1986 FINAL REPORT TO CALIFORNIA PEAR ZONE

PRINCIPAL INVESTIGATOR:

S. E. Lindow, Department of Plant Pathology, University of California, Berkeley

OTHER PERSONNEL INVOLVED:

D. Okamoto and G. Anderson, Staff Research Associates, Department of Plant Pathology, University of California, Berkeley; J. Joos, Cooperative Extension, Santa Rosa; B. Bearden, Cooperative Extension, Ukiah; W. Reil, Cooperative Extension, Fairfield; W. Vogel, Coopertive Extension, Stockton; J. Beutell, Cooperative Extension, Davis

RESEARCH PROJECT:

Control of fireblight, frost injury, and fruit russet of pear using chemical and biological agents.

SUMMARY

Much progress was made in understanding both the causes and control of pear fruit russeting as well as in developing effective integrated control methods for frost injury and fireblight to pear. Cool early season temperatures mimimized the occurrence of fireblight as well as epiphytic populations of the fireblight pathogen Erwinia amylovora in all of seven different plot locations established during 1986. subfreeezing temperatures were observed only in one plot location and significant frost control resulted from several different treatments. Populations of ice nucleation active bacteria, responsible for inciting frost damage to pear, were reduced by several different biological and chemical treatments. Synergistic effects between certain antagonistic bacteria and registered bactericides such as streptomycin and Terramycin were observed in all field trials in reducing both the population size of ice nucleation active bacteria, reducing frost injury to plants, and in reducing the incidence of russeting to pear fruit. The freezing temperature of pear tissues treated with various bacterial antagonists as well as bactericides and mixtures of these two agents were obtained in all trials. The freezing temperature of pear tissues was reduced from 1 - 4°F by various treatments. Of considerable importance was the finding that certain bacteria which produce plant growth regulators appear to be important causes of fruit russeting to pear. Application of bacteria that produce 3-indole acetic acid to pears in each of three plot locations during 1986 significantly increased the incidence and severity of fruit russeting. Certain antagonistic bacteria and bactericides reduced the incidence of russeting, presumably by controlling auxin producing bacteria. A survey of russet-free and russet-prone orchards revealed that indole acetic acid producing

bacteria are more prevalent in russet-prone orchards and produce higher levels of this plant growth regulator. The identification of bacteria as a probable cause of fruit russeting explains the irregular appearance of this problem and suggests methods by which fruit russeting might be controlled.

More specific information regarding progress in several general areas is provided below:

INTEGRATED BIOLOGICAL AND CHEMICAL CONTROL OF FIREBLIGHT AND FROST INJURY TO PEAR

Meteorological features for 1986 were exceptional during the growing season for pear. Flowering was prolonged and was initiated 2-3 weeks earlier than average. Flowering occured largely during periods of cool, rainy weather during 1986. Because of the very cool temperatures during the blossom period, populations of the fireblight pathogen Erwinia amylovora, did not develop during 1986. No significant epiphytic populations of E. amylovora were detected in any of eight plot locations in all major pear growing regions of the state during 1986. Only occasional fireblight strikes were observed in our plots and these were generally associated with hold over cankers from 1985. Because of the rainy weather in the early spring of 1986 however, populations of ice nucleation active bacteria, principally Pseudomonas syringae, were common in all plot locations (Table 1, and Figures 1-6). However, only in Scotts Valley, CA were significant sub-freezing temperatures encountered. Despite a lack of frost injury in most plots, extensive measurements of plant freezing temperatures and bacterial populations in laboratory studies revealed the effects of various biological and chemical treatments for the control of frost injury to pear.

The population size of ice nucleation active bacteria on untreated trees in all plot areas in 1987 increased from shortly after flowering to in excess of approximately 100,000 bacteria per gram of pear to flower, fruit, and leaf tissue (Table 1, Figures 1 and 4). Repeated application of bactericides such as Terramycin (Table 1, Figure 3) or mixtures of streptomycin and Terramycin to trees starting at bud break significantly reduced the population size of ice nucleation active bacteria. The magnitude of the reductions in the numbers of ice nucleation active bacteria varied greatly from plot to plot. Previous results during 1985 had shown that the populations of Pseudomonas syringae in different geographical regions in California varied greatly in their incidence of streptomycin and/or Terramycin resistance. It appears likely that differences in control by bactericide sprays is due to the escape of resistant populations in some cases and might be expected in commercial fields as well. Antagonistic bacteria applied in a single application at approximately 10% bloom colonized energizing vegetative tissues of pear anywherefrom 1 - 3 months. Antagonistic bacteria comprised from 90 - 95% of the total population of bacteria on treated trees (Figures 2, 3, 5, 6, Table 1). This was particularly impressive in light of the fact that the vegetative growth of pear exhibited an approximately 100 fold increase in mass during these periods of time. Not only did the

population size of antagonistic bacteria not decrease during this rapid growth of plant tissue, but increased over this period. Thus antagonistic bacteria appeared to grow rapidly on pear tissue and continued to colonize newly expanding pear tissues as trees grow. This very likely accounts for their efficacy in controlling populations of Pseudomonas syringae as well as E. amylovora to be discussed later.

Certain antagonistic bacteria isolated from pear trees were shown to be naturally resistant to both commonly used antibiotics, streptomycin and Terramycin (oxytetracycline). Antagonistic bacteria A506 and A526, both strains of the saprophytic bacterium Pseudomonas fluorescens, grew well on pear trees which did not receive applications of antibiotics (Figure 2 and 5) as well as on trees that received, in addition, repeated applications of streptomycin and/or Terramycin (Figure 3, Table 1). The population sizes of these antagonistic bacteria were not significantly reduced and were frequently higher on trees treated with antibiotics in addition to antagonistic bacteria. Reductions in the numbers of ice nucleation active bacteria was proportional to the numbers of antagonistic bacteria found on plants. The higher the populations of antagonistic baceteria on plants the lower the population size of ice nucleation active bacteria that could be supported on these plants (Figure 2, 3, 5, 6).

The supercooling point of pear tissues (the coldest temperature to which the flowers and fruit of pear could be cooled before ice formation occurs) was decreased both by bactericides and antagonistic bacteria during 1986. Tissue from untreated pear trees generally supercooled very little (the supercooling point was between -2.2 and -2.8 C) (28 F). Such tissue would likely freeze as soon as the air temperature dropped to a temperature of no more than 28-29 F. However, such tissues treated with a single application of various antagonistic bacteria at approximately 10% bloom, caused the freezing temperature of the plant tissues to be reduced from 1 - nearly 3 C (Tables 2 - 5). Thus pear tissues treated with antagonistic bacteria such as a mixture A506 and A526 as well as other non-ice nucleation active strains of Pseudomonas syringae such as P56, P767, P1016, and P40 caused the freezing temperature to drop from 2 - 3 F compared to untreated control plants. While treatment with antibiotics such as Terramycin or a mixture of streptomycin and Terramycin also caused a significant decrease in the supercooling point of pear tissues (Tables 2 - 4), a mixture of bactericide-resistant antagonistic bacteria with such bactericides caused an even greater reduction in the freezing point of the plant tissues (Table 2 - 4). These reductions in the supercooling point (freezing temperature) of pear tissue are largely correlated with the reductions in the numbers of ice nucleation active bacteria on such pear tissue. Antagonistic bacteria, and mixtures of antagonistic bacteria such as A506 and A526 mixed with antibiotics such as Terramycin caused the greatest reductions in both the numbers of ice nucleation active bacteria and the freezing temperature of pear tissue. Freezing temperatures were encountered in a pear trial near Scotts Valley, CA during 1986. Minimum air temperatures at this plot were estimated at between -3 and -4 C. Frost injury at this plot was

reduced by antagonistic bacteria such as A506 and A526 which reduced numbers of ice nucleation active bacteria at this and other plots (Table 6) as well as by a mixture of streptomycin and Terramycin (Table 6). Comparisons of Table 6 with Tables 1 - 4, reveal that the reduction of frost damage to plants under field conditions at Scotts Valley was highly correlated with the reduction of the supercooling point shown in other trials. Therefore, we would expect that had freezing temperatures been encountered at other plot locations, reductions in freezing damage would also have occurred.

Antagonistic bacteria were established in a large, well replicated field trial using commercial "speed sprayer" application equipment during 1986. Most previous trials had involved a relatively small number of trees (from 8 - 20 per treatment). A large replicated trial of two bacteria were established and applied with a speed sprayer and received repeated applications of Terramycin also applied by speed sprayer application. Antagonistic bacteria A506 and A526 both survived speed sprayer application well (Table 1). The effectiveness of these antagonistic bacteria either singly or in combination with antibiotic applications both in reducing the numbers of ice nucleation active bacteria (Table 1) and in reducing the supercooling point of treated tissues (Table 2), both indicate that the effectiveness of antagonistic bacteria when applied in a large acerage under a commercial situation may be larger than that seen in replicated experimental plots. In large trials such as that at Steamboat Orchards, relatively few trees within the experimental plot are near untreated trees on the "outside" of the plot which are contributing inoculum of either ice nucleation active of E. amylovora to the treated trees. Therefore, the "edge" effect is diminished when using large trials. We might then surmise that the effectiveness of antagonistic bacteria or bactericides should both increase with the increasing size of the treated plots.

Toxicological information on antagonistic bacteria A506 and A526 has now been obtained through the service of a private laboratory. Such toxicological information will be necessary to obtain an experimental use permit for these organisms. In addition, during 1986 residue analysis of these two antagonistic bacteria on untreated plots were obtained in three different locations in California. No detectable populations of either strain A506 or A526 were found on fruit at harvest. Since no significant toxicity of these saprophytic bacteria was obtained, and since no detectable residue of these bacteria remain at harvest, little difficulty in obtaining an experimental use permit or their use on large acerages is foreseen. This information is now being collated and is being provided to the California Department of Food and Agriculture to obtain an experimental use permit for the testing of these organisms on large commercial size acerages within California. Hopefully, such an experimental use permit may be available for use during the 1987 growing season.

INVOLVEMENT OF PLANT HORMONE PRODUCING BACTERIA IN PEAR FRUIT QUALITY

Considerable information was obtained during 1986 confirming the involvement of a group of bacteria which live on healthy pear leaves and fruit in causing fruit russeting. Preliminary work during 1985 had indicated that bacteria which produced 3-indole acetic acid could increase the severity of fruit russeting to pear. This was confirmed by extensive trials during 1986. Several bacteria were characterized from pear tissue during 1985 that produced very high levels of a plant growth regulator (3-indole acetic acid) in laboratory studies. Since such strains were thought to be much better colonizers of pear than were the bacteria used during 1985 to elucidate the role of this hormone in russet formation, these bacteria were characterized and used during 1986 in more extensive studies. All bacteria that produced 3-indole acetic acid and only bacteria that produced 3-indole acetic acid increase the severity of fruit russeting when applied to young pear fruit at approximately 10-20% bloom (Table 7 - 9). The severity of fruit russeting varied greatly from the three different geographical regions where fruit russeting was tested. Fruit russeting was highest near Healdsburg (Table 7) of intermediate severity near Ukiah (Table 8) and lowest near Marysville, CA (Table 9) during 1986. However, at all three locations, IAA producing bacteria significantly increased the severity of fruit russeting to treated fruit. Fruit russeting was from 2 - 3 times higher on IAA bacteria colonized fruit than on untreated fruit or on fruit colonized by various other antagonistic bacteria that did not produce IAA (Tables 7 - 9). Tryptophan is an intermediate in the synthesis of 3-indole acetic acid in most bacteria that have been studied. Generally bacteria in culture produce more 3-indole acetic acid in the presence of tryptophan than in the absence of tryptophan. For this reason tryptophan was added to inoculum of some IAA producing bacteria. The severity of fruit russeting was increased from some to a great deal in the presence of added tryptophan giving additional indications that IAA production per se was the cause of russeting on these fruit (Table 7). IAA producing bacteria grew well on treated pear trees (Figure 6). Most IAA producing bacteria comprised 90-95% of the total bacteria on treated trees. While untreated control trees almost certainly also had certain IAA producing bacteria on them, the number of IAA producing bacteria was substantially increased on trees treated with IAA producing bacteria. Therefore it seem likely that the severity of fruit russeting is correlated with the population of IAA producing bacteria on these trees. This will also be addressed later.

Certain antagonistic bacteria and bactericides reduced the severity of fruit russeting when applied at 10-20% bloom (Tables 10 - 12). Presumably, these antagonistic bacteria or bactericides, by modifying the numbers and types of other bacteria (including IAA producing bacteria on plants) effected the amount of exogenous IAA being produced on trees. While the reduction in severity of fruit russeting on trees treated with various antagonistic bacteria such as A526, A506 and in some plots treated with a mixture of streptomycin and Terramycin was subtle, these differences were statistically significant and confirmed similar effects during 1984 and 1985. These

results also indicate that biological and chemical control of fruit russeting are also possible. This point will also be addressed later.

Further involvement of the role of IAA producing baceteria in inciting fruit russeting to pear was obtained by a detailed survey of eight orchards from Mendocino County with a well-known and reproducible history of russeting. Four orchards had a high russet severity and four orchards had a low severity of russeting in previous years. Fruit samples were obtained from these orchards shortly after petal fall (when other studies by Jim Beutell, T. Thomas and others had shown that fruit russeting seemed to induced) and evaulated for the types of bacterial populations present. In particular, the IAA production of a subset of this population was evaluated in laboratory studies. Approximately 3,600 bacterial isolates were made randomly from bacteria recovered from russet-free and russet- prone orchards and scored for their ability to produce IAA in culture. Bacteria isolated from russet-prone orchards generally had a higher rate of production of IAA than did bacteria isolated from orchards with a lower severity of fruit russeting (Table 13 - 15). IAA producing bacteria were detected both on the leaves of russet-prone orchards (Table 13) as well as on the fruit of russet-prone orchards (Table 14). When considered in total, the average rate of production of IAA in orchards having a high russet severity was higher than that of orchards having low russet severity (Table 15). This was particularly striking when the average rate of production of IAA by bacteria was multiplied by the incidence of such producing strains on isolates from russet free and russet prone orchards (Table 15). This data also clarifies the role of IAA producing bacteria in inciting russeting. While plant susceptibility to exogenously produced auxins such as IAA probably differs and accounts for difference in overall extent of russeting, other local and regional differences in severity of russeting between orchards (having very similar climates and soil types for example) might readily be accounted for based on differences in the types of bacteria appearing on these trees. Data found in Tables 13 - 15 clearly show that IAA bacteria do differ in their abundance between such orchards within a small geographical region. Further analysis of results from such survey information is being performed to further clarify the role of IAA in stimulating russet formation. Bacterial species causing IAA formation, and therefore fruit russeting, have been at least partially characterized. Several different bacterial species can produce IAA in culture, including strains of Pseudomonas fluorescens, Erwinia, and Flavobacterium. Therefore, bacteria stimulating russeting appear not to be a homogenous group and encompass a diversity of bacteria, all of which produce plant growth regulators. Apparently by producing locally large concentrations of auxins on fruit they alter the development of fruit in early stages of fruit maturation which leads ultimately to fruit russeting and corky cell formation at full maturity.

Among the 3,600 bacterial strain evaluated for their ability to produce IAA and therefore cause russeting, several were identified that did not produce IAA and degraded this compound in culture. Since IAA is found in small quantities on the surfaces of leaves both from plant origin (as well as from bacteria which might produce it in this

habitat) bacteria have apparently evolved the ability to degrade this compound and use it as a source of nutrients. We are further identifying and studying such IAA-degrading bacteria as potential antagonistic bacteria to reduce the severity of fruit russeting. We will be establishing IAA-degrading bacteria on the surfaces of pear fruit during 1987 to determine their ability to reduce fruit russeting.

The role of IAA in inducing fruit russeting appears strong and is consistent with the observations of other workers. For example, variations in fruit russeting between orchards may be due simply to changes in bacterial populations which we have documented between orchards. Increased severity of fruit russeting and wet years are also consistent with higher bacterial populations during these years. The reduction of fruit russeting by applications of Gibberellic and other plant growth regulators is also consistent with a hormonally induced change in fruit development. For example gibberellic acid counteracts the effects of auxins in fruit development. Therefore, having a better indication of a significant cause of fruit russeting allows for several different approaches for its control.

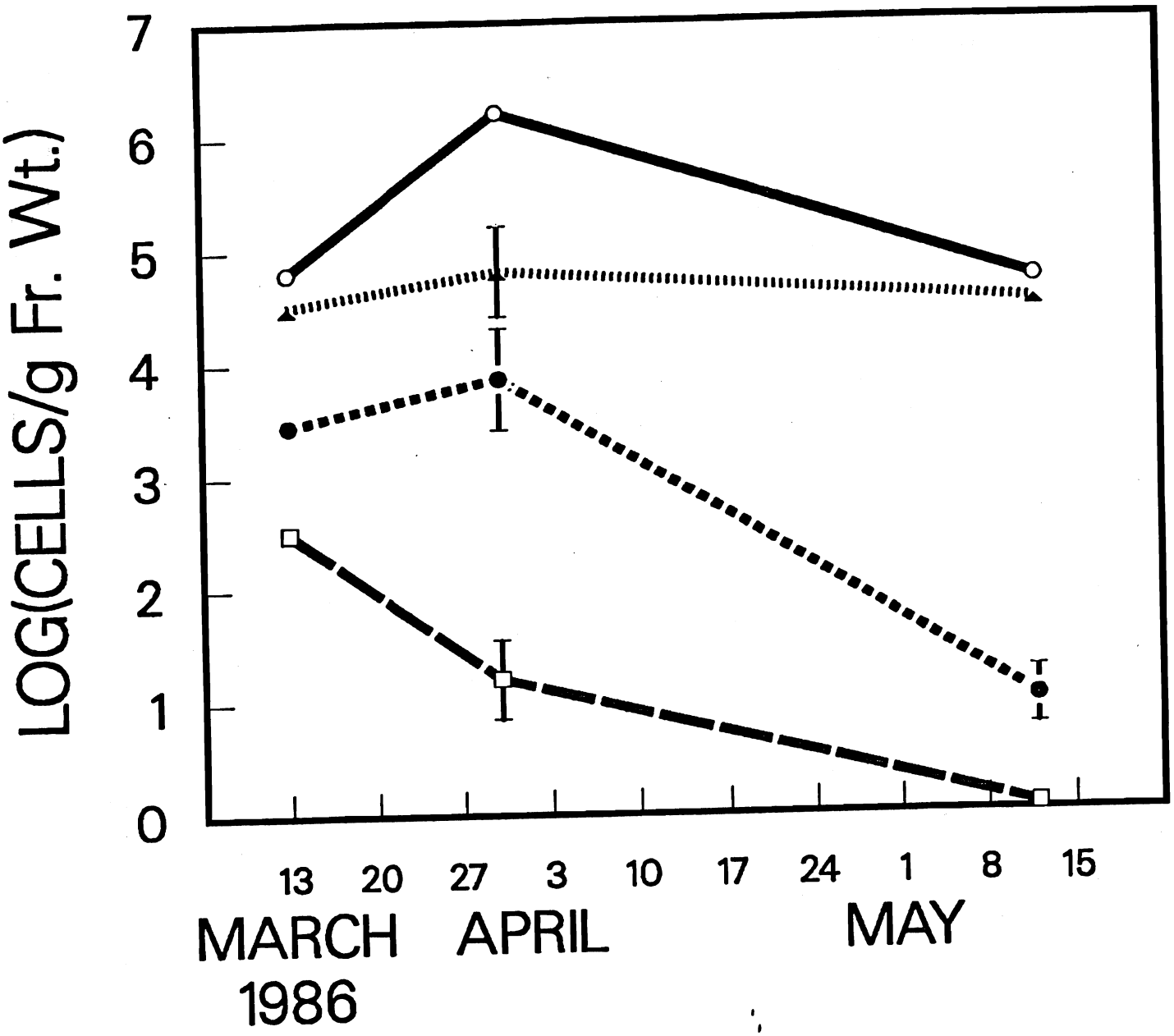

Figure 1. Total numbers of bacteria (circle), population size of ice nucleation active bacteria (triangle), rifampicin resistant antagonistic bacteria (filled circle), and ice nuclei active at -5 C (square) on untreated Bartlett pear trees grown near Marysville, CA. The vertical bars represent the standard error of the determination of the mean.

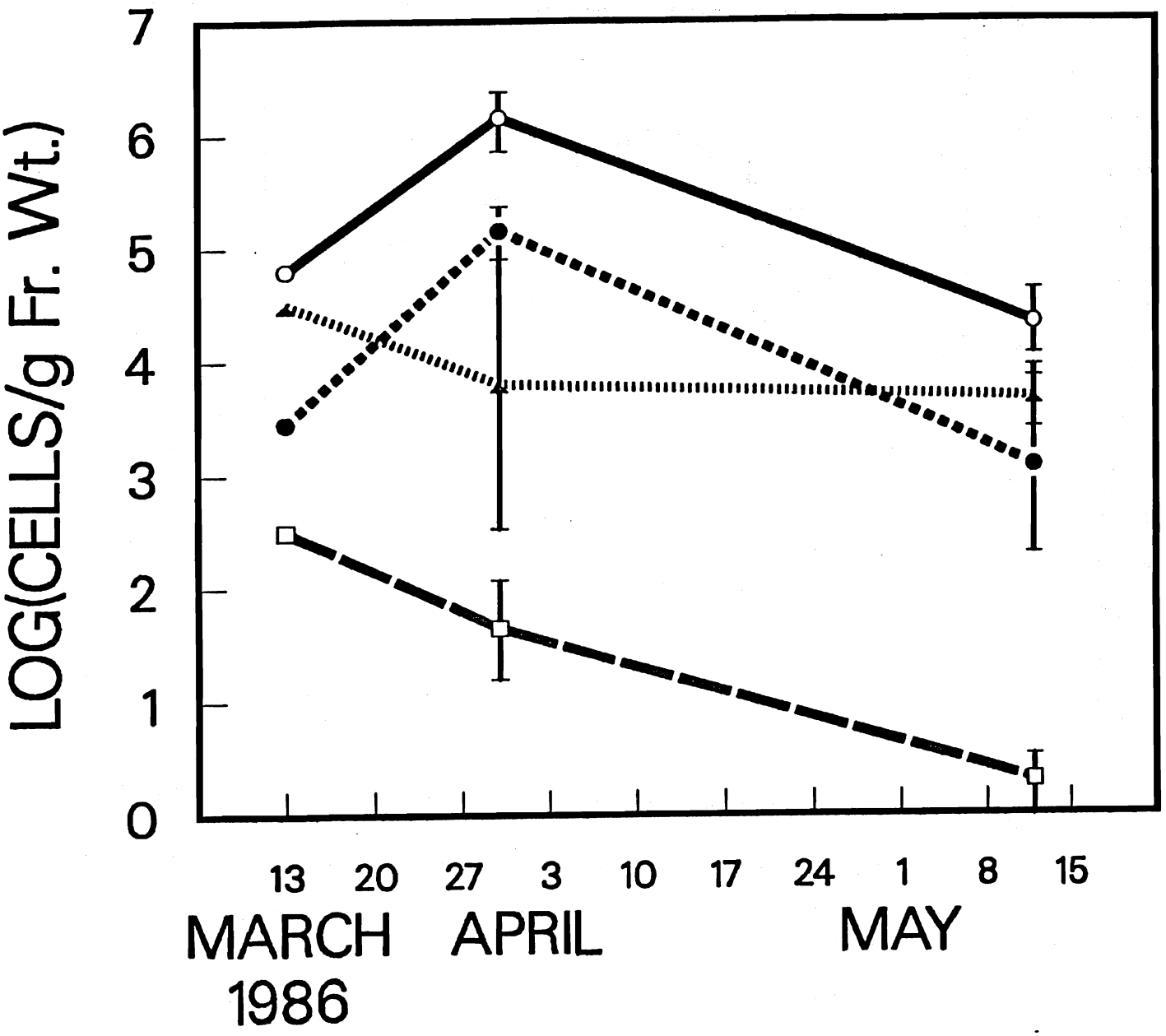

Figure 2. Total bacteria (circle), ice nucleation active bacteria (triangle) and ice nuclei active at -5 C (square) on Bartlett pear trees treated at 10% bloom with antagonistic Pseudomonas fluorescens strain A506 (filled circle) at approximately 10% bloom in a plot near Marysville, CA.

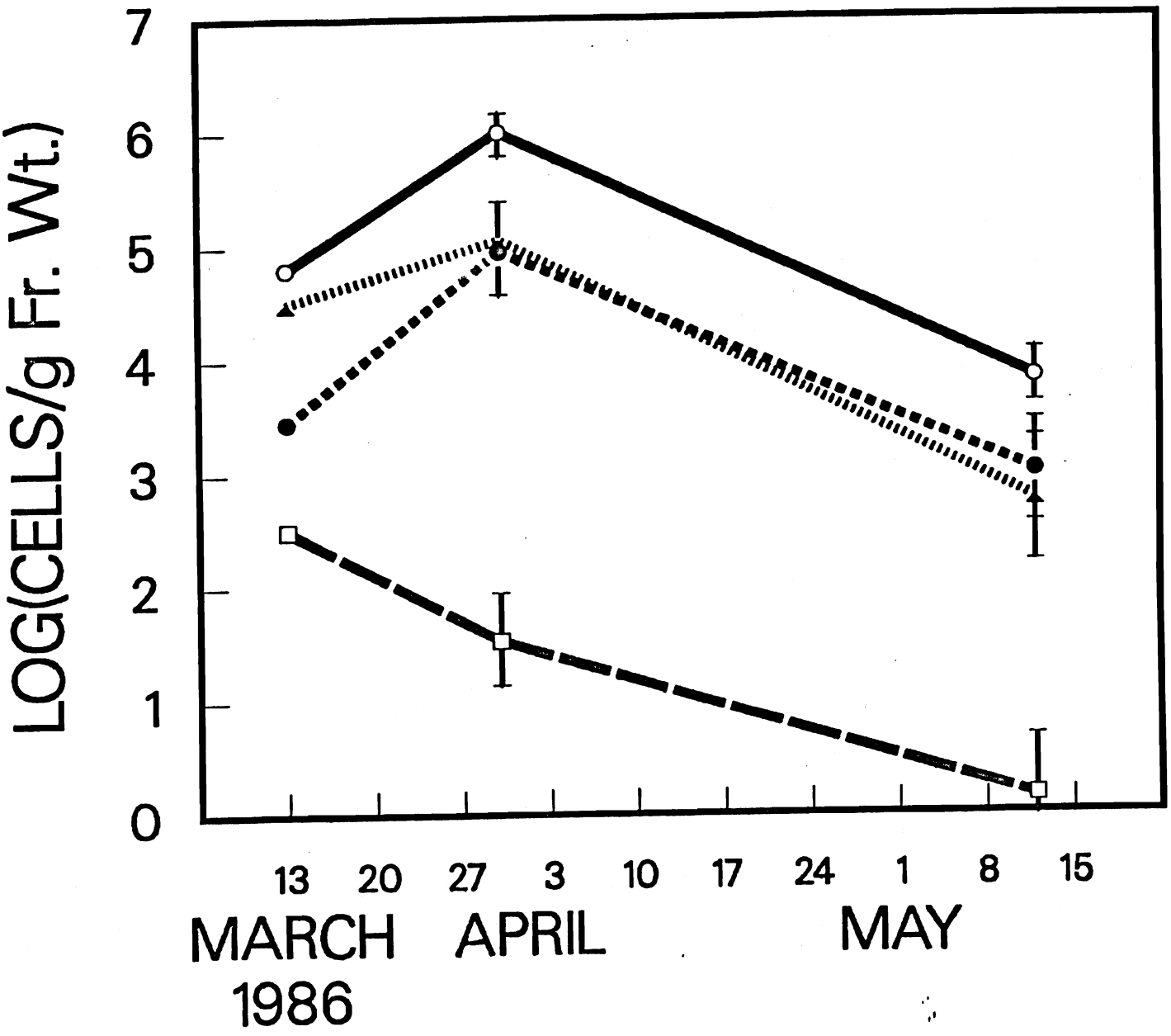

Figure 3. Total bacteria (circle), ice nucleation active bacteria (triangle) and ice nuclei active at -5 C (square) on Bartlett pear trees sprayed with a suspension of antagonistic bacteria A506 at approximately 10% bloom on March 12, and sprayed repeatedly (approximately bi-weekly) with Terramycin until May 15 in a plot near Marysville, CA.

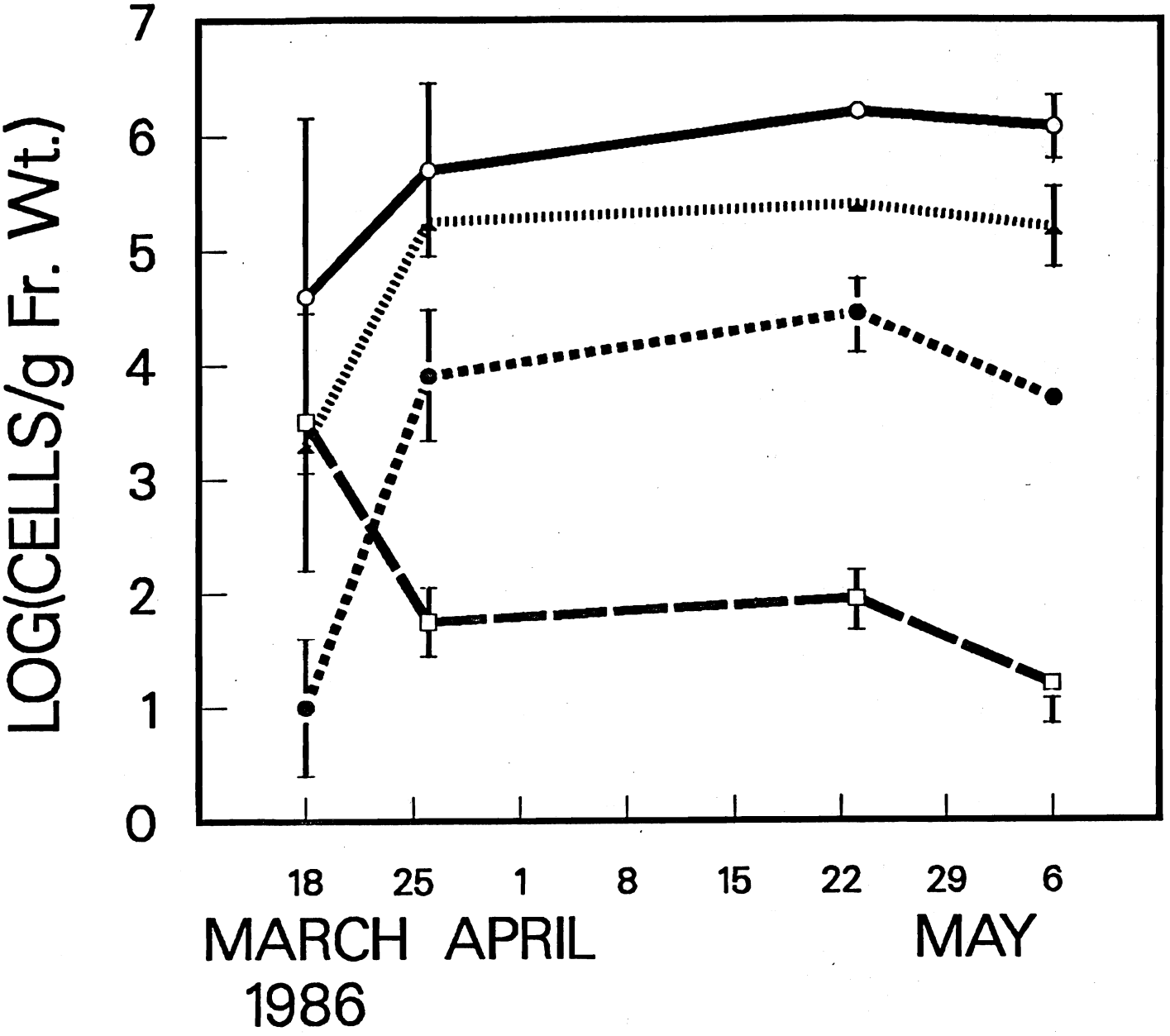

Figure 4. Total bacteria (circles), ice nucleation active bacteria (triangle) and ice nuclei active at -5 C (square), and rifampicin-resistant antagonistic bacteria on untreated pear trees grown near Healdsburg, CA.

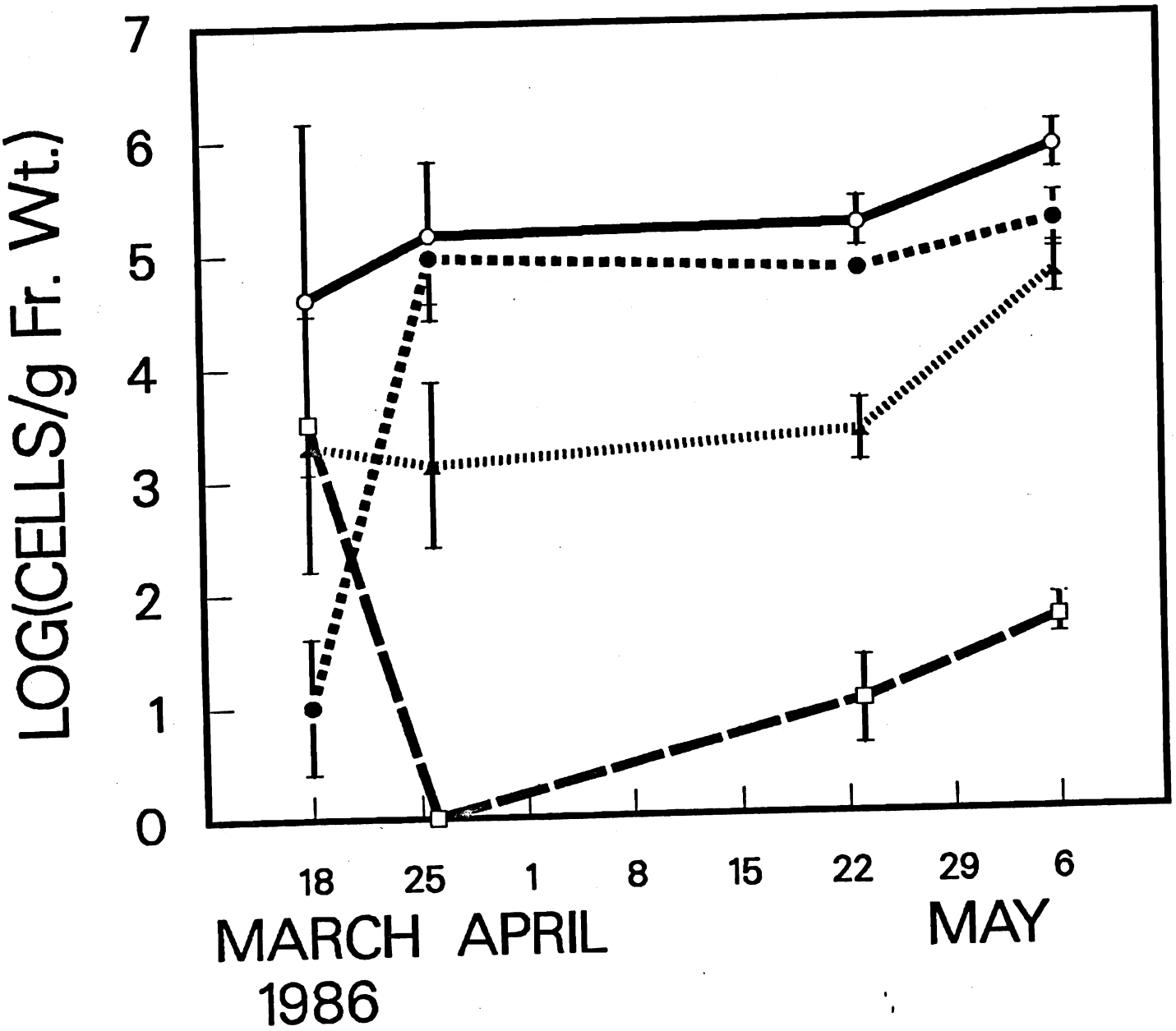

Figure 5. Total bacteria (circle), ice nucleation active bacteria (triangle) and ice nuclei active at -5 C (square) on Bartlett pear trees treated at approximately 10% bloom with a suspension of antagonistic bacteria A506 (filled circle) in a plot near Healdsburg, CA.

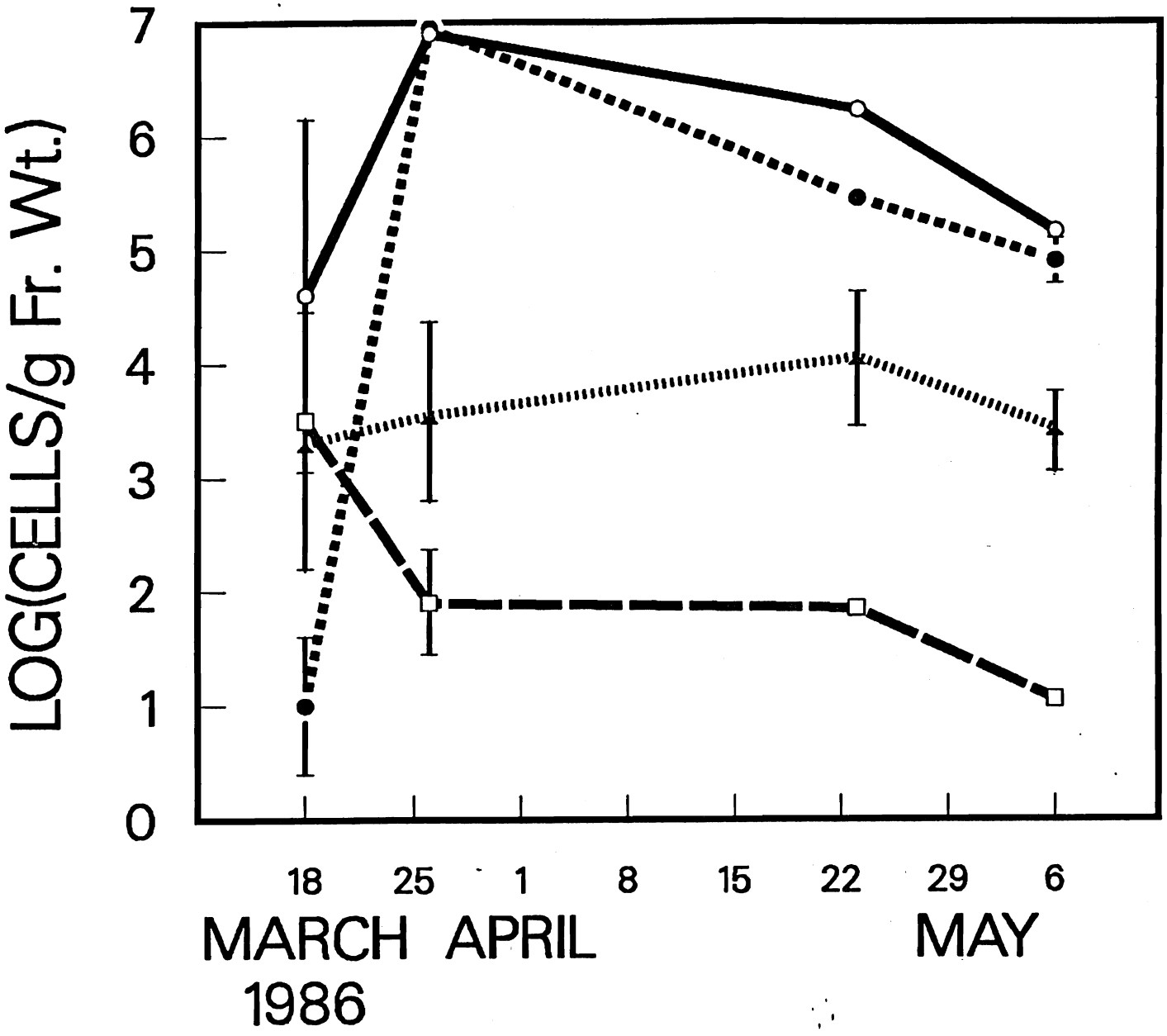

Figure 6. Total bacteria (circle), ice nucleation active bacteria (triangle) and ice nuclei active at -5 C (square) on Bartlett pear trees treated at approximately 10% bloom with bacterial strain 240R which produces copious quantities of 3-indole acetic acid in culture (filled circle) in a plot near Healdsburg, CA.

TABLE 1

BACTERIAL POPULATIONS ON PEAR TREATED WITH A BACTERICIDE AND/OR ANTAGONISTIC BACTERIA
STEAMBOAT ORCHARD 1986

Treatment	Bacteria Recovered (Log(Cells/g fr. wt.)) Total	INA	Antagonist
Control	6.10 a	5.17 a	4.89 c
A506+A526	6.42 a	5.06 a	6.15 a
A506+A526+Terra	5.97 b	1.85 b	5.70 b
Terramycin	5.75 b	0.91 b	5.70 b

TABLE 2

REDUCTION OF SUPERCOOLING POINT OF PEAR BY MIXTURES OF BACTERICIDES AND ANTAGONISTIC BACTERIA
STEAMBOAT ORCHARDS 1986

Treatment	Mean Supercooling Point (C) April 8	May2
Control	-2.84	-2.57
Terramycin	-4.21	-2.95
A506+A526	-2.86	-2.67
A506+A526+Terramycin	-5.44	-3.69

TABLE 3

REDUCTION OF THE SUPERCOOLING POINT OF PEAR
BY A BACTERICIDE AND/OR ANTAGONISTIC BACTERIA
MARYSVILLE 1986

Treatment	Mean Supercooling Point (C)
Control	-2.83
A506	-2.65
A506+Terramycin	-3.67
A526	-3.46
A526+Terramycin	-2.94
Terramycin	-3.29
T565+308R+380R	-3.11

TABLE 4

REDUCTION OF THE SUPERCOOLING POINT OF PEAR
WITH BACTERICIDES AND ANTAGONISTIC BACTERIA
UKIAH 1986

Treatment	Mean Supercooling Point (C)
Control	-2.23
A506+A526	-2.33
Chlorox+A50+A526	-2.13
Strep+Terra+A506+A526	-2.79
Strep+Terra	-2.65
P40	-2.77
A1105	-2.12

TABLE 5

REDUCTION OF THE SUPERCOOLING POINT OF PEAR BY ANTAGONISTIC BACTERIA
HEALDSBURG 1986

Treatment	Mean Supercooling Point (C) March 26	April 23
Control	-2.41	-2.50
240R	-2.61	-3.51
T565	-2.25	-2.70
T565+Tryptophan	-2.78	-2.49
308R	-2.55	-2.61
7SR7	-2.62	-2.77
299R	-2.86	-2.58
P56 Ice-	-3.03	-3.54
P767 Ice-	-2.61	-3.53
P1016 Ice-	-2.92	-3.51

TABLE 6

INCIDENCE OF FRUIT FROST INJURY TO PEAR FROM TREES
TREATED WITH BACTERICIDES OR ANTAGONISTIC BACTERIA

Treatment	Injury (Fraction of Fruit)
Control	0.76 a
Streptomycin + Terramycin	0.56 ab
TS65 + 380R + 308R	0.51 b
A506	0.41 b
A526	0.38 b

SEVERITY OF PEAR FRUIT RUSSETTING ON TREES TREATED WITH IAA PRODUCING BACTERIA AT FLOWERING

Treatment	IAA Production	Russet (% of surface)
T565+Tryptophan	+	18.7 a
240R	+	14.3 cd
30FR+Tryptophan	+	13.9 de
389R	+	13.6 de
308R	+	13.6 de
380R	+	12.9 ef
276R	+	12.9 ef
268R	+	12.9 ef
7SR7	+	12.2 fg
P767	-	11.9 fg
299R	+	11.8 gh
T565	+	10.7 i
Tryptophan	-	10.2 i
P56	-	10.2 i
P1016	-	9.9 i
Control	-	7.7 j

TABLE 8

SEVERITY OF PEAR FRUIT RUSSETTING ON TREES TREATED WITH IAA-PRODUCING AND NON-IAA PRODUCING BACTERIAL STRAINS

Treatment	IAA Production	Russet (% of Surface)
T565	+	7.30 a
380R	+	6.21 b
308R	+	6.18 b
A506	-	5.10 c
Control	-	4.97 c

SEVERITY OF FRUIT RUSSETTING ON PEAR TREES TREATED WITH ANTAGONISTIC BACTERIA AND IAA-PRODUCING BACTERIAL STRAINS MARYSVILLE 1986

Treatment	IAA Production	Russet (% of Surface)
TS65+308R+380R	+	6.04 a
Terramycin	-	4.20 b
A526 + Terramycin	-	4.18 b
A506 + Terramycin	-	3.67 bc
A526	-	3.39 cd
Control	-	3.20 cd
A506	-	2.81 d

TABLE 10

INCIDENCE OF PEAR FRUIT RUSSETTING FROM ORCHARDS TREATED WITH BACTERICIDES OR ANTAGONISTIC BACTERIA

Treatment	Russet (fraction of fruit)
Streptomycin+Terramycin	0.85 a
Control	0.78 a
A506	0.61 b
A526	0.57 b

Severity of Pear fruit russetting on trees treated with antagonistic bacteria and/or bactericides North Orchard 1986

Treatment	Russet (% of surface)
A1105	6.15 a
Control	6.11 a
Chlorox + A506 + A526	5.84 ab
A506 + A526	5.33 bc
P40	5.30 bc
Streptomycin + Terramycin	5.24 c
Streptomycin + Terramycin + A506 + A526	3.34 d

TABLE 12

Severity of Pear fruit russetting on trees treated with antagonistic bacteria and bactericides

Treatment	Russett (% of surface)
Chlorox + A506	12.44 a
Streptomycin + Terramycin + A526	12.35 a
Chlorox + A526	11.05 b
Control	10.92 b
Chlorox	10.54 bc
Streptomycin + Terramycin + A506	10.07 bcd
A506	9.91 bcd
Streptomycin + Terramycin	9.46 cd
A526	9.02 d

TABLE 13

PRODUCTION OF IAA BY BACTERIA ISOLATED FROM PEAR ORCHARDS WITH DIFFERENT SEVERITIES OF FRUIT RUSSET-1986

Orchard	Russet Severity	IAA Production
7	+	0.22
8	+	0.17
3	–	0.16
5	+	0.13
4	–	0.12
6	+	0.10
1	–	0.09
2	–	0.08

TABLE 14

PRODUCTION OF IAA BY BACTERIA ISOLATED FROM PEAR ORCHARDS OF DIFFERENT RUSSET SEVERITY

Orchard	Russet Severity	IAA Production
5	+	0.15
7	+	0.06
6	+	0.05
2	–	0.04
4	–	0.04
1	–	0.04
3	–	0.00
8	+	0.00

PRODUCTION OF IAA BY BACTERIA ISOLATED FROM PEAR ORCHARDS HAVING DIFFERENT RUSSET SEVERITIES

Russet Severity	IAA Production	
	Total per Tree	Total per Strain
High	124.8	0.06
Low	10.8	0.03