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The Scenario of Fire Blight and Streptomycin Resistance

Fire blight disease, caused by the bacterium *Erwinia amylovora*, continues to be one of the more serious disorders of pomaceous fruits, both in its native North America and in other areas of the world. The disease is particularly destructive of pears (Figs. 1 and 2) but also affects apples, quinces, loquats, and ornamental hosts such as hawthorn, cotoneaster, and pyracantha, particularly in Europe. Uncontrolled, fire blight has the potential to destroy an established pear orchard in one growing season.

Streptomycin's Early Success

Copper-containing chemicals such as Bordeaux mixture and the various fixed coppers have been widely used for control in commercial orchards since the early 1930s, and antibiotics such as streptomycin appeared on the scene some two decades later. Streptomycin's earliest use on apples was reported in 1952 by Murneek in an orchard test in Missouri; a spray applied at 50% bloom gave significant control. Numerous reports of success with this antibiotic appeared in ensuing years.

Applied at regular intervals to early-season growth, streptomycin appeared to be superior to copper materials in various parts of the United States. Ark. in California, reported almost 100% control with bloom and postbloom applications of streptomycin dust on pears, and Goodman in Missouri found that 100 ppm streptomycin sprays on apple were effective, nonphytotoxic, and superior to weak Bordeaux mixtures. Various workers throughout the country confirmed the efficacy of the antibiotic in the 1950s and 1960s against fire blight (1,10,15). Streptomycin's localized systemic action and ability to be translocated and apparently remain unaltered within plant tissues were subsequently demonstrated.

Interestingly enough, the development of streptomycin resistance was predicted in 1954. Laboratory tests showed that *E. amylovora* developed resistance to streptomycin in vitro more rapidly in a medium containing streptomycin alone than in one containing a mixture of streptomycin and oxytetracycline (5). This early finding resulted in a mixture of the two antibiotics being formulated for use against fire blight. As time passed, however, oxytetracycline appeared to offer little additional advantage in the field and was eventually deleted from the mixture. The use of streptomycin alone came into common practice and initially was restricted to no later than 90 days before harvest. Further tests during 1968–1969 showed that residues were insignificant even if the antibiotic was used as late as 30 days before harvest. By the end of the 1960s, streptomycin was firmly established as an effective blight control chemical throughout pome fruit-growing regions of the United States.

Detection of Field Resistance

In California in 1970, fire blight disease was unusually severe in pear orchards when a long, wet spring bloom period followed a winter with minimal chilling. It was considered the worst blight season in two decades. Streptomycin was extensively used that year for blight control in the Sacramento Valley and north coast districts. Despite the epiphytotic, the antibiotic appeared to give effective control at 5–10 oz of 17% WP formulations per acre (0.350–0.700 kg/ha) applied in concentrate sprays of 50 gal/A (468 L/ha), 120–240 ppm, every 5 days from the beginning of bloom in late March until after spring rains ceased in early May. Such a regimen entailed a minimum of 10–14 spray applications during spring.

The following year, 1971, the same methods of streptomycin use *failed* to control blight in Sacramento Valley pear (cv. Bartlett) orchards, although the material was effective elsewhere in California. In the first orchard, near Gridley, where the antibiotic failed to give control in early May, desperate measures were immediately adopted. The grower doubled the application rate and frequency of streptomycin use, but disease was not controlled. Field observations in the vicinity revealed that in some pear orchards certain trees were heavily infected (60–300 strikes/tree) while most had few infections (1–5

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Fig. 1. Severe pruning is sometimes necessary on pear trees extensively infected by early-season fire blight.

Fig. 2. Close-up of 2-week-old blight infection on pear tree.

strikes/tree). Both shoot and blossom infections were common, and blight lesions appeared to elongate more rapidly than normal. Close scrutiny of spray schedules did not indicate why streptomycin treatments failed, although in nearby orchards the disease was being suppressed with copper sprays (8).

To determine if the prevalent strain of the bacterium was resistant to streptomycin, samples from blighted Bartlett trees were taken in May 1971 and plated out on an *Erwinia*-selective medium (7) (Fig. 3) to which 200 ppm streptomycin had been added. The first samples taken from two severely infected pear orchards near Gridley showed excellent growth of *E. amylovora*. The bacteria even grew in the presence of 1,000 ppm streptomycin. In most orchards where control was poor, streptomycin-resistant (Str^r) strains predominated, although the common streptomycin-susceptible (Str^s) strains were present. In orchards with good control, only the Str^s strains were detected.

Str^r Strains in Other Areas of California and Other States

During 1971, the number of samples taken from 31 orchards in the Sacramento Valley and Sacramento River pear districts totaled 110; Str^r *E. amylovora* was found in 47 samples from 17 orchards. In 1972, four more orchards with resistance were located, and in the fall an orchard with Str^r *E. amylovora* was found in the Sacramento River area 20–30 miles south of orchards where the previous outbreaks had occurred.

A number of orchards with resistant strains had been on a streptomycin program for only 1 or 2 years. Approximately 30 samples were taken from other pear districts in California, and no Str^r strain was detected. In 1971 and 1972, all Str^r *E. amylovora* was confined to the Sacramento Valley between Yuba City and Hamilton City. By 1973, Str^r bacteria were detected in other pear-growing areas encompassing half of the state pear acreage. The locations of orchards with Str^r blight in central California in 1973 are shown in Figure 4. The situation has stabilized in recent years, and growers in certain areas of the state where the spring weather is cooler still used streptomycin in 1980.

After the outbreaks caused by Str^r strains of *E. amylovora*, fire blight was controlled in commercial orchards with sprays containing 0.5–1 lb of metallic copper in 50–100 gal of water per acre (0.56–1.12 kg in 468–936 L/ha). These

sprays were applied every 5 days from early bloom until early May (Table 1).

In 1972, some growers in the Yakima, Washington, area reported exceptionally poor control of fire blight after extensive use of streptomycin. Surveys revealed that Str^r strains of the pathogen were present in several orchards in Oregon and Washington (4). Sampling in Michigan apple orchards in 1974 (13) revealed no Str^r strains, and surveys in New York pear and apple orchards in 1975 showed that all isolates were Str^s (2). Thus, to date, all detections of Str^r strains in pome fruit orchards have been limited to the Pacific Coast of the United States.

Nature of Streptomycin Resistance

The extent of devastation caused by the streptomycin-resistant *E. amylovora* severely disturbed California pear growers. Emergency meetings of growers, researchers, and chemical company representatives were held during the early summer of 1971 to assess the situation and to formulate a course of action. Explanations to account for the occurrence of Str^r strains in the field and for the epiphytotic ranged widely. Some felt that university researchers had created the situation by advocating lower amounts of streptomycin per acre than those recommended by chemical companies. The rate recommended by the manufacturers of streptomycin in the 1960s and early 1970s was 28 oz/A (1.961 kg/ha), 17% active ingredient. Experiments by California researchers, however, showed that approximately the same level of control could be obtained by applying only 12 oz/A (0.841 kg/ha). Furthermore, applications at the higher rates frequently caused phytotoxicity, especially during periods of cold weather. The application of reduced amounts of streptomycin purportedly caused a stepwise training of fire blight bacteria for resistance to streptomycin. Others suggested that the resistant strains were probably introduced—and soon would spread to all pear-growing regions in California—and that the Str^r strains were much more virulent than the typical sensitive strains.

It became apparent that selective pressure of streptomycin in the field favored the buildup of Str^r strains. The emergence of Str^r strains was not caused by applying less streptomycin than recommended by the manufacturers. This was supported by the finding that the mechanism of resistance was a chromosomal mutation (11). Such mutations occur spontaneously with and without the presence of streptomycin. Accordingly, some Str^r strains were found on pyracantha plants in a southern California nursery where streptomycin was not used and where there had been little chance for contamination by Str^r strains from the northern orchards 800 km (500 miles) away.

The Str^r strains isolated from pears were all high-level mutants. The theoretical mutation rate of Str^s to Str^r

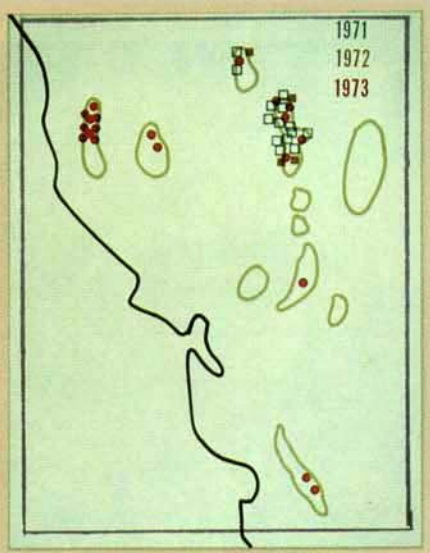

Fig. 4. Spread between 1971 and 1973 of streptomycin-resistant fire blight in central California pear orchards to the north and south of San Francisco.

Fig. 3. Smooth, round, orange colonies of the fire blight bacterium, *Erwinia amylovora*, grown on the Miller-Schroth selective medium.

Fig. 5. Oozing bacteria from fire blight canker in spring.

Table 1. Results of experimental fire blight control tests in California in 1972 after occurrence of Str^r strains in pear trees, showing superiority of some copper treatments over streptomycin[a]

Chemical	Rate/A	Strikes in 16 trees
Streptomycin 17%	28.8 oz	5.3
Tribasic copper	2 lb	10.0
Copper oxychloride sulfate (COCS)	2 lb	1.8
	1 lb	2.2
Copper hydroxide (Kocide)	2 lb	1.2
	1 lb	0.3
Control	···	15.3

[a]All sprays applied at 5-day intervals from early bloom until early May.

Table 2. Results in experimental plots in three California counties during the 1977 season, showing no increase in fire blight when treatment is delayed until temperature prediction line is crossed

Treatment started	Strikes/10 trees in		
	Sacramento	Yuba	Napa
5% Bloom	9	9	17
Temperature line[a] crossed	12	12	18
Bacteria in blossoms	28	32	35
Untreated control	38	33	31

[a]Drawn from 16.7 C (62 F) on March 1 to 14.4 C (58 F) on May 1.

Fig. 6. Epiphytic populations of *Erwinia amylovora* can be monitored before an infection period by sampling healthy pear blossoms.

strains calculated by laboratory tests revealed a low level of resistance to streptomycin at 10 ppm (10 μg/ml) with a mutation rate of 1×10^{-10} and a mutation rate to resistance to high levels (500 ppm) at 4.1×10^9. These findings were consistent with streptomycin resistance data for aminoglycoside antibiotics. Typical Str^r mutants show single-step, high-level changes with altered ribosomes, although there are bacteria with low or intermediate levels of streptomycin resistance (10). Coyier and Covey (4) in the Pacific Northwest reported finding strains of *E. amylovora* in the field with intermediate susceptibility, and Bennett and Billing (3) found such strains in laboratory-derived mutants. Comparing published accounts on the level of streptomycin resistance is difficult, however, since many laboratories do not use recommended performance standards in antibiotic disk tests, and different media markedly influence the size of inhibition zones (12).

Characteristics of Str^r Strains

The growers' notion in 1971 that Str^r strains of high virulence were present in a small epicenter and would soon spread throughout California was incompatible with scientific knowledge on streptomycin resistance. Because Str^r strains occur spontaneously, logic suggests they were present in all parts of California but selection pressures and other factors were not sufficient to favor their buildup. The subsequent finding of widespread distribution of Str^r strains with different characteristics indicates they arose from a heterogeneous assortment of susceptible strains and the epiphytotic was not caused by spread of a single virulent strain from an epicenter.

When compared, Str^r strains varied in virulence, growth rate, and colony type; the variation was no different from that of susceptible strains. This again is consistent with present knowledge of mutations to drug resistance. There is no evidence that mutations to drug resistance in bacteria are accompanied by increased virulence in animals (6). However, growth rates of resistant bacteria may increase, decrease, or remain the same, depending on the chemical (10). Thus, the epiphytotic in California in 1971 was apparently caused by weather conditions ideal for disease development coupled with extensive but ineffective use of streptomycin. This combination may have helped to disseminate the inoculum.

Stability and Longevity of Resistant Strains

The question of the stability and longevity of Str^r strains was of paramount importance because alternative chemicals for controlling fire blight, aside from copper compounds, were not available. Furthermore, copper compounds frequently caused fruit russet in many regions, particularly when applied early in the season. It was hoped, therefore, that the Str^r bacteria would soon disappear if streptomycin applications were discontinued for a year or so to avoid selective pressures. This seemed to be a reasonable expectation, since mutations to drug resistance frequently are accompanied by other changes, such as a slower growth rate, that do not favor survival (6). These data are primarily based on laboratory and theoretical studies, however, and do not justify confident prediction of population dynamics in the field. Also, most studies have dealt with resistance conferred by extrachromosomal elements in clinical situations.

Isolations in 1971 from the Gridley pear orchard, the discovery site of Str^r strains, indicated that 95% or more of the population of *E. amylovora* was resistant to streptomycin. Because an excessive amount of streptomycin had been applied, finding susceptible strains was difficult. When the resistant mutants were discovered, streptomycin use was immediately terminated. This orchard provided an excellent environment in which to study the stability and longevity of the Str^r bacteria.

By 1973, 75% of the flowers sampled in the orchard still contained Str^r *E. amylovora*; flowers were commonly colonized by mixtures of Str^r and Str^s strains. In 1974, 1975, and 1976, Str^r bacteria were found in 33, 14, and 10%, respectively, of the sampled diseased flowers. The persistence of Str^r strains indicated they were relatively stable. Str^r strains were not detected in 1978, although fewer than 30 infections were examined. No isolations were made in 1979. In 1980, however, 28 active infections were taken from the orchard and examined for Str^r; one sample contained resistant bacteria.

These findings indicate that the Str^r strains had persisted for almost 10 years in the same location despite no applications of streptomycin and also that the Str^r population was much more stable than expected or predicted on the basis of past studies. Although the overall frequency of Str^r bacteria declined over the 10-year period, sufficient bacteria are unquestionably present in the area to cause devastation similar to the 1971 outbreak if ideal weather conditions occur for infection and streptomycin treatments are used as the principal control.

Why Are Str^r Strains Limited to the Pacific Coast?

Two of the most puzzling aspects of streptomycin resistance in California are why was it not discovered earlier and why does it still not occur in some California localities where streptomycin is routinely used? Third, why has resistance to streptomycin not been detected in the Midwest and Northeast where streptomycin is used routinely for control of fire blight? We speculate that this is a reflection of the environment, the disease pattern, and the level of selection pressures exerted by streptomycin.

In California, Str^r strains occur principally in areas where the temperature is warm during flowering. These conditions favor epiphytic multiplication of the bacterium, with resultant flower infection. The opportunity for interaction between streptomycin and bacteria is much less in other California localities where streptomycin is applied because the bacteria are less likely to colonize flowers, and the incidence of disease is correspondingly much lower than in warmer areas. Also, much less streptomycin is used, which lowers the level of selection pressure for Str^r mutants. The 1971 date for detecting Str^r strains in California appears related to the intense use of the antibiotic during that year. Before then, streptomycin was used sporadically and alternately with other chemicals. Also, until 1968 it could not be applied later than 90 days before harvest, which meant fewer applications.

The disease pattern in California is very different from that in the Northeast and Midwest, where fire blight can occur throughout the season and where relatively few applications of streptomycin are made during bloom. The selection pressures for Str^r mutants are much greater on the Pacific Coast, where the disease occurs primarily during the bloom period and where numerous applications of streptomycin are made during a short period of time when the pathogen is extremely active. There is ample opportunity in the Midwest and Northeast for infections to be caused by Str^s *E. amylovora*, and resistant strains probably also occur. However, populations of resistant strains presumably have been too small for detection and the selection pressures have not been sufficient to cause a buildup.

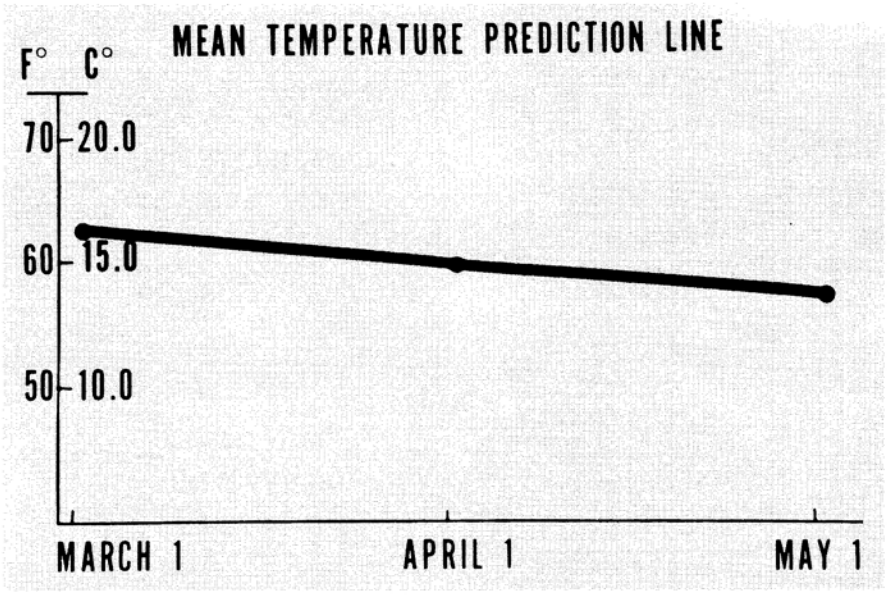

Fig. 7. Under Pacific Coast climatic conditions, epiphytic populations of *Erwinia amylovora* in blossoms do not increase until the daily mean spring temperature exceeds the prediction line; bactericide applications should then be started.

Epidemiology and Measures of Control in California

E. amylovora survives and overwinters in pear and apple trees or other rosaceous hosts. Some of these wood cankers on the tree become active and ooze bacteria in spring (Fig. 5), providing inoculum for a new season of infections. Population monitoring (Fig. 6) has shown that once bacteria are transmitted to new blossoms by rain splash, insects, mites, or wind, epiphytic colonization of pear flowers can proceed at a rapid rate, especially with warmer days. Although under Pacific Coast conditions the bacteria multiply in the flowers, they do not necessarily invade and infect the tree unless periods of high humidity or abundant rainfall coincide with warm temperatures.

During cool springs in California (14) and Washington (R. P. Covey, *personal communication*), bacteria buildup in the blossoms sometimes does not occur until well past the principal bloom period, which means growers can withhold sprays until the need is more critical—a management decision now possible as a result of intensive blossom monitoring studies. Consistently, in California, bacteria are first found after bloom has commenced and the daily mean temperature in the orchard (average of high and low temperatures from midnight to midnight) crosses a temperature line

drawn from 16.7 C (62 F) on March 1 to 14.4 C (58 F) on May 1 (Fig. 7) (14).

By contrast, if the daily mean temperatures exceed the prediction line during bloom and are soon followed by rain (or irrigation), the orchard probably will be severely diseased—unless chemical treatments are started. Results in experimental plots in three California counties during the 1977 season confirmed this approach to blight management on the West Coast (Table 2). A review of old blight records and corresponding temperature data further validated the concept (9).

Blight control will continue to rely on the time-tested methods of orchard cleanup plus careful chemical treatment. Recent research clearly shows that in those years when temperatures remain low through bloom, big savings are possible by reducing the number of chemical treatments.

In orchards where streptomycin has failed because of resistance, coppers or oxytetracycline (Terramycin) is the alternative. Terramycin, which has been registered for use on the Pacific Coast during the past few years, has been effective in orchards where streptomycin has failed and is less phytotoxic than copper.

Acknowledgments

We thank James A. Beutel and Wilbur O. Reil for the significant roles they played in developing some of the data and concepts described in this article.

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