>Significance level; levels of  $P \leq 0.050$  indicate a nonrandom distribution of disease.

- duction to Plant Disease Epidemiology. John Wiley & Sons, Somerset, NJ.
11. Campbell, C. L., and van der Gaag, D. J. 1993. Temporal and spatial dynamics of microsclerotia of *Macrophomina phaseolina* in three fields in North Carolina over four to five years. *Phytopathology* 83:1434-1440.
  12. Crosse, J. E., Goodman, R. N., and Shaffer, W. H., Jr. 1972. Leaf damage as a predisposing factor in the infection of apple shoots by *Erwinia amylovora*. *Phytopathology* 62:176-182.
  13. Dueck, J., and Morand, J. B. 1975. Seasonal changes in the epiphytic population of *Erwinia amylovora* on apple and pear. *Can. J. Plant Sci.* 55:1007-1012.
  14. Falkenstein, H., Bellemann, P., Walter, S., Zeller, W., and Geider, K. 1988. Identification of *Erwinia amylovora*, the fireblight pathogen, by colony hybridization with DNA from plasmid pEA29. *Appl. Environ. Microbiol.* 54:2798-2802.
  15. Gibbons, J. D. 1976. *Nonparametric Methods for Quantitative Analysis*. Holt, Rinehart, and Winston, New York.
  16. Gottwald, T. R., and Graham, J. H. 1990. Spatial pattern analysis of epidemics of citrus bacterial spot in Florida citrus nurseries. *Phytopathology* 80:181-190.
  17. Gottwald, T. R., Graham, J. H., and Egel, D. S. 1992. Analysis of foci of Asiatic citrus canker in a Florida citrus orchard. *Plant Dis.* 76:389-396.
  18. Gottwald, T. R., Miller, C., Brlansky, R. H., Gabriel, D. W., and Civerolo, E. L. 1989. Analysis of the spatial distribution of citrus bacterial spot in a Florida citrus nursery. *Plant Dis.* 73:297-303.
  19. Gottwald, T. R., Reynolds, K. M., Campbell, C. L., and Timmer, L. W. 1992. Spatial and spatiotemporal autocorrelation analysis of citrus canker epidemics in citrus nurseries and groves in Argentina. *Phytopathology* 82:843-851.
  20. Gottwald, T. R., Richie, S. M., and Campbell, C. L. 1992. LCOR2-Spatial correlation analysis software for the personal computer. *Plant Dis.* 76:213-215.
  21. Gottwald, T. R., Timmer, L. W., and McGuire, R. G. 1989. Analysis of disease progress of citrus canker in nurseries in Argentina. *Phytopathology* 79:1276-1283.
  22. Graham, D. C., and Harrison, M. D. 1975. Potential spread of *Erwinia* spp. in aerosols. *Phytopathology* 65:739-741.
  23. Ishimaru, C., and Klos, E. J. 1984. A new medium for detecting *Erwinia amylovora* and its use in epidemiological studies. *Phytopathology* 74:1342-1345.
  24. Keil, H. L., and van der Zwet, T. 1972. Recovery of *Erwinia amylovora* from symptomless stems and shoots of Jonathan apple and Bartlett pear trees. *Phytopathology* 62:39-42.
  25. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *J. Lab. Clin. Med.* 44:301-307.
  26. Kuan, T.-L., Minsavage, G. V., and Schaad, N. W. 1986. Aerial dispersal of *Xanthomonas campestris* pv. *campestris* from naturally infected *Brassica campestris*. *Plant Dis.* 70:409-413.
  27. Lewis, S., and Goodman, R. N. 1965. Mode of penetration and movement of fire blight bacteria in apple leaf and stem tissue. *Phytopathology* 55:719-723.
  28. Lightner, G., and Steiner, P. W. 1990. Computerization of a blossom blight prediction model. *Acta Hort.* 273:159-162.
  29. Lindemann, J., and Upper, C. D. 1985. Aerial dispersal of epiphytic bacteria over bean plants. *Appl. Environ. Microbiol.* 50:1229-1232.
  30. Madden, L. V., Louie, R., Abt, J. J., and Knoke, J. K. 1982. Evaluation of tests for randomness of infected plants. *Phytopathology* 72:195-198.
  31. Madden, L. V., Louie, R., and Knoke, J. K. 1987. Temporal and spatial analysis of maize dwarf mosaic epidemics. *Phytopathology* 77:148-156.
  32. McInnes, T. B., Gitaitis, R. D., McCarter, S. M., Jaworski, C. A., and Phatak, S. C. 1988. Airborne dispersal of bacteria in tomato and pepper transplant fields. *Plant Dis.* 72:575-579.
  33. McManus, P. S., and Jones, A. L. 1994. Epidemiology and genetic analysis of streptomycin-resistant *E. amylovora* from Michigan and evaluation of oxytetracycline for control. *Phytopathology* 84:627-633.
  34. Miller, P. W. 1929. Studies of fire blight in Wisconsin. *J. Agric. Res.* 39:579-621.
  35. Miller, T. D., and Schroth, M. N. 1972. Monitoring the epiphytic population of *Erwinia amylovora* on pear with a selective medium. *Phytopathology* 62:1175-1182.
  36. Modjeska, J. S., and Rawlings, J. O. 1983. Spatial correlation analysis of uniformity of data. *Biometrics* 39:373-384.
  37. Quinn, C. E., Sells, A., and Graham, D. C. 1980. Soft rot *Erwinia bacteria* in the atmospheric bacterial aerosol. *J. Appl. Bacteriol.* 49:175-181.
  38. Schroth, M. N., Thomson, S. V., Hildebrand, D. C., and Moller, W. J. 1974. Epidemiology and control of fire blight. *Annu. Rev. Phytopathol.* 12:389-412.
  39. Southey, R. F. W., and Harper, G. J. 1971. The survival of *Erwinia amylovora* in airborne particles: Tests in the laboratory and in the open air. *J. Appl. Bacteriol.* 34:547-556.
  40. Steiner, P. W. 1990. Predicting apple blossom infections by *Erwinia amylovora* using the MARYBLYT model. *Acta Hort.* 273:139-148.
  41. Steiner, P. W. 1990. Predicting canker, shoot and trauma blight phases of apple fire blight epidemics using the MARYBLYT model. *Acta Hort.* 273:149-158.
  42. Stewart, V. B. 1913. The fire blight disease in nursery stock. N.Y. (Cornell) Agric. Exp. Stn. Bull. 329:315-371.
  43. Sutton, T. B., and Jones, A. L. 1975. Monitoring *Erwinia amylovora* populations on apple in relation to disease incidence. *Phytopathology* 65:1009-1012.
  44. Teviotdale, B. L., Wiley, M., and Harper, D. H. 1991. How disinfectants compare in preventing transmission of fire blight. *Calif. Agric.* 45:21-23.
  45. Thomson, S. V., Schroth, M. N., Moller, W. J., and Reil, W. O. 1975. Occurrence of fire blight of pears in relation to weather and epiphytic populations of *Erwinia amylovora*. *Phytopathology* 65:353-358.
  46. van der Zwet, T. 1968. Recent spread and present distribution of fire blight in the world. *Plant Dis. Rep.* 52:698-702.
  47. van der Zwet, T. 1983. Occurrence of fire blight in commercial pear seedling rootstocks following budding with symptomless scionwood. (Abstr.) *Phytopathology* 73:969.
  48. van der Zwet, T., and Keil, H. L. 1979. Fire blight. A bacterial disease of rosaceous plants. *U.S. Dep. Agric. Agric. Handb.* 510.