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Development of Methods for Use of Antibiotics to Control Fireblight
(Erwinia amylovora)

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SUMMARY

Laboratory experiments found *E. amylovora* inhibited *in vitro* by Terramycin, Streptomycin, and Thiolutin. Greenhouse studies disclosed that foliar sprays with Terramycin and Streptomycin, alone and in combination, were capable of controlling fireblight in artificially innoculated one-year-old apple trees. The mode of action of these materials was established definitely to be that of "systemic protectants," providing a period of immunity of at least 24 hours duration. The addition of methyl cellosolve and Carbowax 4000, each at 1 percent concentration, increased the effectiveness of the antibiotic sprays. The theoretical function of these additives was to act as penetrating agents, facilitating ingress of the plant tissues by he antibiotic molecule.

Orchard experiments substantiated laboratory and greenhouse findings, in that antibiotic sprays provided excellent control of the fireblight organism. Complete and lasting inhibition was obtained with concentrations as low as 100 ppm Streptomycin. However, this high degree of protection was afforded only where antibiotic spray schedules were initiated early in the blooming period. Schedules initiated later, at early petal fall and at calyx, permitted some blossom blight to develop. In spite of this primary infection, the late sprays did reduce secondary infection significantly. This suggested that the antibiotics possessed an "erradicative potential" as well as the ability to provide temporary immunity. There also was evidence that antibiotics applied at 3 to 5 day intervals could provide adequate control.

This bulletin is a report on Department of Horticulture research project number 69, entitled "New Sprays and Spraying Methods, Fungicides, Eradication Spraying and Spray Injury."

Development of Methods for Use of Antibiotics to Control Fireblight
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INTRODUCTION

A disease of apples and pears, commonly known as fireblight, caused by the bacterium *Erwinia amylovora,* has been epiphytotic in Missouri five out of the past six years. Many Missouri apple orchards have been affected severely through the loss of crops and serious damage to trees. Losses from fireblight by no means have been restricted to Missouri, nor have they been limited to recent years. This disease sporadically wreaks havoc wherever apples are grown in the United States. It is directly responsible for the almost complete destruction of pear culture in the eastern and central states. No adequate control measure for fireblight has been available to the orchardist up to the present time.

Recent investigations have indicated that antbiotics could reduce blight infections. In the light of these developments and the pressing need for an effective control for fireblight in Missouri orchards, the effectiveness of antibiotics as blight control agents was studied.

This investigation was executed in three distinct phases; the first in the laboratory, the second in the greenhouse, and the final in the orchard. The experimental procedures and results are presented with respect to these phases.

PREVIOUS WORK

Literature pertaining to fireblight, its etiology and control, is exceedingly extensive and excellent reviews appear elsewhere (5, 8, 11, 12, 14, 15). A fairly complete bibliography of numerous investigations concerning *Erwinia amylovora* also is available (3).

Recently, antibiotics, the wonder drugs of human and veterinary medicine, have found application in plant disease control (1, 2, 7, 9, 10, 13).

The earliest investigation wherein these materials were employed successfully to control fireblight in apples was made in 1951 by Murneek (10), who found Thiolutin and Streptomycin reduced blight infections 75 and 50 percent, respectively. In 1952 Hueberger and Poulos obtained similar results with Streptomycin (7).

EXPERIMENTAL PROCEDURES AND RESULTS

I. Laboratory Experiments

(a) Activity of Antibiotics Against *Erwinia amylovora in vitro.*

Procedures—The organism used in these experiments was isolated from a typical canker of a naturally infected Jonathan apple tree. The isolate was maintained in a nutrient broth.

The antibiotics tested were Terramycin (crude 50%), Streptomycin (crude 30%) and Thiolutin (crude 66%).*

Activity of the antibiotics was ascertained by the cylinder plate technique (4). The Agar was seeded, while it was still fluid, with a 24-hour broth culture of the bacterium.

Results—The minimum concentrations at which the characteristic antibiotic-induced zones of inhibition were observed were:

Terramycin—2.5 ppm
Streptomycin—50.0 ppm
Thiolutin—100 ppm

(b) Methods of Formulating Antibiotic Sprays.

Procedures and Results.—The extreme solubility of Streptomycin in water facilitated its formulation in the activity experiments and spray preparations. Some difficulty was encountered, however, with Terramycin and Thiolutin since both are insoluble in water.

Terramycin was found to be exceedingly soluble in ethyl alcohol, acetone and methyl cellosolve. However, upon dilution with water, this antibiotic prepared with the above solvents formed a fine colloidal suspension. The suspension was dispersed easily and a clear solution prepared by the addition of a few drops of concentrated hydrocloric acid.

Thiolutin was much less soluble than Terramycin in the conventional organic solvents. Of the several organic solvents and combinations thereof, ethyl alcohol appeared to be the best.

Dissolution of all antibiotics in their appropriate solvents was accomplished with the aid of a Waring Blendor. This step was especially desirable for Terramycin as it permitted dissolving large quantities of this antibiotic in small amounts of solvent.

In preparing combination sprays of Terramycin and Streptomycin, best results were obtained when a definite order of procedure was followed. The Terramycin was dissolved first in alcohol in a blendor. From stock solution thus prepared, the desired concentration was obtained by dilution with water. Resulting suspension was clarified with HC1 and the Streptomycin was added.

*The author wishes to express his appreciation for the large quantities of antibiotics made available for this study by the Chas. Pfizer Co., Inc.

II. Greenhouse Experiments

Procedures—One year old trees of the extremely blight-susceptible Jonathan variety were used exclusively in the greenhouse-spray experiments. Sprays were applied with a continuous stream Jaeckh hand sprayer. Care was taken to assure complete coverage of both the top and undersides of leaves.

The innoculum was prepared by adding 10 cc of sterile water to a 48-hour slant culture.

In an effort to ascertain whether or not these antibiotics were systemic in their action, the following procedure was adopted:

(1) Antibiotic sprays were applied to the foliage of vigorous shoots.
(2) Twenty-four hours after the spray was applied the innoculum was administered, subepidermally.
(3) The point of innoculation was mid-way between the apex and the first node of the vigorous shoot.

Methyl cellosolve was added to the antibiotic spray formulations to facilitate entry of the comparatively large antibiotic molecule into the leaf tissue. This material was employed specifically as a cuticle solvent, the intent being to reduce the barrier imposed by the cuticle.

Results—Results of the first experiment (Table 1) showed Streptomycin and Terramycin capable of providing considerable resistance at the 1000

TABLE 1 -- GREENHOUSE EXPERIMENT 1

Antibiotic	Conc. ppm	Penetrant	Plants Protected	Plants Suscept.
Thiolutin	250	Methyl Cellosolve	0	9
Thiolutin	500	Methyl Cellosolve	0	9
Thiolutin	1000	Methyl Cellosolve	0	9
Streptomycin	250	Methyl Cellosolve	2	7
Streptomycin	500	Methyl Cellosolve	7	2
Streptomycin	1000	Methyl Cellosolve	6	3
Terramycin	250	Methyl Cellosolve	2	7
Terramycin	500	Methyl Cellosolve	3	6
Terramycin	1000	Methyl Cellosolve	5	4
Control	H_2O	Methyl Cellosolve	0	27

and 500 ppm concentrations. From these data it is assumed that the antibiotics acted as "systemic protectants." There is little doubt about the validity of this assumption, since the antibiotics were applied as foliar sprays 24 hours prior to a subepidermal administration of the innoculum.

Thiolution, under the conditions of these experiments, did not provide "lasting protection".[1] Even though "lasting protection" was not furnished by Thiolution or in some instances by Streptomycin and Terramycin, a

[1]Lasting protection implies that the antibiotics treated shoots did not display symptoms of the disease for the duration of the experiment (7 days after the last check tree reacted positively).

Figure 1—Apple shoots showing positive symptoms of fireblight 72 hours after artificial innoculation.

"temporary protection"[2] was observed. Control trees usually demonstrated positive symptoms (Figure 1) 72 to 96 hours after innoculation.

From this experiment it also was apparent that Streptomycin was directly responsible for a marginal and veinal chlorosis (Figure 2). This injury was rather unique in that the chlorotic regions failed to become necrotic and remained very much the same until normal leaf abscission took place.

During the course of the spraying operation it was noticed that the spray material dried rapidly on the leaf surface. An attempt was made to remedy this situation in the second greenhouse experiment (Table 2). The

TABLE 2 -- GREENHOUSE EXPERIMENT 2

Antibiotic	Conc. ppm.	Penetrant	Plants Protected	Plants Suscept.
Thiolutin	250	Methyl Cellosolve + Lanolin	0	9
Thiolutin	500	Methyl Cellosolve + Lanolin	0	9
Thiolutin	1000	Methyl Cellosolve + Lanolin	0	9
Streptomycin	250	Methyl Cellosolve + Lanolin	0	9
Streptomycin	500	Methyl Cellosolve + Lanolin	0	9
Streptomycin	1000	Methyl Cellosolve + Lanolin	0	9
Terramycin	250	Methyl Cellosolve + Lanolin	0	9
Terramycin	500	Methyl Cellosolve + Lanolin	0	9
Terramycin	1000	Methyl Cellosolve + Lanolin	0	9
Control	H_2O	Methyl Cellosolve + Lanolin	0	9

antibiotics in this experiment were formulated in lanolin emulsions (6); the intent being to reduce the rate of drying of the spray material on the leaf surface, thereby allowing the antibiotics a longer period for entry into the leaf tissues.

[2]Temporary protection implies symptoms of the disease were delayed from 1-4 days after the check tree reacted positively.

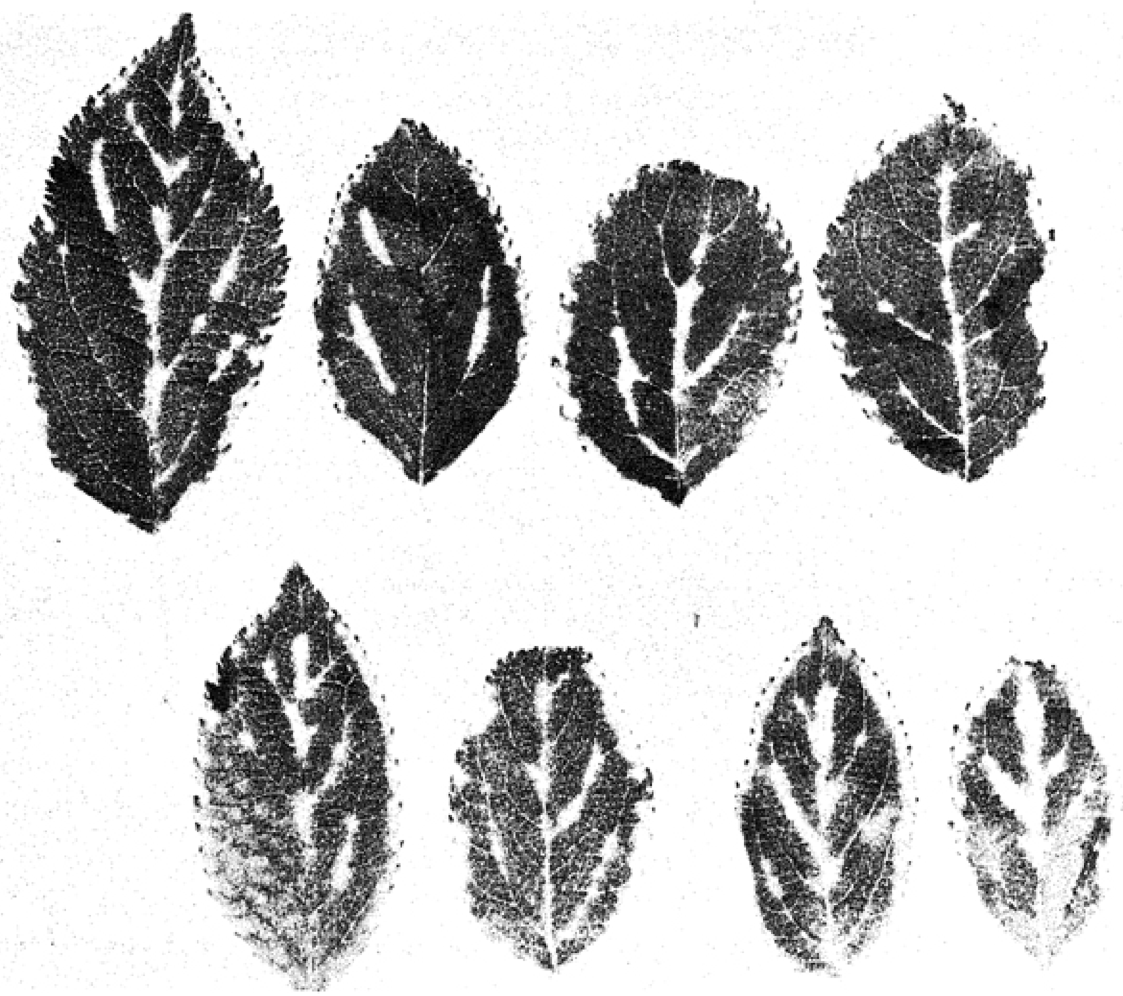
Although the spray material remained in a "wet" condition for a longer period of time than in the previous experiment, the data indicate that lanolin emulsion prevented rather than facilitated the ingress of the antibiotics. The lack of control obtained suggests that the lanolin either blocked entry of the antibiotics or permitted only a small amount of the material to penetrate the leaf tissues. This contention was supported by the absence of the Streptomycin-induced chlorosis observed in previous experiments.

The third experiment (Table 3) embodied two new modifications in the spray formulation. First, in another effort to reduce the rate of drying

TABLE 3 -- GREENHOUSE EXPERIMENT 3

Antibiotics	Conc. ppm.	Penetrants	Plants Protected	Plants Suscept.
Terramycin	500	Methyl Cellosolve + Carbowax 4000	7	3
Streptomycin	500	Methyl Cellosolve + Carbowax 4000	9	1
Terramycin + Streptomycin	250 + 250	Methyl Cellosolve + Carbowax 4000	10	0
Control	H_2O	Methyl Cellosolve + Carbowax 4000	0	10

Figure 2—Marginal and veinal chlorosis resulting from foliar spray with 500 ppm Streptomycin.



of the spray, and to reduce its surface tension, carbowax 4000, at 1 percent concentration was added. Secondly, the Streptomycin content of one of the spray formulations was reduced to 250 ppm. The total antibiotic concentration, however, was maintained at 500 ppm by the addition of Terramycin at 250 ppm.

Results indicated that carbowax increased effectiveness of the antibiotic-methyl cellosolve formulation. Equally significant was the excellent control, 100 percent, provided by the 250-250 ppm Streptomycin-Terramycin combination. This combination gave less Streptomycin injury. A reduction in Streptomycin content in the spray apparently would minimize the injury caused by this antibiotic.

The fourth experiment (Table 4) was conducted to establish the necessity of methyl cellosolve in the spray formulation. It differed from the previous one in that methyl cellosolve was removed from the spray formulation.

TABLE 4 -- GREENHOUSE EXPERIMENT 4

Antibiotic	Conc. ppm.	Penetrants	Plants Protected	Plants Suscept.
Terramycin	500	Carbowax 4000	3	7
Streptomycin	500	Carbowax 4000	1	9
Terramycin + Streptomycin	250 + 250	Carbowax 4000	1	9
Control	H2O	Carbowax 4000	0	10

The data clearly suggest that methyl cellosolve, under the conditions of these experiments, is a necessary component of the antibiotic spray formula.

In the fifth experiment (Table 5), further reduction of the Streptomycin to 125 ppm decreased the effectiveness of the combination spray in controlling fireblight infections. However, it was observed that Streptomycin injury was no longer visible. Thus it was apparent that the reduction of Streptomycin concentration in the spray was accompanied by a decrease of foliar chlorosis caused by this material.

TABLE 5 -- GREENHOUSE EXPERIMENT 5

Antibiotics	Conc. ppm.	Penetrants	Plants Protected	Plants Suscept.
Terramycin + Streptomycin	250 + 250	Methyl Cellosolve + Carbowax 4000	14	1
Terramycin + Streptomycin	250 + 125	Methyl Cellosolve + Carbowax 4000	6	9
Control	H2O	Methyl Cellosolve + Carbowax 4000	0	6

There was, however, an additional justification for reducing the Streptomycin content of these sprays; the greenhouse type of artificial innoculation was probably much more severe than would be encountered under orchard conditions. Any significant decrease in antibiotic concentration of the spray would improve the economic feasibility of this method of plant disease control.

III. Orchard Experiments

(a) **Plot I**—Determining the Time to Initiate the Antibiotic Spray Schedule and the Frequency of Application.

Procedures—The 72 trees in Plot I (Table 6) were nine-year-old Jonathans which had suffered severely from blight ravages during the previous

TABLE 6 -- PLOT I - DETERMINING TIME TO INITIATE ANTIBIOTIC SPRAY SCHEDULE AND FREQUENCY OF APPLICATION

Treatment	*Average No. Blossom Clusters	*Average No. Clusters Blighted	*Average No. Shoots Blighted
H7	102	0	0
C7	211	9	17
L7	293	0	0
H6	314	0	0
C6	421	18	16
L6	255	0	0
H5	175	4	0.5
C5	95	4	15.0
L5	432	8	1.5
H4	262	11	2
C4	227	20	31
L4	318	29	4

*Averages of 6 trees per treatment

Spray Schedule: First spray at ballon stage, April 22; second at 30-50% of full bloom, April 27; third at early petal fall, April 30; fourth at calyx, May 4 and three cover sprays on May 9, 14, and 21.

Key to Data:
H = 250 ppm Streptomycin + 250 ppm Terramyin + penetrants
L = 125 ppm Streptomycin + 250 ppm Terramycin + penetrants
C = Water + penetrants

7 = Received 7 sprays starting at balloon stage (April 22)
6 = Received 6 sprays starting at 30-50% of full bloom (April 27)
5 = Received 5 sprays starting at early petal fall (April 30)
4 = Received 4 sprays starting at calyx (May 4)

three years. These trees were separated into four distinct groups, with each group receiving its initial spray at a different stage of floral development: ballon stage (April 22), 30 to 50 percent of full bloom (April 27), early petal fall (April 30), and calyx (May 4). All of the trees received three cover sprays which were applied, May 9, 14, and 21. Thus there were trees that received as many as 7 and as few as 4 sprays.

The antibiotic concentrations evaluated in this block were the Streptomycin-Terramycin combinations that proved so successful in the greenhouse experiments, 250 ppm Streptomycin + 250 ppm Terramycin and 125 ppm Streptomycin +250 ppm Terramycin. These sprays also contained the two penetrants, methyl cellosolve and carbowax 4000, each at 1 percent concentration.

There was a check tree adjacent to each antibiotic sprayed tree. These check trees were sprayed with water and penetrants to neutralize any dissemination of innoculum by the spray *per se.* Each treatment was replicated 6 times.

The spray equipment used in all of the orchard experiments was a 300 gallon high pressure sprayer. Application of spray was made from the ground with a hand gun which delivered approximately 10 g.p.m. under 500 to 600 pounds pressure.

Results—Although fireblight was less severe than it had been previously, only one of 24 control trees failed to show blight injury. Blossom blight was first observed on May 9 and shoot blight was noted on May 16.

Perhaps the outstanding result of this experiment was that fireblight control was complete, regardless of concentration, where the spray schedule was initiated at either the ballon stage (April 22) or 30 to 50 percent of full bloom (April 27). This high degree of control was maintained throughout the growing season.

Where the spray schedule was initiated at either early petal fall (April 30) or calyx (May 4) some blossom and shoot blight was evident. Although blight developed in these late bloom-sprayed trees, the amount of shoot blight (secondary infection) was significantly less than that observed in the control trees, the order of reduction being approximately 1:15.

These results indicate that the application of early bloom sprays is imperative for blossom blight control. The marked reduction in shoot blight provided by the late bloom sprays suggests that the antibiotics act not only as "systemic protectants," but also may be effective as erradicants. The probability that antibiotics possess an "erradicative potential" is supported by the fact that complete control was obtained where the spray schedules were initiated at either ballon stage or 30 to 50 percent of full bloom. There was an interval of five days between these two spray periods, an interval during which considerable infection could possibly have been established, yet none was observed.

The data also suggest that a three to five day interval between spray applications should provide adequate control.

The relative importance of the cover sprays cannot be deduced from the data. However, it seems logical to assume that if blossom blight is controlled, then shoot blight (secondary infection) should not be a major problem.

The characteristic Streptomycin-induced foliage injury was observed with both antibiotic formulations. The effect on foliage and fruit was severe at the 250-250 ppm Streptomycin-Terramycin combination. At the 125-250 ppm concentration, foliage injury was drastically reduced and there seemed to be little, if any injury on the fruit.

It was apparent also that a large portion of the injury to foliage and fruit was caused by the cover sprays.

(b) **Plot II**—Effectiveness of Antibiotic Sprays in Controlling Fireblight in Mature Trees.

Procedures—Four Jonathan trees were selected to receive antibiotic sprays in this plot. The trees were 18 years old and were in a block that had consistently suffered severe fireblight damage during the previous six-year period.

The spray formulation was the 250-250 ppm Streptomycin-Terramycin combination with methyl cellosolve and carbowax 4000, each at 1 percent.

The spray schedule was identical with that followed in Plot I.

There were control trees located adjacent to and across from each antibiotic sprayed tree. As in Plot I the check trees were sprayed with water and penetrants.

Results—As had been noted in previous years, fireblight was most severe in this block and in an adjacent one. All of the control trees exhibited considerable blight injury. The data for this experiment is recorded in Table 7. It was evident that blight control was excellent in the antibiotic sprayed trees. Again it was observed that the control obtained was lasting, since the treated trees remained free of infections throughout the growing season.

TABLE 7 -- PLOT II - EFFECTIVENESS OF ANTIBIOTIC SPRAYS IN CONTROLLING FIREBLIGHT IN MATURE TREES

Treatment	Average No. Blossom Clusters	Average No. Clusters Blighted	Average No. Shoots Blighted
*H7	855	0	0
**C7	---	31	90

*Average of 4 trees
**Average of 6 trees

Spray Schedule: First spray at balloon stage, April 22; second at 30-50% of full bloom, April 27; third at early petal fall, April 30; fourth at calyx, May 4 and three cover sprays on May 9, 14 and 21.

Key to Data:
H7 = Sprayed 7 times with 250 ppm Streptomycin + 250 ppm Terramycin + penetrants
C7 = Sprayed 7 times with water + penetrants.

Sreptomycin injury was severe in this group of sprayed trees. Much of the early foliage showed the typical marginal chlorosis and some of the fruit also took on a yellowish cast. The injured foliage remained chlorotic. Necrosis of the injured tissue was not observed. Premature defoliation of the injured leaves was not observed, but many of the young fruit that yellowed abscissed prematurely. Fruit remaining on the tree showed varying amounts of russet.

Although blight control was excellent, it would appear that the 250-250 ppm Streptomycin-Terramycin was too phytotoxic. As in the previous experiment, it was noted that much of the fruit and foliage injury was caused by the cover sprays.

(c) **Plot III**—Fireblight control with Streptomycin at a low concentration.

Procedure—A row of 12 nine-year-old Jonathan trees was selected for this experiment. Previous blight injury in this plot had been light. The antibiotic spray formulation contained Streptomycin at 100 ppm and the penetrants methyl cellosolve and carbowax 4000, each at 1 percent concentration.

The spray schedule differed from those in the previous experiments in that the last two cover sprays (May 14 and 21) were omitted. Therefore, the trees in this experiment received only 5 sprays.

Every third tree in the row was a control. The controls were sprayed with water and penetrants.

Results—Although the incidence of blight was rather light in this plot (Table 8) each of the four check trees displayed blight infections. The antibiotics treated trees, however, remained completely free of infection throughout the growing season.

TABLE 8 -- PLOT III - FIREBLIGHT CONTROL WITH STREPTOMYCIN AT A LOW CONCENTRATION
Nine-Year-Old Jonathans

Treatment	Average No. Blossom Clusters	Average No. Clusters Blighted	Average No. Shoots Blighted
*S5	474	0	0
**C5	415	5	11

*Average of 8 trees per treatment
**Average of 4 trees per treatment

Spray Schedule: First spray at balloon stage, April 22; second at 30-50% of full bloom, April 27; third at early petal fall, April 30; fourth at calyx, May 4; and at first cover, May 9.

Key to Data:
S5 = Sprayed 5 times with 100 ppm Streptomycin + penetrants
C5 = Sprayed 5 times with water + penetrants

Of decided importance is the fact that Streptomycin injury was not apparent on either foliage or fruit.

(d) **Plot IV**—Effectiveness of Bordeaux Mixture as a Fireblight Control Agent.

Procedure—It has been reported frequently that a weak Bordeaux mixture 1-3-100, applied during the bloom effectively controls fireblight. A row of 14 nine-year-old Jonathan trees was selected to receive this treatment. Alternate trees were controls and received a water spray. Previous blight damage of these trees had been light.

The Bordeaux was applied twice during the bloom, at 30 to 50 percent of full bloom and at early petal fall.

Results—The results are recorded in Table 9. The incidence of blight was light, yet it is apparent that Bordeaux effected little, if any, control. The characteristic fruit russet caused by this material was observed on most of the fruit.

There is little doubt that, under the conditions of these experiments, antibiotics proved far superior to Bordeaux.

TABLE 9 -- PLOT IV - BORDEAUX MIXTURE AS A FIREBLIGHT CONTROL AGENT
Nine-Year-Old Jonathans

Treatment	*Average No. Blossom Clusters	*Average No. Clusters Blighted	*Average No. Shoots Blighted
B2	326	5	11
C2	403	13	14

*Averages of 7 trees per treatment

Spray Schedule: First spray at 30-50% of full bloom, April 27; and second at early petal fall, April 30.

Key to Data:
B2 = Sprayed twice with Bordeaux 1-3-100
C2 = Sprayed twice with water

DISCUSSION

Laboratory and Greenhouse Experiments

Mode of Action of Antibiotics. Through laboratory and greenhouse experiments it was found that Streptomycin and Terramycin were effective inhibitors of *E. amylovora,* both *in vitro* and *in vivo.* Although it was not evident from this investigation whether their effect was bacteriacidal or bacteriastatic, there seemed little doubt that their mode of action was truly systemic. Excellent control was obtained (in greenhouse experiments) where the innoculum was administered subepidermally 24 hours after a foliar spray with antibiotics. This clearly demonstrated that the antibiotic materials were present in effective concentration within the plant tissues. Thus it may be said that, under the conditions of these experiments, the antibiotics functioned as "systemic protectants," furnishing a temporary immunity from infection by the fireblight organism.

Penetrants—The greenhouse experiments indicated that two organic chemicals, methyl cellosolve and carbowax 4000, were essential components of the antibiotic spray formulation. Their specific function was to facilitate penetration of the leaf tissue by the antibiotic molecule. The manner in which they accomplished this end can only be theorized at this time.

It was the contention of the writer that the cuticle would present a considerable barrier to the comparatively large antibiotic molecule. Therefore methyl cellosolve, an organic solvent, was selected to partially dissolve or perforate this waxy layer of the leaf surface. Appreciable quantities of antibiotic molecules could undoubtedly pass through the stomatal and other natural openings. Yet it seemed questionable that lateral passage from these natural openings to a major portion of the plant cells could be effected without a concomitant dilution of antibiotic in the tissues. However, if in some way the cuticle could be made more pervious, the number of cell surfaces exposed to the antibiotic spray would be increased. Extensive translocation of these materials would, as a result, become unnecessary since countless numbers of cells would be directly infused with the antibiotic.

Carbowax 4000 was added to the spray formulation to reduce the surface tension of the spray liquid, permitting the leaf to be wetted uniformly and completely. Greenhouse experiments found this material to be ineffecttive as the sole penetrant, but when combined with methyl cellosolve the effectiveness of the antibiotics were materially increased.

Methyl cellosolve and carbowax 4000 are members of two rather extensive groups of organic chemicals. It seems reasonable to assume that other members of these two families of compounds would function equally as well if not better as penetrating agents. Their use as penetrants need not be limited to spray chemicals for plant disease control, but might also find application in other phases of plant science; for example, the field of plant growth regulators.

Orchard Experiments

Early bloom sprays important. The orchard experiments confirmed results of the laboratory and greenhouse studies, demonstrating that antibiotics could effectively inhibit the fireblight organism. Furthermore, control was complete and lasting, at concentrations as low as 100 ppm Streptomycin, where the spray schedules were initiated at either the balloon stage or at 30 to 50 percent of full bloom. These early bloom sprays appear to be most important if blossom blight is to be eliminated. Spray schedules initiated later in bloom period, early petal fall, and calyx permitted both blossom and shoot blight to develop.

Erradicative potential of antibiotic sprays. The orchard studies suggest that antibiotics function not only as "systemic protectants" but that they also possess an "erradicative potential." This is indicated in the data by the highly significant reduction in secondary blight obtained with the late bloom sprays, despite the fact that primary infection had occurred. The existence of this potential is also validated by the fact that complete exclusion of primary and secondary infection was accomplished where the spray schedule was initiated at either the ballon stage or at 30 to 50 percent of full bloom. A five-day interval elapsed between these two periods, an interval during which numerous blossoms were open and susceptible to infection. It seems reasonable to assume that some innoculum was disseminated during this period, yet none was observed to develop.

Frequency of spray applications. To accurately determine the frequency at which antibiotic sprays should be applied, the length of time that these materials remain active within the plant tissue must be known. The greenhouse experiments conclusively demonstrated that the antibiotics remain effective *in situ* for at least a 24-hour period. The field experiments suggest that a three to five-day interval between applications provides excellent control of primary and secondary blight. However, it is possible that the antibiotics do not remain effective during the entire three to five day period. Should this be the case, it would appear that any infections incurred after the antibiotics had lost their potency would be erradicated by the succeeding spray.

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