Monitoring Erwinia amylovora Populations on Apple in Relation to Disease Incidence

T. B. Sutton and A. L. Jones

Research Associate and Associate Professor, respectively, Department of Botany and Plant Pathology, Michigan State University, East Lansing 48824. Present address of senior author: Department of Plant Pathology, North Carolina State University, Raleigh 27607.

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ABSTRACT

The Miller-Schroth medium was used for detecting and isolating *Erwinia amylovora* from infected and from apparently healthy apple tissues. *E. amylovora* was not detected in samples from 12 apple orchards before fire blight infections were common in the orchards, and, in those orchards where fire blight became severe, not until numerous additional infections were initiated. It is suggested that fire blight outbreaks in Michigan result from the rapid development of temporary epiphytic populations of *E. amylovora* following infrequent, but widespread, dissemination. Possible modifications of the sampling technique and the need for a predictive model are discussed.

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Fire blight, caused by the bacterium *Erwinia amylovora* (Burrill) Winslow et al., can be a very serious problem one year, but unimportant in others. Growers either follow a diligent but excessive chemical control program, as in California (3), or, as an apparent result of its sporadic occurrence, neglect chemical control and experience serious losses in certain years and locations. Michigan growers generally practice the latter control strategy. In 1972, nine of the 206 orchards surveyed in the Cooperative Michigan Apple Pest Management Pilot Project received one to four bactericide sprays, while in 1973, 25 of the 217 orchards surveyed received one to four sprays. Because of the year-to-year variation in disease incidence, some method is needed for forecasting disease outbreaks in sufficient time to employ effective, economic, and ecologically sound control measures.

In 1972, Miller and Schroth (1) developed a selective medium suitable for monitoring orchard populations of *E. amylovora* on pears in California. Later, it was reported that *E. amylovora* could be detected before symptoms of fire blight were observed in the orchard and that information gained from the monitoring was useful for the timing of sprays (3).

The purpose of this investigation was to evaluate the Miller-Schroth monitoring method as a possible pest management tool for timing fire blight control measures on apples in Michigan.

MATERIALS AND METHODS.—*Monitoring sites.*—Orchards with a history of fire blight problems were chosen for study. In 1973, we monitored two orchards in southwest Michigan (No. 1 and 4) and five orchards near Grand Rapids (No. 5, 6, 7, 8, and 9). Tree age varied from 4 years in orchard 5, to 25-30 years in orchards 4 and 8. Cultivars sampled included Fenton (orchards 1, 5, 6), Idared (orchards 5, 8, 9), and Jonathan (orchard 4).

In 1974, we monitored four orchards in southwest Michigan (No. 1, 2, 3, 4) and three orchards near Ludington (No. 10, 11, and 12). Tree age varied from 6 years in orchard 2 to 25-30 years in orchards 4, 10, 11, and 12. Cultivars sampled were Fenton (orchard 1), Jonathan (orchards 3, 4, 10, 11), R. I. Greening (orchards 2, 10, 11), and crabapple (orchard 12). All orchards were in medium vigor except for orchard 12 which was in low vigor. A 0.8 to 1.6-hectare (ha) (2- to 4-acre) block of trees was selected in each orchard for sampling purposes.

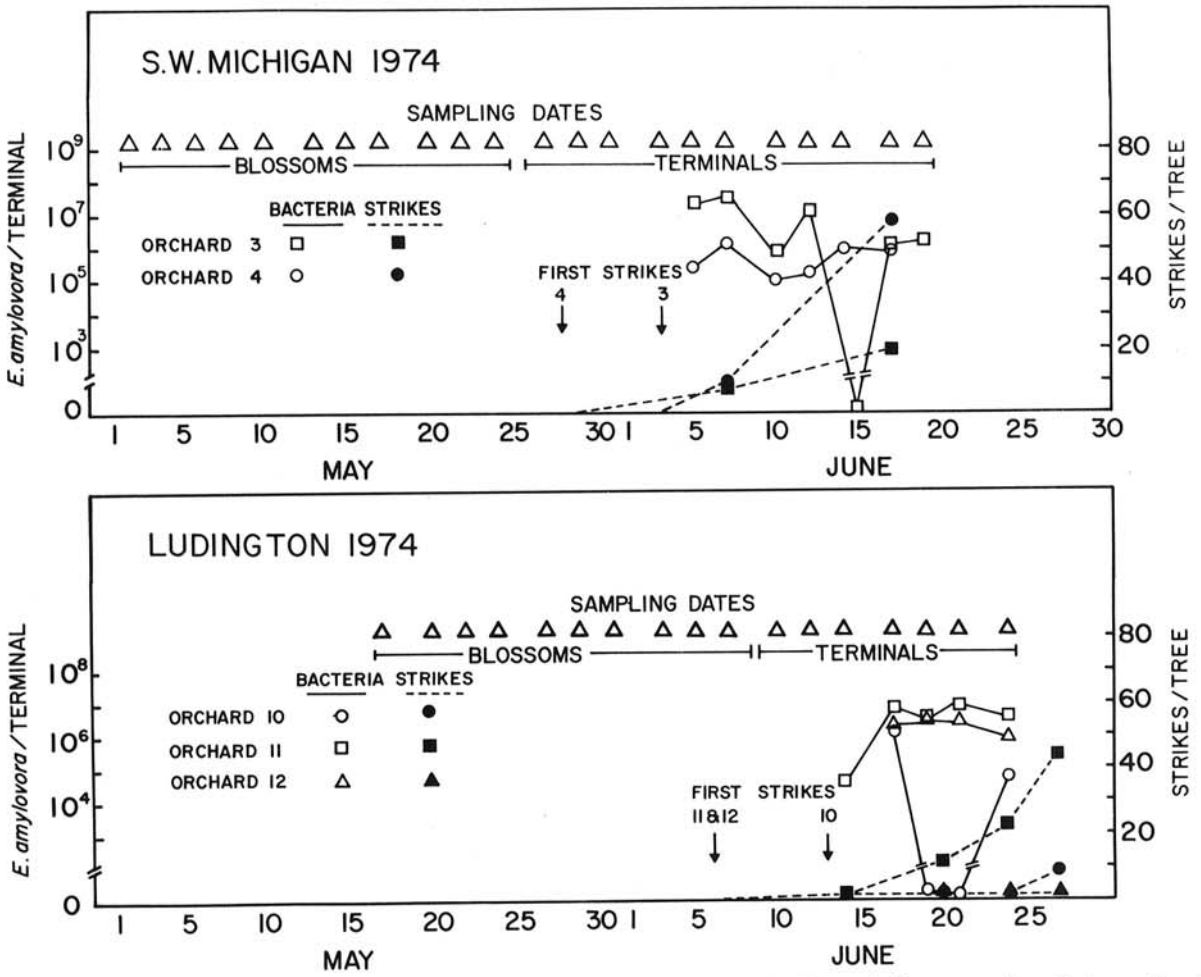

Fig. 1. Relationship between the detection of *Erwinia amylovora* on apparently healthy apple blossoms and terminal growth and disease severity in two orchards in southwest Michigan (upper) and in three orchards near Ludington, Michigan (lower). Dates fire blight bacteria were detected are sampling dates, but in practice results were not available until about 60 hours later.

Streptomycin at 100μg/ml (100 ppm) was applied at the cooperating growers' discretion in orchards 1 and 4 on 11 May 1973, and in orchard 1 on 22 May 1974.

Sampling procedure.—Sampling, three times weekly, was initiated in the early pink stage and continued through the third week in June. Ten blossoms were collected from each of 20 randomly selected trees per block. When blossoms were no longer present, similar numbers of terminal samples were collected by breaking out the first expanded leaf and growing point. Samples were placed in plastic bags and kept in a cooler until processed.

In 1973, blossom and terminal samples were sent to Michigan State University (MSU) for washing, plating, and incubation. In 1974, samples were washed and plated at two field stations, located near Watervliet and Scottville, Michigan, and the culture plates were sent to MSU for incubation.

Monitoring techniques.—The selective medium (MS) was prepared as described by Miller and Schroth (1) except that 20 ml per liter, rather than 10 ml, of the 2% aqueous solution of nitrilotriacetic acid was used. Fresh medium was prepared and used each week. Preliminary laboratory tests were conducted with Michigan isolates of *E. amylovora* and with mixtures of *E. amylovora*, *E. herbicola*-like strains, and certain other bacterial plant pathogens to insure the accuracy of distinguishing *E. amylovora* from other bacteria.

A procedure developed in California to sample pear blossoms for *E. amylovora* (4) was used to sample each 200-blossom or terminal sample. Each sample was placed in a 2-liter flask and washed with 100 ml distilled water for 30 seconds. Aliquot samples (0.1 ml) from the original and from 10^{-2} and 10^{-4} dilutions of the original washings were plated (two replications) on the MS medium and distributed evenly with an L-shaped glass rod. Petri plates were incubated in an inverted position at 28 C for 40, 48, or 60 hours.

Colonies of *E. amylovora* were identified by their characteristic reddish-orange color and colony morphology on MS media (1) and by pathogenicity tests (2). Three apple seedlings were inoculated with bacteria from representative suspect colonies, and results were compared to inoculations with known isolates. Over 200 pathogenicity tests were conducted over the 2 years of this study.

Disease incidence.—Each time samples were taken, observations were made to establish when the first strikes or primary infections appeared. Usually one or two blighted blossoms or terminals were noted initially in an orchard. Later, blight counts were made by examining all the trees in each block and counting the number of blossom and terminal strikes visible from the ground.

Streptomycin resistance.—Representative isolates of *E. amylovora* obtained through the monitoring program in 1973 and 1974, and from blight samples collected from problem orchards in the Michigan Apple Pest Management Project were screened for possible streptomycin resistance on MS media in which was incorporated 25, 100, or 200 μg per ml streptomycin.

RESULTS.—The incidence of fire blight was moderate to severe in apple orchards within the study areas used in 1974, but was light or absent in 1973. Blight was also a problem in nearby pear orchards in 1974.

Monitoring.—High populations of bacteria other than *E. amylovora* were often present in blossom and terminal samples. Initially they were low; however, with warmer weather, populations rapidly increased to 10^6 to 10^8 per blossom or terminal. When *E. amylovora* populations became high, numbers of other bacteria decreased. High populations of *Erwinia* sp. other than *E. amylovora* often made identification of *E. amylovora* difficult before 60-hours incubation.

In 1973, blossom blight was not observed in any of the seven monitored orchards, but terminal infections were noted in orchards 1 and 4 in southwest Michigan. One blighted terminal was observed in orchard 1 on 13 June and terminal washings made on that date revealed a low concentration of *E. amylovora* (2 per terminal). *E. amylovora* was not detected in subsequent samples from this orchard. Little spread of blight occurred in this block and only 0.38 blighted terminals per tree were noted on 26 June. No *E. amylovora* were detected in a series of 24 samples from orchard 4 although an average of 0.56 blighted terminals per tree was recorded on 26 June. No *E. amylovora* were found in 120 samples taken from the five orchards near Grand Rapids and no fire blight was observed in any of the orchards.

In 1974, 5% bloom was recorded in southwest Michigan on 12 May, with full bloom a week later. There was considerable variation in the results obtained from the four orchards in this area. *E. amylovora* were not detected in blossom or in terminal washings from orchard 1 although one infected terminal was observed there on 19 June. No fire blight bacteria were detected in either blossom or terminal samples from orchard 2, where no blight developed.

In orchard 3, *E. amylovora* was first detected on 5 June (2.5×10^7 per terminal) and except for 15 June, populations of *E. amylovora* remained high in subsequent samplings (Fig. 1). Detection of bacteria came 2 days after blight symptoms were observed in the orchard. The number of blight strikes to fruiting spurs or terminals on 14 June was estimated at 5-10 per tree and on 17 June actual counts averaged 20 strikes per tree with two trees having over 100 strikes each.

In orchard 4, *E. amylovora* was first detected on 5 June, 7 days after scattered blossom blight was observed on late blooms in the orchard (Fig. 1). Populations of *E. amylovora* remained high throughout the remainder of the sampling period. Strikes increased from 10 to 15 per tree on 7 June to almost 60 per tree on 17 June, with over 200 strikes recorded on some trees.

In the Ludington area, fire blight bacteria were detected in the three sampled orchards, but only after blight was visible in each orchard. Only samples from terminal growth yielded blight bacteria. In orchard 10, *E. amylovora* were first detected in a terminal sample taken on 17 June, 4 days after two or three strikes were observed in the orchard (Fig. 1). The population of fire blight bacteria fluctuated from 1.25×10^6 per terminal on 17 June, to 15 per terminal on 19 June, to 0 per terminal on 21 June, to 6.2×10^4 per terminal on 24 June. Trees averaged three strikes per tree on 24 June and nine per tree on 27 June.

In orchard 11, three strikes were observed on 6 June and *E. amylovora* were detected in a terminal sample on 14 June (Fig. 1) (3.3×10^4 per terminal). Populations of *E.*

amylovora remained high throughout the sampling period and the number of fire blight strikes per tree increased rapidly from 11.5 (20 June) to 44 (27 June).

In orchard 12, one strike was observed on 6 June, but *E. amylovora* were not detected until 17 June (1.8×10^6 per terminal) (Fig. 1). Though the populations of *E. amylovora* remained high for the remainder of the sampling period, little fire blight developed and less than one fire blight strike per tree was present on 27 June.

Streptomycin resistance.—Of the 26 isolates tested in 1973 and 16 isolates tested in 1974, none grew on MS medium containing streptomycin.

DISCUSSION.—We found the MS medium very useful for detecting and isolating *E. amylovora* from infected tissues and from apparently healthy terminal samples. No *E. amylovora* were detected in apparently healthy blossom samples. Although large numbers of miscellaneous bacteria were often present in the samples, with experience, *E. amylovora* could be identified with confidence. However, in diluting samples to obtain discrete colonies for identification, *E. amylovora*, particularly if populations were low, may have been reduced below detectable levels during periods when populations of miscellaneous bacteria were high.

With our monitoring technique we were unable to detect *E. amylovora* before scattered strikes were visible in the orchard, and, where fire blight became severe, not until numerous additional infections were initiated. Although the sensitivity of the technique precludes the detection of scattered strikes, its success depends on detection of *E. amylovora* before extensive infections occur. The monitoring program is effective in California because the appearance of fire blight is apparently associated with a gradual buildup of resident populations of *E. amylovora* prior to infection (1, 3). Our results suggest that under the moist conditions which often exist in Michigan during and following bloom, epiphytic populations are often temporary, and may develop very rapidly following widespread dissemination from overwintered cankers and scattered primary infections. In the four orchards where severe fire blight developed, *E. amylovora* was detected only after visible strikes were common in the orchards, and only after many additional infections occurred. Only in orchard 12 were high resident populations of *E. amylovora* not correlated with a severe outbreak of the disease. Infection could have occurred on scattered blossoms in orchard 12 as well, but bacteria may have failed to invade the fruit spurs before the blossoms fell. This phenomenon has been observed on several occasions when artificial inoculations were made to blossoms and weather conditions were unfavorable for naturally occurring fire blight infections (Jones, *unpublished*).

Because we were unable to detect populations of *E. amylovora* in orchards before numerous infections were initiated, the monitoring technique must be improved before it can be used to advantage in the Michigan apple pest management program. Our results indicate the method is not detecting populations less than 10^4 per terminal. Modification of the monitoring technique by increasing sample size and the wash time might increase the sensitivity and enable detection of *E. amylovora* in advance of infection. Because of the rapid development of *E. amylovora* under favorable conditions, monitoring of the surface of cankers might provide a more reliable method of predicting spread in the eastern United States.

Disease prediction based on monitoring inoculum potential or weather data alone is probably most effective in the geographical areas in which it was developed. Schroth et al. (3) have suggested that through computer simulation of the disease it should be possible to eliminate the monitoring program and still provide blight forecasts to fruit growers. We concur in this philosophy, but suggest that the model or simulator should be accurate for all geographic areas. A national effort to accomplish this goal would be desirable.

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