Project Number: 82-L4

82-L-4

TITLE: 1982 ANNUAL REPORT PROJECT: EPIDEMIOLOGY AND CONTROL OF FROST INJURY TO ALMOND INCITED BY LEAF SURFACE ICE NUCLEATION ACTIVE BACTERIA.

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PRINCIPAL INVESTIGATOR:

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OBJECTIVES DURING 1982:

1. To investigate the epidemiology of colonization of almond leaves, flowers, and nutlets with ice nucleation bacteria including the assessment of sources of inoculum of species and populations of ice nucleation active bacteria predominating on leaves.

2. Evaluate possible methods of application of antagonistic bacteria for use as biological control agents of frost injury.

3. Determine the most effective bactericides, antagonistic bacteria, and ice nucleation active bacterial inhibitors to control frost injury and to determine the enviornmental parameters which influence their effectiveness.

4. To determine the supercooling of almond tissue as a function of time and treatments that alter the populations or ice nucleation activity of ice nucleation active bacteria.

5. To quantitate reductions of frost damage to almond in relation to reductions of populations of ice nucelation active bacteria on leaves of almond trees following application of bactericides and antagonostic bacteria to almond trees.

6. To select antagonistic bacteria from healthy almond leaves and flowers and to evaluate their efficacy for use as biological control agents of frost injury to almond.

7. To evaluate chemicals which inactivate ice nucleation active bacteria as frost control agents.

INTERPRETIVE SUMMARY:

Although no natural field frost occurred in any of three field plot locations during the spring of 1982 significant progress was made in measuring the differences and frost sensitivity of almond trees treated with different chemical and biological agents to reduce the numbers or

the ice nucleation activity of ice nucleation active bacteria on almond trees. The bacterium, Pseudomonas syringae was found to be the most common ice nucleation active bacteria on almond in all growing areas. Erwinia herbicola and Pseudomonas florescens were only occasionally found on almond flowers, leaves, or nutlets and were of lower ice nucleation activity than was Pseudomonas syringae. Standard applications of non-copper containing fungicides or insecticides did not reduce the populations of these ice nucleation active bacteria in commercially managed orchards. The standard growing procedures are not conducive to the control of Pseudomonas syringae. Populations of ice nucleation active bacteria were lowest (about 100 bacteria/spur) on almond at bud break but increased rapidly at flowering and reached populations of over 10 million bacteria/spur after full bloom.

A new technique was developed to measure the supercooling point of almond tissue (lowest temperature below 32 F (0 C) that a plant part could be cooled before ice nucleation and therefore ice formation in the plant occurred). The supercooling point of untreated almond increased from about -3.5 C (25 F) at bud break to only about -2 C (28 F) after full-bloom. Ice nucleation active bacteria therefore were responsible for the decreased ability of almond to supercool and also to contributing to the increase frost sensitivity to almond during maturation in the spring. Treatment of almond with label rates of cupric hydroxide on a 7-10 day interval starting at bud break reduced the populations of ice nucleation active bacteria significantly and also reduced the supercooling point of almond about 2-4 F. Antagonistic bacteria applied to almond at 5 percent bloom colonized almond for over 50 days and reduced the numbers of ice nucleation active bacteria, the numbers of bacterial ice nuclei, and the supercooling point of treated almond trees during this period. Approximately 2-4 F frost protection was also seen following treatment of trees early in the season with non-ice nucleation active bacterial antagonists. Tank mixes of cupric hydroxide and bis-dithiocarbamates including Maneb significantly increased the effectiveness of copper materials in controlling bacterial populations and should be investigated further for frost and bacterial blast control.

EXPERIMENTAL PROCEDURE:

Many of the experimental procedures used in this study during 1982 were similar to those reported in the 1980 and 1981 annual reports or in the attached technical publication to be submitted in February of 1983. Bactericides were applied at label rates in the case of cupric hydroxide or at 150 parts/million and 75 parts/million active ingredient in mixes of streptomycin and Terramycin, respectively. All bactericide sprays were applied with added surfactant triton CS 7 with either a handgun sprayer at approximately 300 gal/acre or with a speed sprayer at approximately 200 gal/acre. Antagonistic bacteria were applied to almond trees at about 5 percent bloom at a concentration of about 10^8 bacterial cells/milliliter with a back-pack mist blower. Approximately 1-2 gal/tree of bacterial suspension was applied. Other chemicals including phosphoric acid and Hyamin[e] 2389 were applied within 24 hours of expected freezing conditions with a handgun sprayer to run-off to almond trees.

Bacterial populations on almond leaves, flowers, and nutlets were quantitated by removing bacteria from the surface of the leaves by immersion in sterile phosphate buffer and sonication for 8 minutes. Dilution plating of bacterial suspensions was then done on Kings Medium B or Kings Medium B containing appropriate antibiotics for identification of antibiotic marked antagonistic bacteria. The numbers of ice nuclei on almond tissue was measured from these same leaf washings by placing a collection of 40 or more droplets of leaf washing solution on the surface of aluminum sheets held at -5 C or -9 C or appropriate dilutions of these bacterial suspensions were placed on these sheets. From the fraction of droplets which remained unfrozen at these temperatures the number of ice nuclei active per ml and thus per gram of leaf tissue could be determined. The supercooling point of almond leaf, flower, and nutlet tissues was also determined during 1982. The distribution of freezing temperatures of almond tissue was determined by placing 40 or more plant parts in tubes of ice nucleus free water at -2 C or warmer. The temperature of the tubes was then slowly cooled at 0.5 C temperature intervals and at each interval the number of tubes which froze was determined. From the normal distribution of freezing temperatures observed the median supercooling point of a given population of leaves, flowers, or nutlets was determined by computer directed algorithms.

RESULTS:

Almond leaves, flowers, and nutlets were colonized with very high numbers of ice nucleation active bacteria during 1982. Over 100 thousand ice nucleation active bacteria per gram of leaf and flower tissue were observed on trees during periods of maximum frost hazard on untreated trees (Figure 1). Populations of ice nucleation active bacteria on untreated trees were at their lowest point at the pink bud and popcorn stage but increased rapidly to over 10^5 bacteria/gram fresh weight after full bloom. Total populations of bacteria remained fairly constant and were generally higher than about 10^6 cell/gram fresh weight. The number of ice nuclei on untreated almond trees parallel the increase in the numbers of ice nucleation active bacteria on almond tissue (Fig. 1). Approximately 1 cell in 20 ice nucleation active bacteria contributed in ice nucleus active at -5 C or warmer while on almond trees (Fig. 1).

The populations of ice nucleation active bacteria were reduced approximately 100 fold from 100,000 bacteria/g to about 1,000/g by treatment with cupric hydroxide (Kocide 101). The number of ice nuclei active at either -9 C or at -5 C were also reduced approximately 100 fold by treatment with cupric hydroxide (Fig. 2). However, when Maneb at label rates was added to a tank mix of Kocide 101, the reduction of ice nucleation active bacteria and bacterial ice nuclei ranged from 1,000 to 10,000 fold compared to untreated control plants (Fig. 3). This addition of maneb to Kocide 101 dramatically increased the reductions in populations of ice nucleation active bacteria on these plants. The total numbers of bacteria on plants treated with Kocide plus Maneb was also significantly less than on plants treated with Kocide 101 only. Thus it appears that the synergistic effect of applications of maneb with cupric hydroxide is not specific to that of *Pseudomonas syringae* but in general increases the bactericidal effect of cupric hydroxide. A mixture of streptomycin and Terramycin was nearly as effective as a mixture of Maneb and cupric hydroxide in controlling populations of ice nucleation active bacteria (Fig. 4). Populations of ice nucleation active bacteria were reduced approximately 1,000 fold at all times compared to untreated control of trees. The number of bacterial ice nuceli was also reduced approximately 1,000 fold compared to untreated trees.

The non-ice nucleation bacterial mixture of A506 and A526 readily colonized almond tissue for 40 days or more following a single application at 5 percent bloom (Fig. 5). The populations of ice nucleation active bacteria and bacterial ice nuclei were reduced approximately 100 fold on trees treated with these non ice nucleation active bacteria. However the most effective bacterial antagonists were the combination spray of Cit 13-12 and Cit 30-11, which were ice nucleation deficient mutants of Pseudomonas syringae and Erwinia herbicola, respectively (Fig. 6). These bacteria accounted for nearly all of the bacteria found on almond trees for 50 days following a single application and the populations of ice nucleation active bacteria and bacterial ice nuclei found on trees were reduced at least 100 fold on trees treated with these non ice nucleation active bacteria.

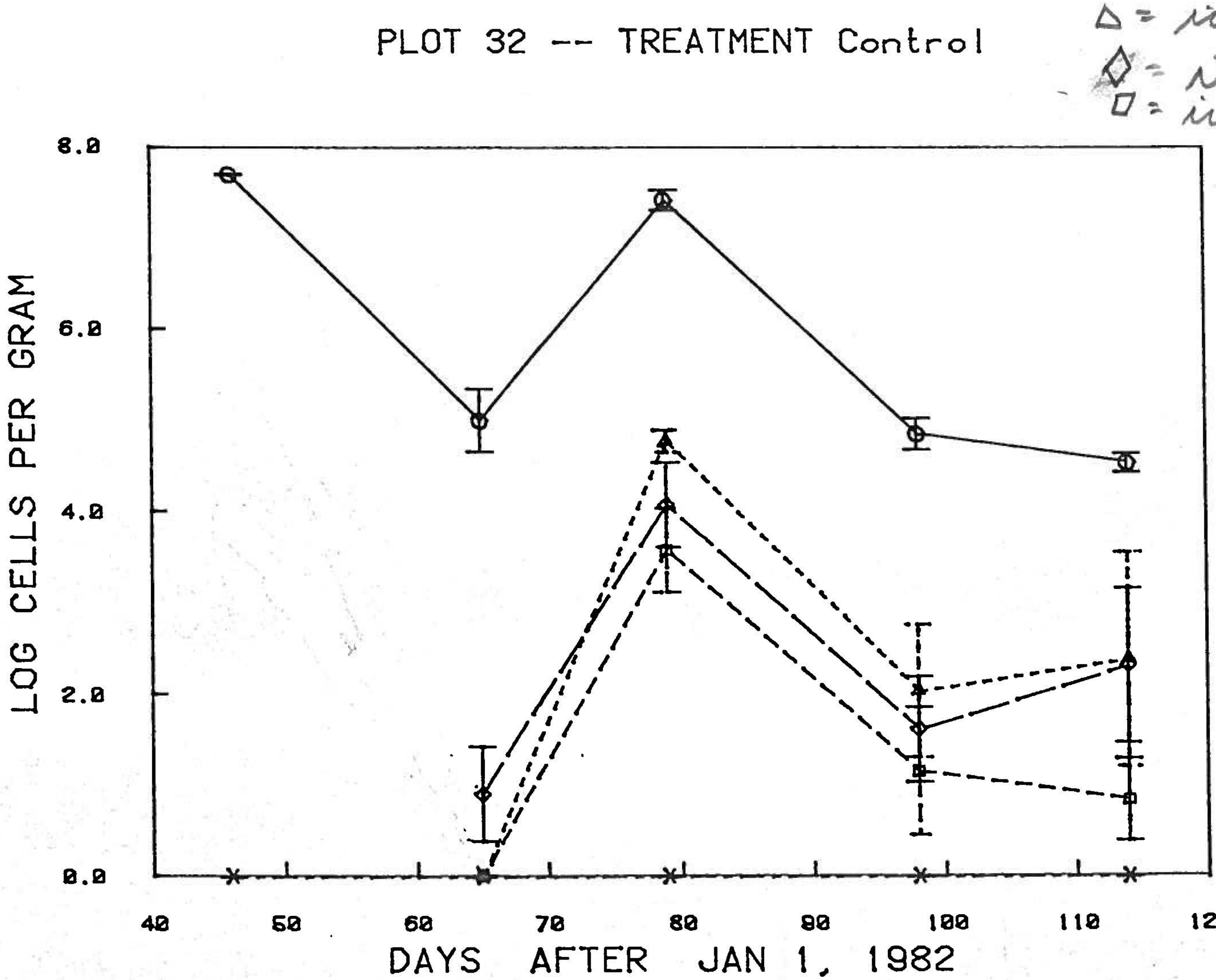
Trees treated with a dilute solution of phosphoric acid also exhibited a significantly lower population of both ice nucleation active bacteria but primarily populations bacterial ice nuclei active at -5 C. The numbers of bacterial ice nuclei were reduced approximately 100 to 1000 fold on trees treated with a dilute solution of phosporic acid twice during the growing season (Fig. 7).

The supercooling points of almond spurs closely followed the reductions in populations of bacterial populations or of bacterial ice nuclei on almond tissue. Untreated control trees of almond had the highest supercooling point of only -3.65 C (Table 1). Trees treated with three different non-ice nucleation active bacteria had supercooling points approximately 1 C colder than that of untreated trees. In addition, plants treated with bactericides including Kocide 101 and streptomycin had supercooling points approximately 2 C colder than that of untreated almond spurs. Trees treated with a mixture of Kocide plus Maneb which was the most effective at reducing populations of ice nucleation active bacteria and bacterial ice nuclei on almond had the lowest supercooling point in of the treated trees examined and was approximately 2.2 C colder than untreated almond trees.

DISCUSSION

Work done during 1982 indicates the feasibility of significantly reducing the supercooling point of almond and thus the ability of almond to escape damaging ice formation under field conditions by reducing the populations of ice nucleation active bacteria by both biological and chemical means. Several non-ice nucleation active bacteria were particularly adept at colonizing almond tissue following a single spray early in the spring and reduced the supercooling point of almond significantly. Because of the requirement for their single application these bacteria appear promising in reducing the cause of frost control. Several bactericidal sprays however also look quite promising in reducing populations of ice nucleation active bacteria. Whereas copper containing fungicides alone are fairly effective at reducing populations of ice nucleation active bacteria, a mixture of Kocide 101 and Maneb was significantly better at reducing these populations. These results suggest that some level of copper tolerance among ice nucleation active bacteria may exist among field populations of ice nucleation active bacteria. These bacteria probably do not represent true resistance to copper but have adapted to tolerate the very low levels of free copper ion available after application of cupric hydroxide. Other workers have shown that the addition of chelating agents such as bis-dithiocarbamate fungicides can increase the availability of copper to copper tolerant strains of bacteria. This phenomenon of copper tolerance needs to be further investigated in almond as is the use of chelating agents such as Maneb to improve the control of bacterial populations for frost control and control of bacterial blast and canker of almond. Since Maneb also represents a fairly effective fungicide which can be substituted for several of the fungicides now used on almond this would seem to represent no increased cost to growers. Bacterial nucleation inhibitors such as dilute solutions of phosphoric acid also appear promising as frost control agents and have significantly reduced the frost hazard of almond trees as measured by their supercooling point. Therefore although in 1982 no natural field frost was observed to test the field behavior of plants treated with these agents to reduce populations of ice nucleation active bacteria, it is expected that the supercooling points as measured by the technique described here should fairly well represent their field behavior. Thus it appears that a significant reduction in frost hazard has been achieved by several different procedures to control ice nucleation active bacteria on almond.

Fig 1



O = total Bacteria
Δ = ice nucleation active Portion
◇ = ice nuclei - 9C
□ = ice nuclei - 5C

FIG 2

PLOT 32 -- TREATMENT Kocide

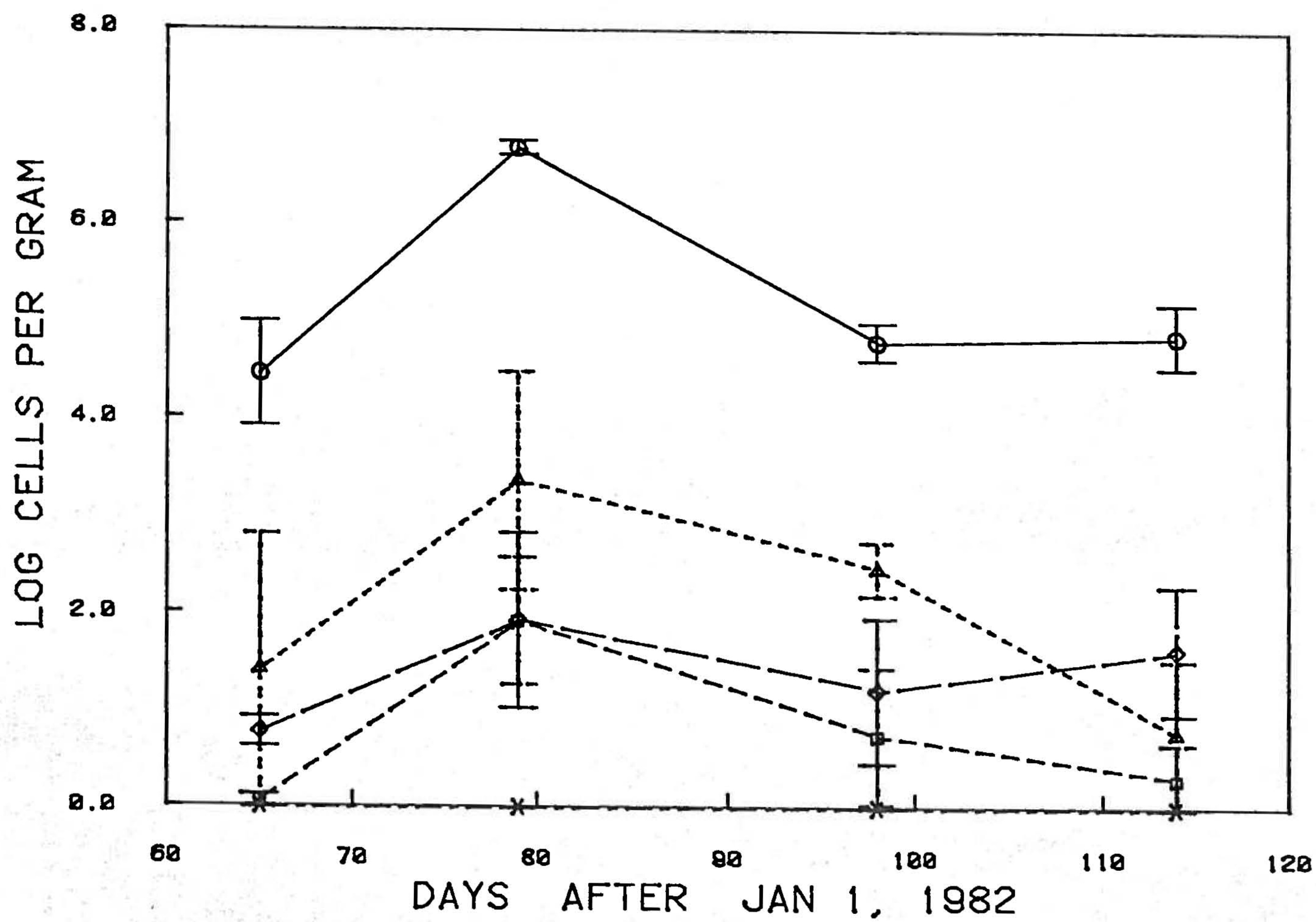


Fig 3

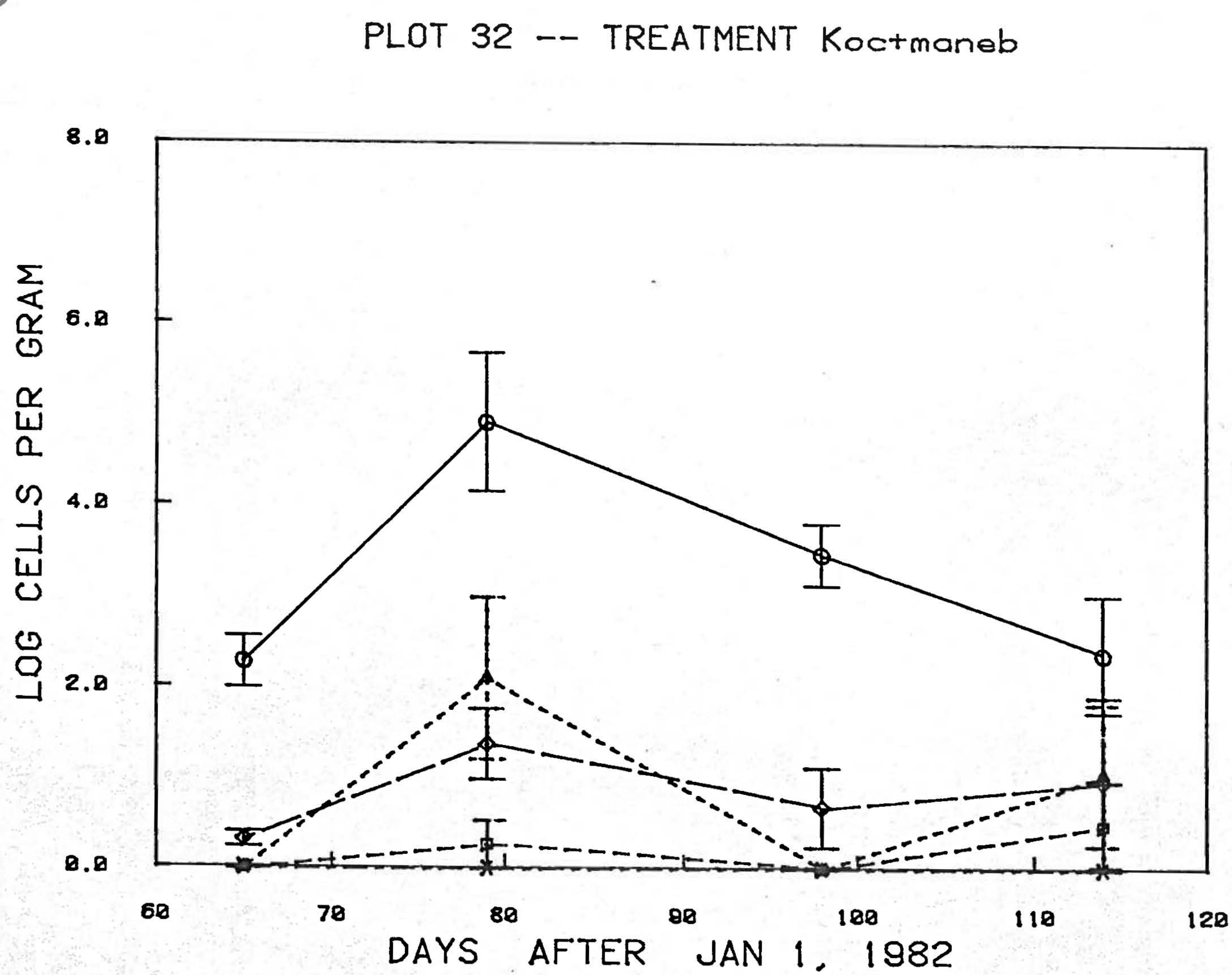
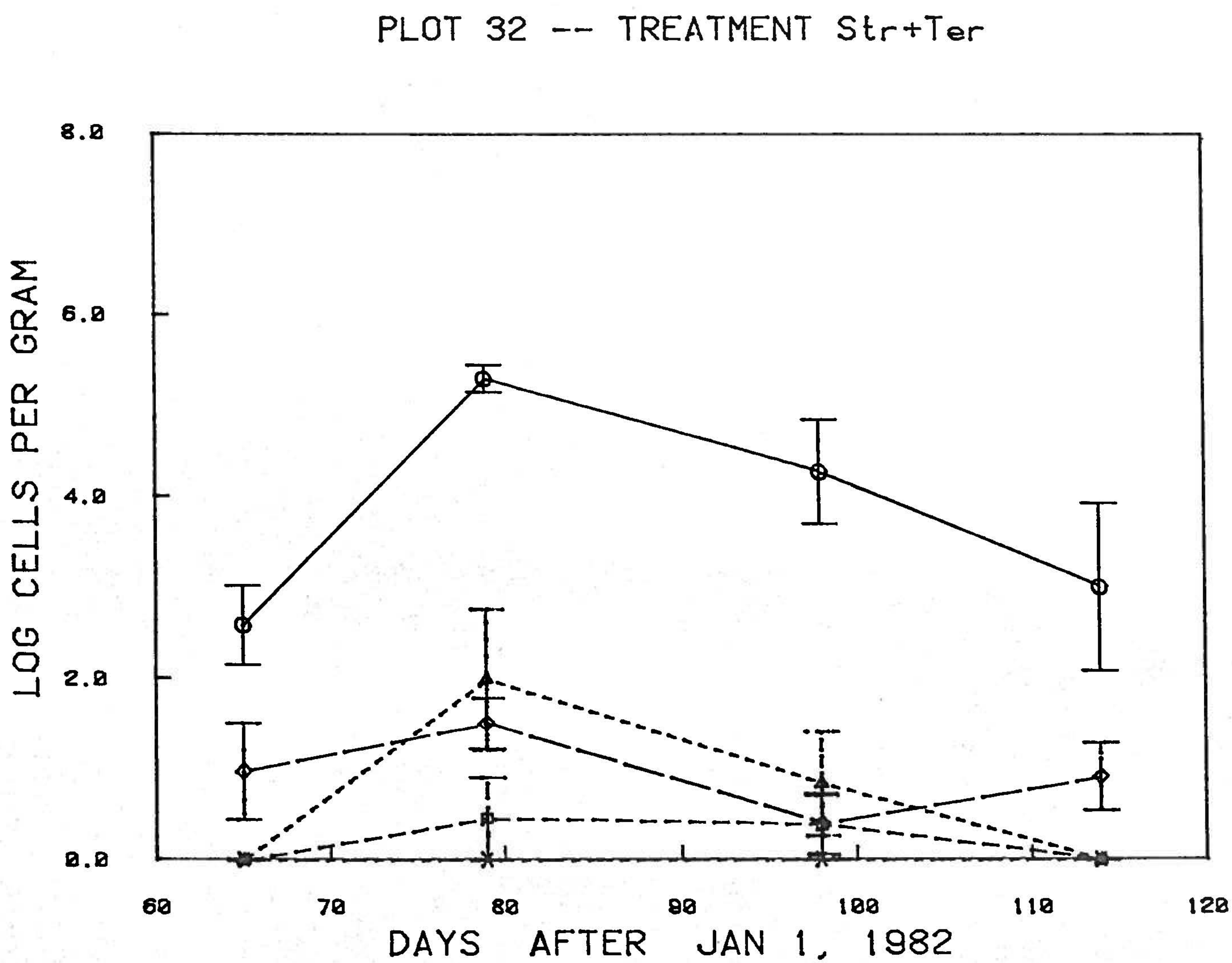

PLOT 32 -- TREATMENT Koctmaneb
LOG CELLS PER GRAM
8.0
6.0
4.0
2.0
0.0
60
70
80
90
100
110
120
DAYS AFTER JAN 1, 1982

Fig 4



PLOT 32 -- TREATMENT Str+Ter
LOG CELLS PER GRAM
0.0
2.0
4.0
6.0
8.0
60
70
80
90
100
110
120
DAYS AFTER JAN 1, 1982

Fig 5

PLOT 32 -- TREATMENT A506+A526

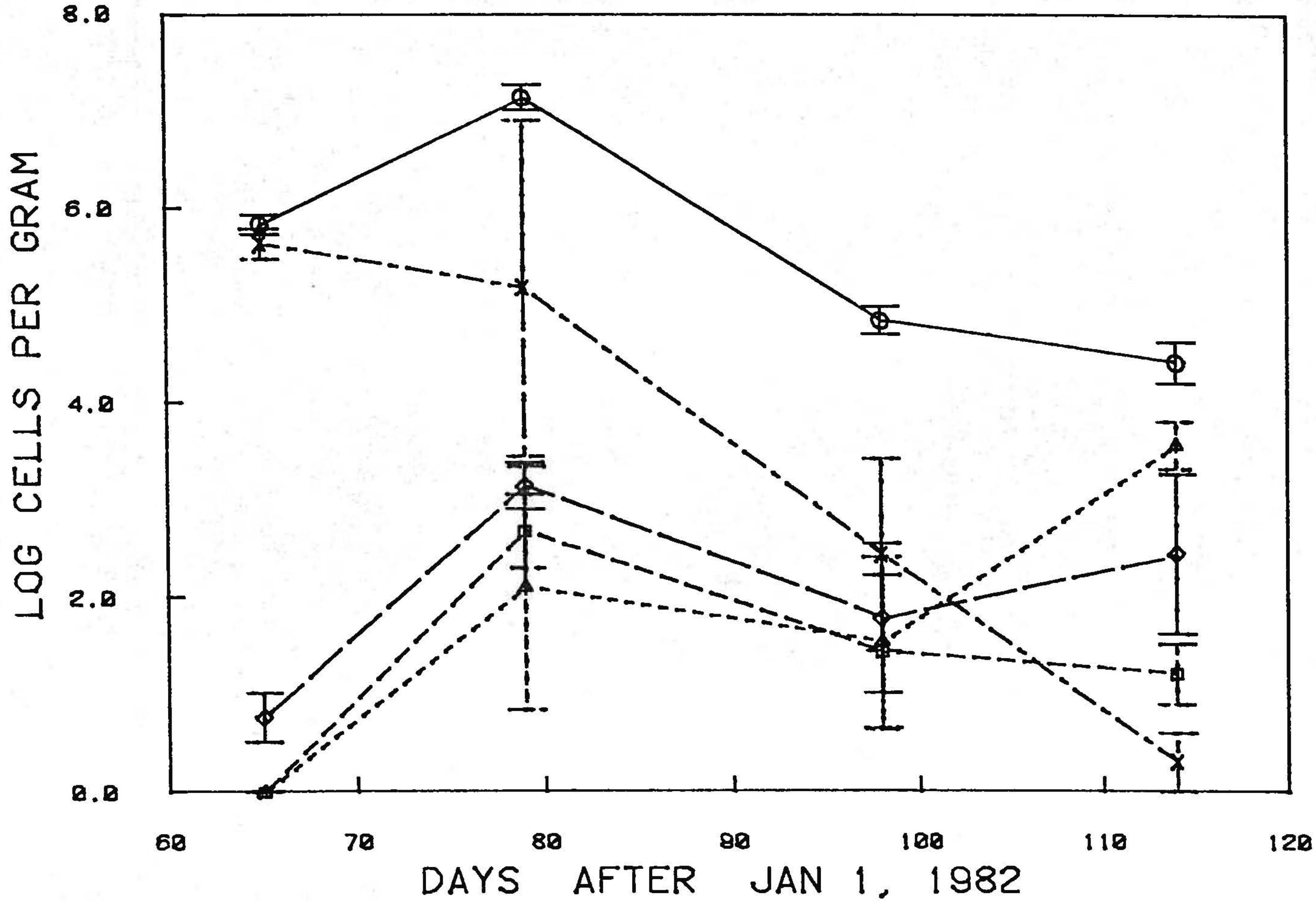


Fig 6

PLOT 32 -- TREATMENT C1312+C3011

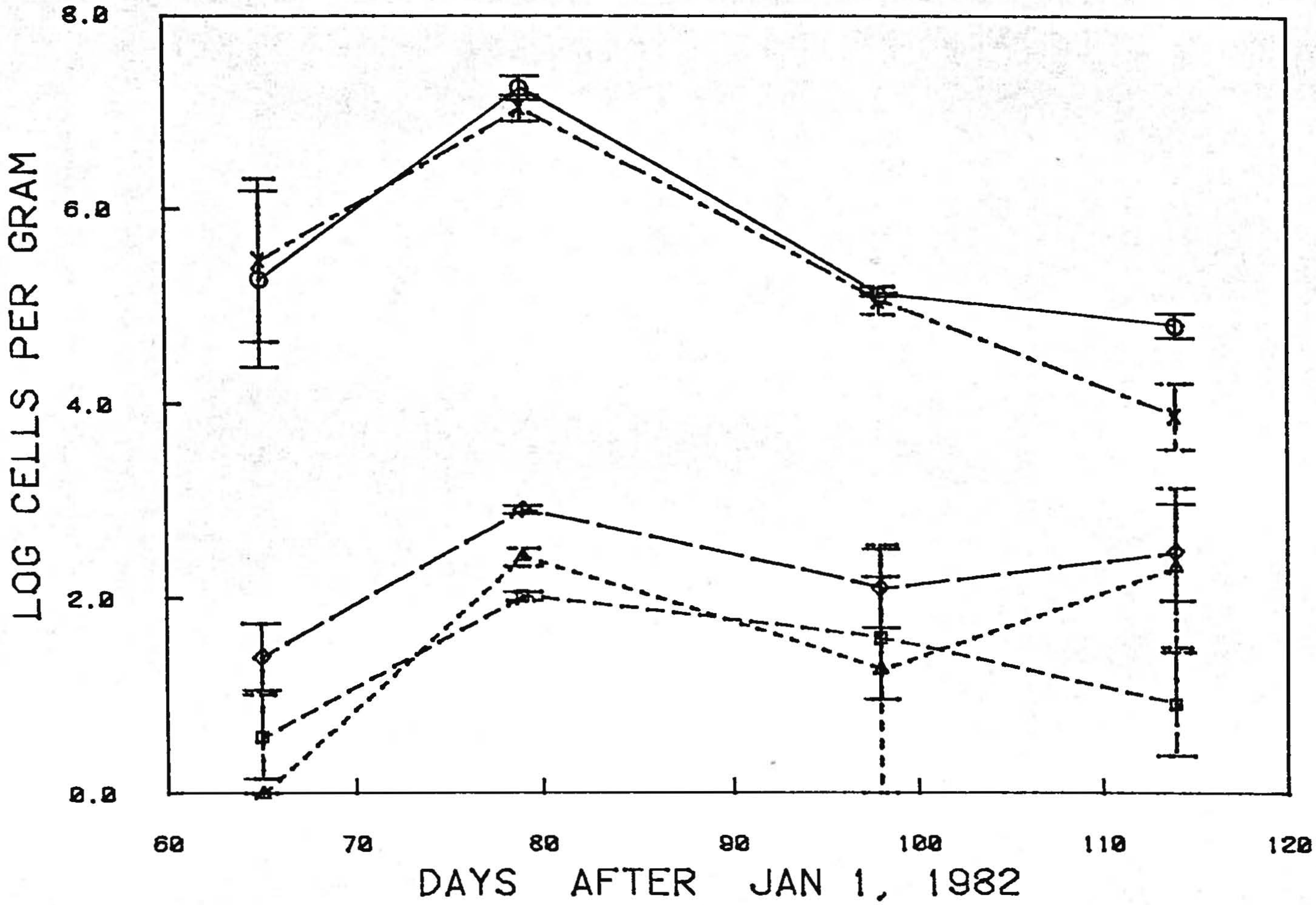
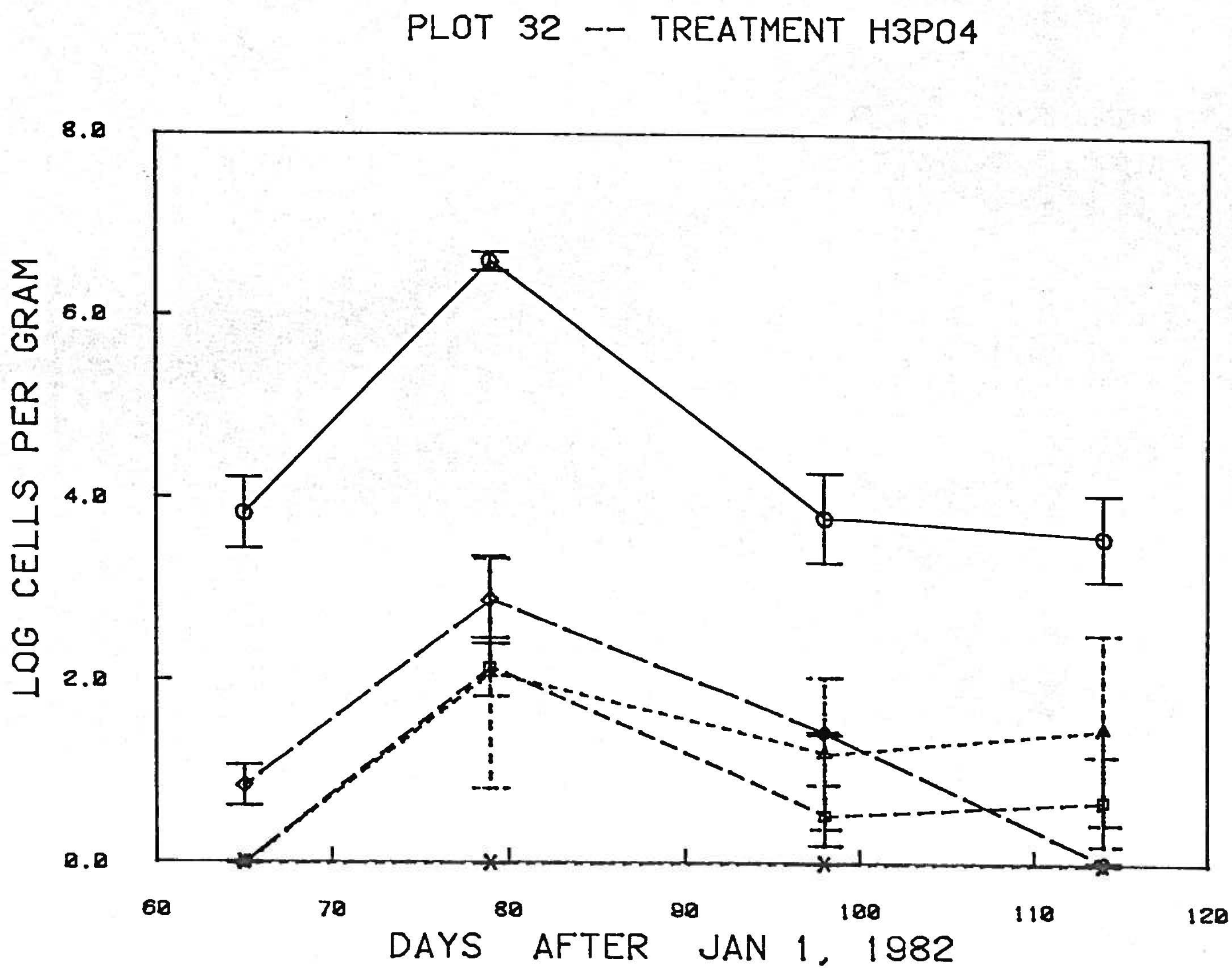


Fig 7


PLOT 32 -- TREATMENT H3PO4
8.0
6.0
4.0
2.0
0.0
LOG CELLS PER GRAM
60
70
80
90
100
110
120
DAYS AFTER JAN 1, 1982

Effects of bactericides and antagonistic bacteria on the supercooling point of almond spurs

Treatment	Supercooling Point	
	50%	10%
Control	-3.65	-1.83
426-10	-3.95	-1.92
C13-12 + C30-11	-4.63	-2.61
A506 + A526	-4.97	2.07
H_3PO_4	-4.98	2.91
Kocide 101	-5.54	-3.73
Kocide + Hyamine 2339	-5.61	-3.95
Streptomycin + Terramycin	-5.70	-4.72
Kocide + Maneb	-5.74	-4.12