84-N6

1984 ANNUAL REPORT

PROJECT:

Epidemiology and Control of Frost Injury to Almond Caused by Surface Ice Nucleation Active Bacteria

PRINCIPAL INVESTIGATOR:

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

OTHER PERSONNEL INVOLVED:

J. Papp, D. Okamoto, and G. Andersen, Staff Research Associates, Department of Plant Pathology, UC Berkeley.
Wesley Asai, Cooperative Extension, Stanislaus County, Modesto, California.
Mark Freeman, Cooperative Extension, Fresno County, Fresno, California.
Joseph Connell, Cooperative Extension, Butte County, Oroville, California.
Clarence Downing, Tenaco West, Chowchilla, California.
Richard Snyder, Cooperative Extension, University of California, Davis, California.

OBJECTIVES DURING 1984:

1) To investigate the epidemiology of colonization of almond leaves, flowers and nuts with ice nucleation active bacteria, including the assessment of sources of inoculum, of species involved in ice nucleation, and seasonal populations of ice nucleation active bacteria predominating on almond and surrounding crops.

2) Determine the most effective bactericides, antagonistic bacteria, and ice nucleation active bacteria to control frost injury to almond and to determine the environmental parameters which influence their effectiveness.

3) Evaluate rates and frequency of application of existing copper containing bactericides for control of frost injury under field conditions.

4) Evaluate possible methods of application of antagonistic bacteria for use in biological control of frost injury to almond.

5) To determine the supercooling point of almond tissue as a function of time and treatments that alter the populations of ice nucleation active bacteria or their activity as ice nuclei.

6) To evaluate chemicals which inactivate ice nucleation active bacteria as frost control agents under field conditions.

7) To quantitate reductions of frost damage to almond under field conditions in relation to reductions of populations of ice nucleation active bacteria on leaves and nuts of almond trees following applications of bactericides, antagonistic bacteria, and nucleation inhibitors to almond trees.

8) To determine the environmental effects of bacterial nucleation inhibitors to control of bacterial ice nuclei for control of frost injury under field conditions.

9) To determine the distribution of freezing temperatures of treated and untreated almond flowers and nuts to predict the effects of frost control in terms of degrees of frost protection.

10) To determine the numbers and activity of ice nucleation active bacteria on almond tissue and to test the effects of nucleation inhibiting chemicals on reducing the supercooling point of treated tissues.

11) To determine the tolerance of a collection of ice nucleation active bacteria from various almond growing areas to copper sulfate and chelated forms of copper and to determine the effectiveness of additional copper chelaters to enhance the bactericidal effects of copper fungicides.

INTERPRETIVE SUMMARY:

Extensive field trials were established at 5 different locations within California during the Spring of 1984. A large field trial which included 10 treatments was established in Modesto, California. Field trials including 18 different treatments were established at the West Side Field Station of the University of California. More extensive numbers of trees were treated with bactericides and antagonistic bacteria on property owned by Tenaco West near Snelling, California. Plots on cooperating grower orchard trees are also established in Fresno County. Temperatures in the Spring of 1984 approached 32 F (0 C) in all locations, but in our plots no significant frost injury resulted. Populations of ice nucleation active bacteria (entirely the species Pseudomonas syringae) in all field trial locations were high during the Spring of 1984 (greater than 10^4 ice nucleation active bacteria per gram fresh weight of tissue) but lower than in previous years. Populations of ice nucleation active bacteria exceeded 100,000 cells per gram fresh weight on almond tissue in all locations. Populations were approximately 100 times lower than during 1982 and 1983 respectively, probably because of the very dry weather encountered during the Spring of 1984. A mixture of Kocide 101 and Bis-dithiocarbamate fungicides conferred significantly better protection against bacterial populations and, therefore, frost sensitivity than Kocide alone in some but not all tirals during 1984. Maneb alone conferred no sigificant reductions of bacterial populations on our trials. The supercooling point, the lowest temperature below 0 C than any plant part can be cooled before ice nucleation and, therefore, frost damage to the plant will occur, has been directly related to the frost sensitivity of these plants under field conditions. The supercooling point of untreated almond tissues varied within a range of about -2 C to -5 C in different locations and at

different times during the Spring of 1984. Variations in the supercooling point of untreated tissues appears to be correlated with previous day time temperatures. The increase in the supercooling point also in many cases paralleled the increase in populations of ice nucleation active bacteria, indicating that they are responsible for the initiation of freezing of almond tissue. Approximately 2-3 degrees Farenheit frost protection (1.5 C) has been observed on plants treated with bactericides or antagonistic bacteria as measured by an analysis of the supercooling points of almonds tissue.

A large collection of ice nucleation active bacteria were made from all locations and have been tested for their sensitivity to copper ions. Data indicates that significant differences in resistance to copper ions exist among bacteria isolated from sprayed and non-copper sprayed almond trees. Highest levels of copper resistance appears to exist among isolates of ice nucleation active bacteria from orchard's with a long history of copper usage. Further work is in progress that will determine whether levels of resistance of copper among isolated bacteria are sufficient to explain the ineffectiveness of copper containing compounds in disease or frost control. Enhanced bactericidal activity of copper containing compounds when tank mixed with bis-dithiocarbamates fungicides such as Maneb may indicate that this is the case.

Small numbers of ice nuclei, apparently associated with non-viable bacteria on almond tissue exist in dormant tissues. The populations of viable ice nucleation active bacteria on dormant buds was very low and could not account for the numbers of ice nuclei observed. Ice nuclei associated with dormant buds could be almost entirely erradicated with dormant applications of nucleation inhibitors including copper sulfate, sodium hypochloride, and guanidine chloride. Those dormant applications of nucleation inhibitors appear important to maximize early season frost control. Nucleation inhibitors applied during the dormant season may also reduce early season populations of viable ice nucleation activity active bacteria and reduce subsequent population increases on vegetative tissue at bud break.

EXPERIMENTAL PROCEDURES:

Most of the experimental procedures used in this study during 1984 were similar to those reported in 1982 to 1983. An article in the Journal of the American Society for Horticultural Science 109:48-53 also details these procedures. Copper containing bactericides were applied at label rates (1-1/2 lb. per 100 gal), or at 100 ppm active ingredient of a mixture of streptomycin and Terramycin respectively. All bactericides were applied with Triton CS-7 or Triton B1956 as a surfactant at label rates. Bactericides are applied with a handgun sprayer at approximately 300 gallons per acre or with a speed sprayer at approximately 100 gallons per acre. Antagonistic bacteria were applied to almond trees at about 2% bloom at a concentration of 10^8 cells/ml with a backpack mist blower. Approximately 1-1/2 gallon bacterial suspension per tree was applied. Nucleation inhibitors were applied with a handgun sprayer to run off. Bacterial populations on almond trees, flowers, and nuts quantified by removing bacteria from the surface of the leaves by emersing them in sterile phosphate buffer followed by sonication for 7 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 in and on almond tissues was measured from these same leaf washings by placing a collection of 40 or more droplets of leaf washings on the surface of aluminum foil held at -5 C. The number of ice nuclei active per ml and thus per gram of leaf tissue could be determined from the fraction of droplets which remained unfrozen at these temperatures. The supercooling point of almond leaves were also determined by placing 40 fruiting spurs individually in tubes of ice nucleus free water at -2 C. The temperature of the tubes was then slowly cooled at 0.5 C temperature intervals and at each interval the number of tubes which had frozen was determined. The median supercooling point of a given population of leaves was determined by computer directed algorithms from the normal distribution of freezing temperatures observed. The copper sensitivity of randomly isolated ice nucleation active bacteria from almond tissue was determined by growth on yeast extract glycerol medium containing various concentrations of copper sulfate in distilled water and assayed by a radial diffusion assay.

RESULTS:

Although almond leaves, flowers and nuts were colonized with high populations of ice nucleation active bacteria during 1984 these populations were lower than in previous years. Approximately 10,000 ice nucleation active bacteria per gram of almond tissue was observed on trees in several plot areas during periods of maximum frost hazard on untreated trees (Figure 1, Figure 2, Table 1). For unknown reasons, populations of ice nucleation active bacteria had increased very rapidly on even the youngest vegetative tissue during early 1984. Populations on untreated tissues subsequently increased tenfold after a period of 20% bloom and remained rather constant at that level till mid-April. The numbers of ice nuclei active at -5 or -9 C on untreated almond trees paralleled the populations of ice nucleation active bacteria on these trees (Figure 1). Approximately one cell in 250 were active in ice nucleation temperatures at -5 C or warmer while on almond trees at the Modesto plot (Figure 1). The somewhat lower fraction of cells (less than about 1 cell in 1000) was active as an ice nucleus at -5 C on the Fresno plot (Figure 2).

The populations of ice nucleation active bacteria were reduced approximately three fold by treatment with cupric hydroxide (Kocide 101) (Figure 3). The numbers of ice nuclei active at either -9 C or especially at -5 C were also reduced approximately 20 to 40 fold by treatment with Kocide 101 (Figure 1, Table 1). When Maneb at label rate (1 lb per 100 gallons) was added as a tank mix to Kocide 101, the reduction of ice nucleation active bacteria and bacterial ice nuclei was roughly the same in the Modesto trial (Figure 5) or greater than with Kocide 101 alone (Table 1) at the Fresno trial. The total populations of bacteria on plants sprayed with Kocide 101 or a mixture of Kocide 101 and Maneb were both reduced significantly compared to untreated plants.

Unlike previous years, a mixture of streptomycin and Terramycin was more effective than Kocide 101 or a mixture of Kocide 101 and Maneb in reducing both total numbers of bacteria on almond tissue as well as ice nucleation active bacteria and bacterial ice nuclei active at -5 and -9 C

(Figure 4). Numbers of ice nuclei and populations of ice nucleation active bacteria were reduced over 100 fold at all dates on plants treated with a mixture of streptomycin and Terramycin.

Several non-ice nucleation active bacteria applied to almond trees at 1 to 5% bloom (only once at this time) colonized almond tissue well and reduced subsequent colonization of this tissue by ice nucleation active bacteria (Figure 6, Figure 8, Figure 9). All three bacterial treatments evaluated in the Modesto trial were nearly equally effective in reducing potential population increases of Pseudomonas syringae on almond tissue. Over 90% of the total bacteria found on almond trees treated with a mixture of non-ice nucleation active Pseudomonas fluorescens strains A506 and A526 were composed of these applied bacteria for the entire duration of the trials (3 months). Populations of ice nucleation active bacteria were generally reduced from 20 to 100 fold on trees treated with these two bacterial species (Figure 6). The populations of isolates 31R1-28 and C30-11 which are ice nucleation deficient mutants of Pseudomonas syringae and Erwinia herbicola respectively were somewhat more variable on treated plants than were other bacterial treatments (Figure 8). However, the populations of ice nucleation active bacteria and of bacterial ice nuclei on plants treated with these two bacterial species were reduced significantly compared to untreated plants (approximately 30 to 150 fold) (Figure 8). Almond trees treated with an ice nucleation deficient mutant of Pseudomonas syringae Cit 13-12 (Figure 9) were composed of approximately 10% of this bacterial species for two months following one application at budbreak. The populations of ice nucleation active bacteria were also reduced significantly (10 to 300 fold) on plants treated with this ice nucleation deficient mutant Pseudomonas syringae. The reductions of populations of Pseudomonas syringae on plants treated a single time with these antagonistic bacteria were reduced to a similar extent to that of most bactericide treatments evaluated and look very promising at this time.

The supercooling points of almond tissues generally were correlated with the reductions in populations of ice nucleation active bacteria on almond tissue. Untreated control trees generally had the highest supercooling point, approximately -2 to -3.5 C (Tables 2-5). The supercooling points of almond tissue varied from sampling to sampling however, and was generally inversely correlated with the mean air temperature for the week prior to sampling. The enhancement of supercooling with treatments of antagonistic bacteria generally ranged from less than 0.5 C to more than 1 C (Table 2, Table 3). Bactericides increased the supercooling point of almond tissue from 0.5 to over 1 C. In some (Table 3, Table 4, Table 5) but not all (Table 2) trials a mixture of Kocide 101 and Maneb reduced the supercooling point of almond tissue more than did application of Kocide 101 alone.

The supercooling point of almond spurs generally were increased from 0.5 to over 2 C when treated with nucleation inhibitors (Table 6, Table 7). Largest increases in enhancement of supercooling occurred when almond spurs were detached and submerged in nucleation inhibitors prior to laboratory assays. However, significant reductions in supercooling point of almond spurs was observed following field applications of nucleation inhibitors with handgun applications to dormant almond tissue. The populations of ice nucleation active bacteria on dormant almond twigs was generally less about

30 to 100 bacteria per gram fresh weight at all samplings during 1984. It can be seen however that untreated dormant almond twigs have a rather high supercooling point (-2.5 to -3 C) (Table 6, Table 7). These nuclei are unlikely of plant origin and more likely represent contaminants of either non viable bacterial cells, or bacterial cells which are difficult to culture or have an altered ice nucleation activity while residing within woody tissues of almond twigs. Greenhouse grown almond wood which was grown free of bacteria supercooled to -5 to -6 C (Data not shown). Thus the supercooling point of almond wood is not determined by the plant material itself but by other contaminants associated with the wood. It is also clear that bacterial nucleation inhibitors, which eliminate the ice nucleation activity of bacterial cells, also substantially increase the ability of almond to supercool and avoid damaging ice formation.

DISCUSSION:

Work done during 1984 indicates that the ability to significantly enhance the supercooling point of almond tissue and thus the ability of almond to escape damaging ice formation under mild field frost conditions. The enhancement of the supercooling ability of almond is achieved by reducing ice nucleation active bacterial populations by both biological and chemical means. Several non-ice nucleation active bacteria were particularly efficient at colonizing almond tissue following single applications early in the spring. Colonization of these trees with bacteria reduced potential epiphytic colonization of this tissue with subsequent ice nucleation active bacterial populations presumably coming from nearby crops. Limited surverys during 1984 indicated that the potential increases in populations of ice nucleation active bacteria differed from orchard to orchard and was most likely correlated with the sources of ice nucleation active bacteria on nearby crops or weeds as has been seen in other crops. It is also clear that reducing overwintering populations of ice nucleation active bacteria is important in any management program aimed at reducing potential population increases. A priori populations must be low at the start of the season to prevent subsequent increases. Thus dormant applications of bactericides still appear necessary to achieve frost protection and certainly to achieve early season frost protection to young flowers. It is also clear that overwintering dormant tissues of almond contain sources of ice nuclei that are not associated with viable bacteria. Either ice nucleation active bacteria have very high nucleation frequencies (a very high ratio of ice nuclei to bacterial cells) compared to vegetative tissues or likely, ice nuclei are associated with nonviable bacterial cells in these woody tissues. It is also apparent however that these sources of ice nuclei can be eliminated with dormant applications of nucleation inhibitors. It is also important to note that dormant applications of nucleation inhibitors also apparently reduce the overwintering populations of viable bacterial ice nucleating species thus having two beneficial effects in terms of frost protection early in the spring. Since certain almond orchards apparently have higher populations of ice nucleation active bacteria than others, these orchards must be considered to be of a higher frost hazard than orchards that have lower populations. Therefore more work is needed to determine the variability of epiphytic populations of ice nucleation active bacteria in different almond orchards and different geographical areas. Management practices such as orchard floor maintenance, nearby crops, etc.

may have a very large effect on the potential frost sensitivity of these orchards. For example, management of orchard floor vegetation which has generally been considered to effect the heat balance of the orchard, may have a similar deleterious effect on the frost sensitivity of almond by serving as reservoirs for ice nucleation active bacteria that would then colonize almond flowers.

Copper tolerance has been observed among strains of almond colonizing Pseudomonas syringae. Current studies are underway to determine whether these populations have sufficient copper tolerance to avoid being eliminated by bactericide sprays. It is also clear however that copper bactericide sensitivity of Pseudomonas syringae is not qualitative, i.e. a quantitative resistance to these bactericides is occurrring. Since the concentrations of copper ions on almond tissues will vary with climatic conditions, rates of deposition, etc. the incomplete control of bacteria with standard copper applications can be largely explained based on an interaction of copper tolerant bacterial populations and inadequate concentrations of copper on leaves to achieve control. It is also clear however that application of Maneb or other copper chelating agents with copper fungicides will achieve greatest increase in control of epiphytic populations of these bacterial species in orchards for which high levels of copper tolerance exist. Thus further study of the distribution of copper tolerance among these bacterial epiphytes is needed.

PUBLICATIONS:

See attached reprint from Journal of American Society of Horticultural Science.

Table1

Bacterial Populations and ice nucleus concentrations on Na plus almond treated with bactericides Fresno 1984

Treatment	Bacterial Populations (log cfu/g) Total	Yellow	Fluor.	INA	Ice Nuclei (log nuclei/g) -5C	-9C
Control	5.41	4.41	4.46	4.42	1.87	2.17
Kocide	5.39	3.56	1.11	1.01	0.82	2.52
Kocide + Maneb	4.26	4.01	0.88	1.16	0.83	2.35

Table 2

SUPERCOOLING POINT OF ALMOND SPURS

MODESTO, FEBRUARY 14, 1984

TREATMENT	SP-50 (C)
CONTROL	-2.78
A006 & A526	-2.87
KOCIDE	-3.11
KOCIDE & MONEB	-2.96
MONEB	-3.00
H_3PO_4	-3.31

Table 3

SUPERCOOLING POINT OF ALMOND SPURS TREATED WITH BACTERICIDES AND ANTAGONISTIC BACTERIA

MODESTO, APRIL 3, 1984

TREATMENT	SP-50 (C)
CONTROL	-2.25
A506 & A526	-2.85
KOCIDE & MONEB	-3.14
STREPTOMYCIN & TERRAMYCIN	-2.61
KOCIDE	-3.01
31R1-28 & CIT 30-11	-3.04
MONEB	-2.16
CIT 13-12	-3.14
H_3PO_4	-2.37
CIT 7 & 31R1	-3.15

Table 4

Supercooling point of Almond spurs treated with bactericides Fresno March 7, 1984

Treatment	SP-50 (C)
Control	-3.49
Kocide	-3.76
Kocide+Maneb	-4.56

Table 5

SUPERCOOLING POINT OF ALMOND SPURS

MODESTO, MARCH 7, 1984

TREATMENT	SP50 (C)
CONTROL	-1.91
STREPTOMYCIN & TERRAMYCIN	-2.13
KOCIDE	-2.14
KOCIDE & MONEB	-2.38

Table 6

Supercooling point of almond spurs treated for 30 minutes with nucleation inhibitors

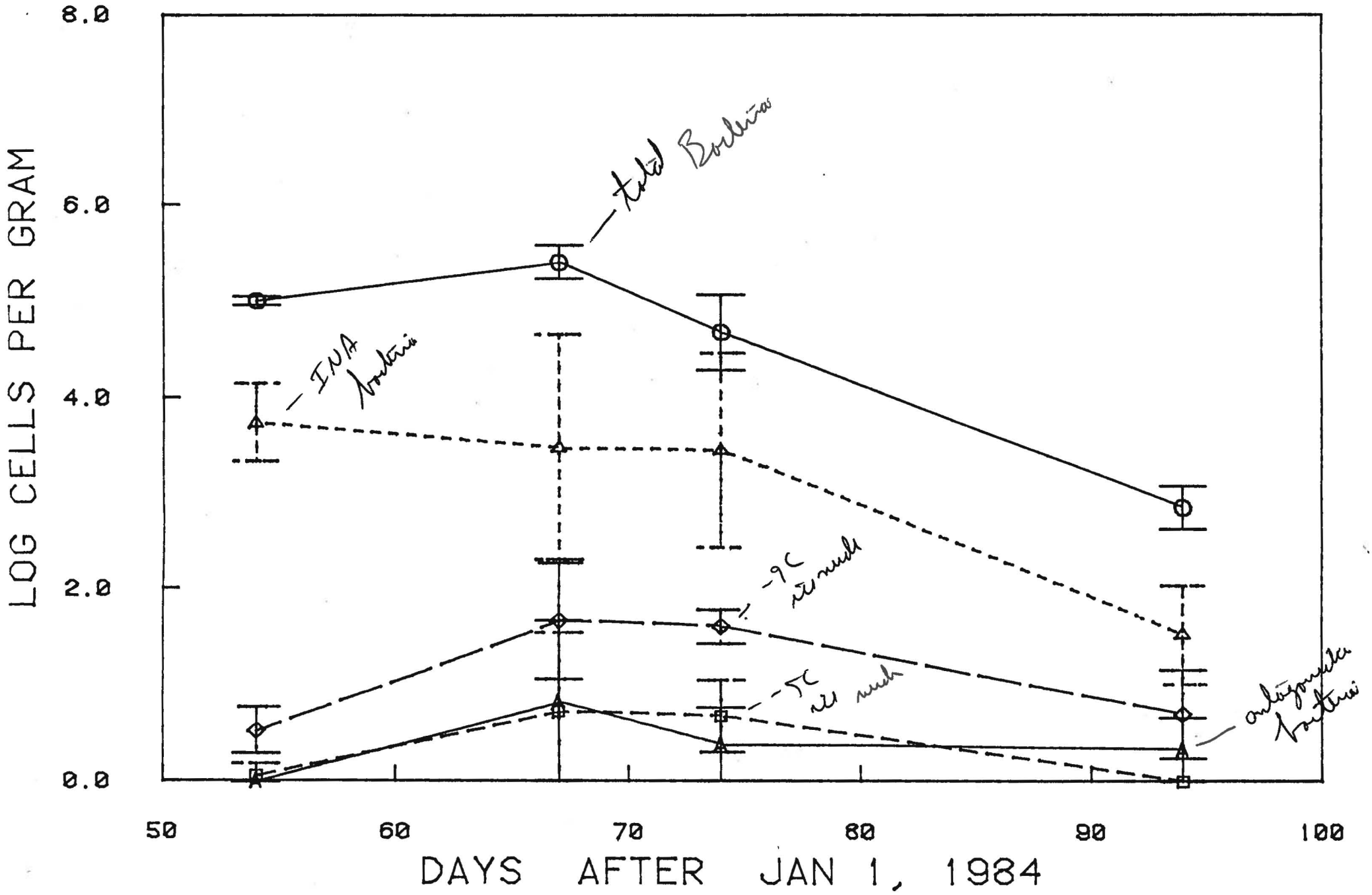
Treatment	SP-50 (C)	SP-10 (C)
Control	-2.42	-1.72
NaOCl	-2.75	-2.04
H_3PO_4	-3.17	-2.00
$CuSO_4$	-3.20	-1.90
Na_2CO_3	-3.05	-2.16
Methyl Benzethonium OH	-2.47	-2.00
Guanidine HCl	-4.58	-3.66

Table 7

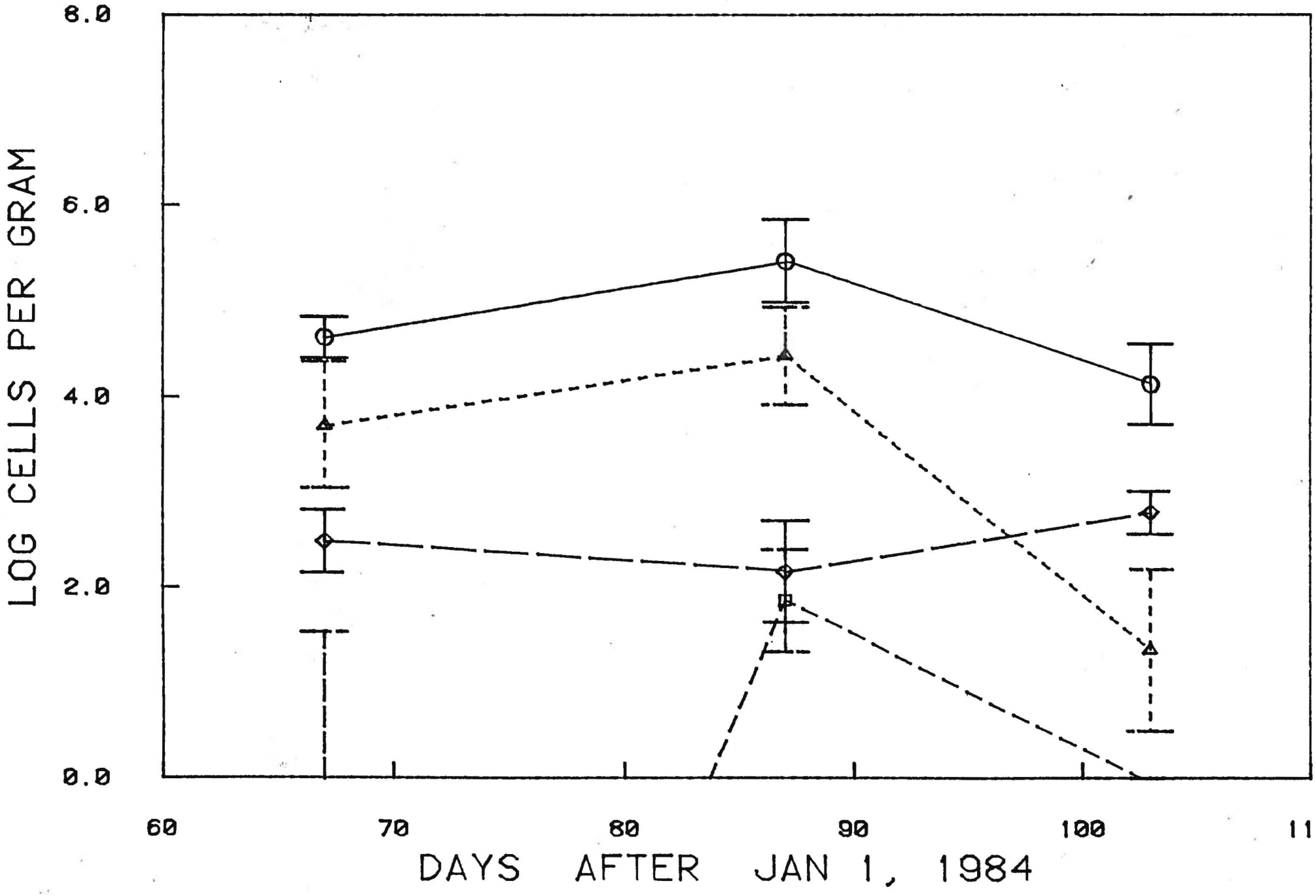
Supercooling point of 3 cm almond spurs treated with nucleation inhibitors for 30 min. LAB SUBMERSION
January 13, 1983

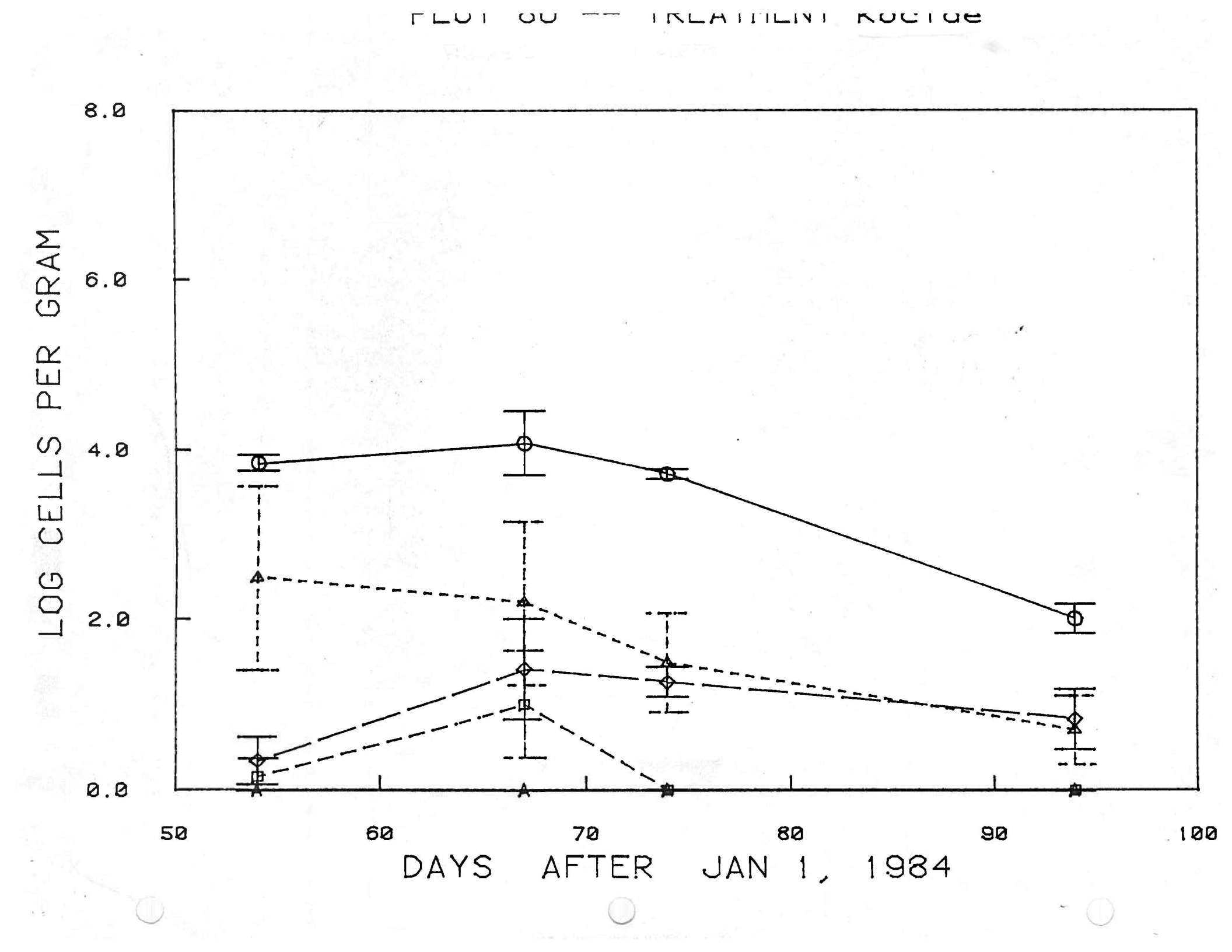
Treatment	SP-50 (C)	SP-10 (C)
Control	-2.56	-1.66
NaOCl (0.2%)	-2.46	-1.90
Guanidine HCl (2.0 M)	-5.60	-4.55
$CuSO_4$ (0.1 M)	-3.36	-2.37
Methyl Benzethonium OH (0.2%)	-3.02	-2.14
H_3PO_4 (2.0%)	-3.39	-2.50

PLOT 30 -- TREATMENT control

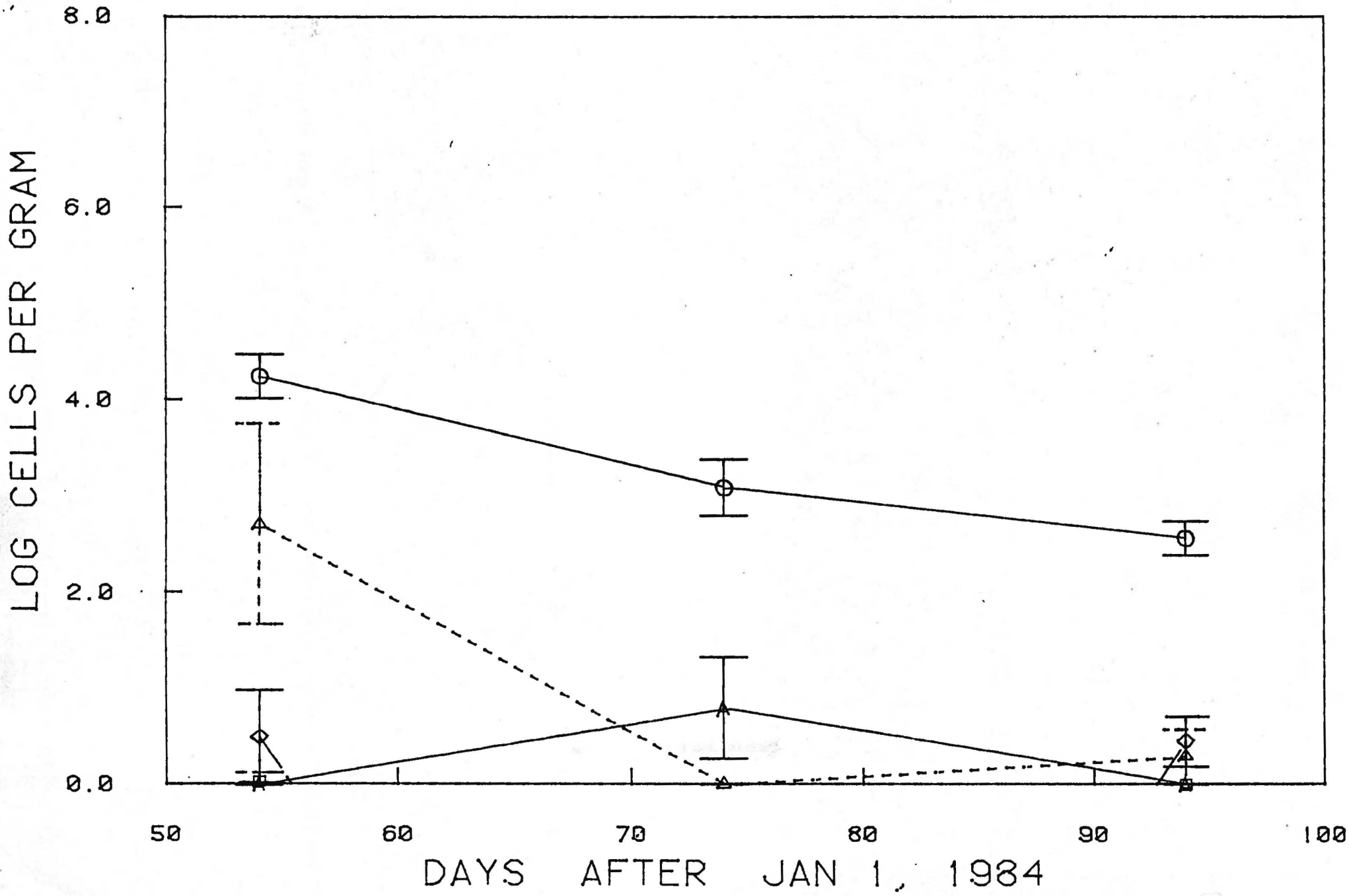


PLOT 85 -- TREATMENT control

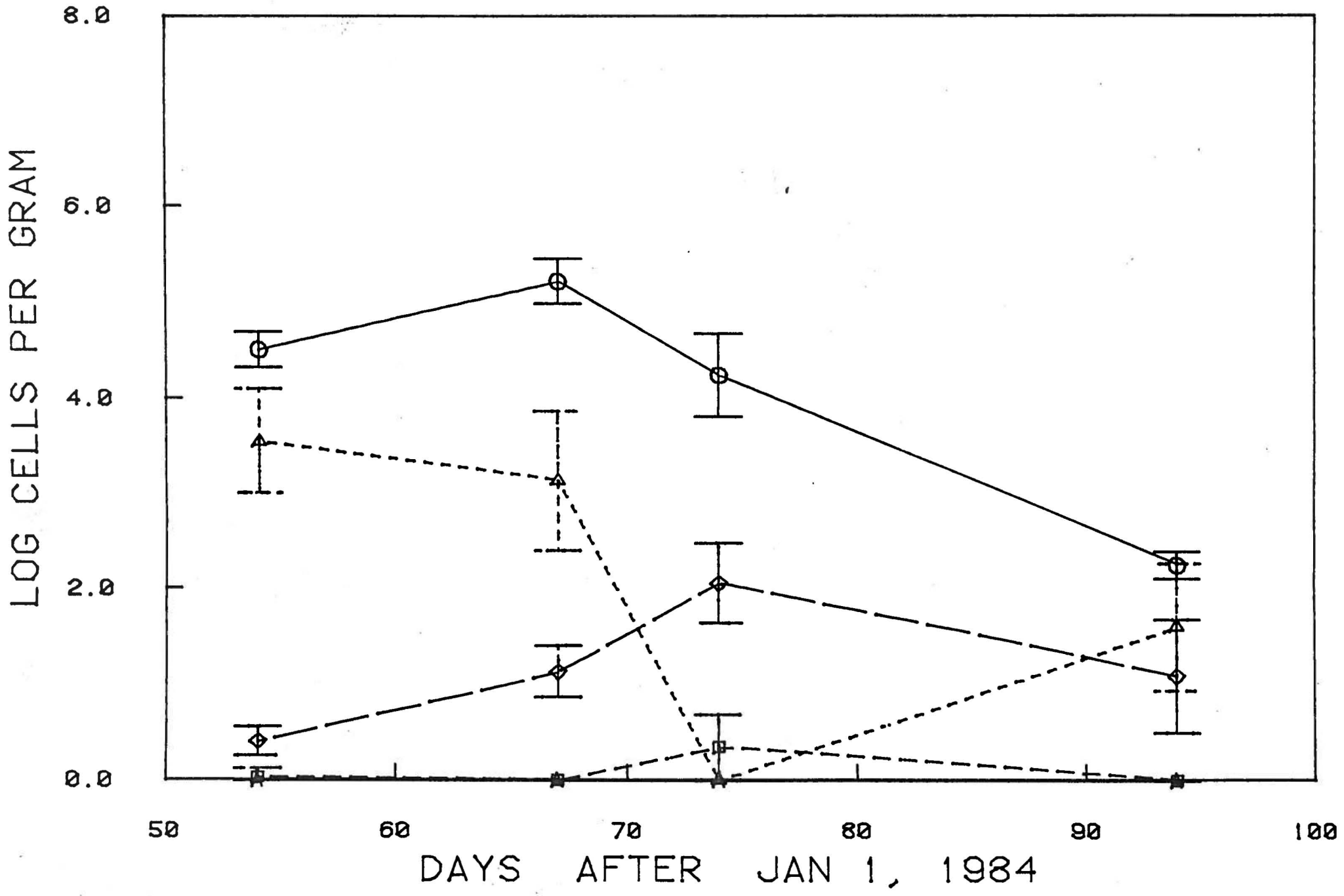



PLOT 60 -- TREATMENT Roctde
8.0
6.0
4.0
2.0
0.0
LOG CELLS PER GRAM
50
60
70
80
90
100
DAYS AFTER JAN 1, 1984

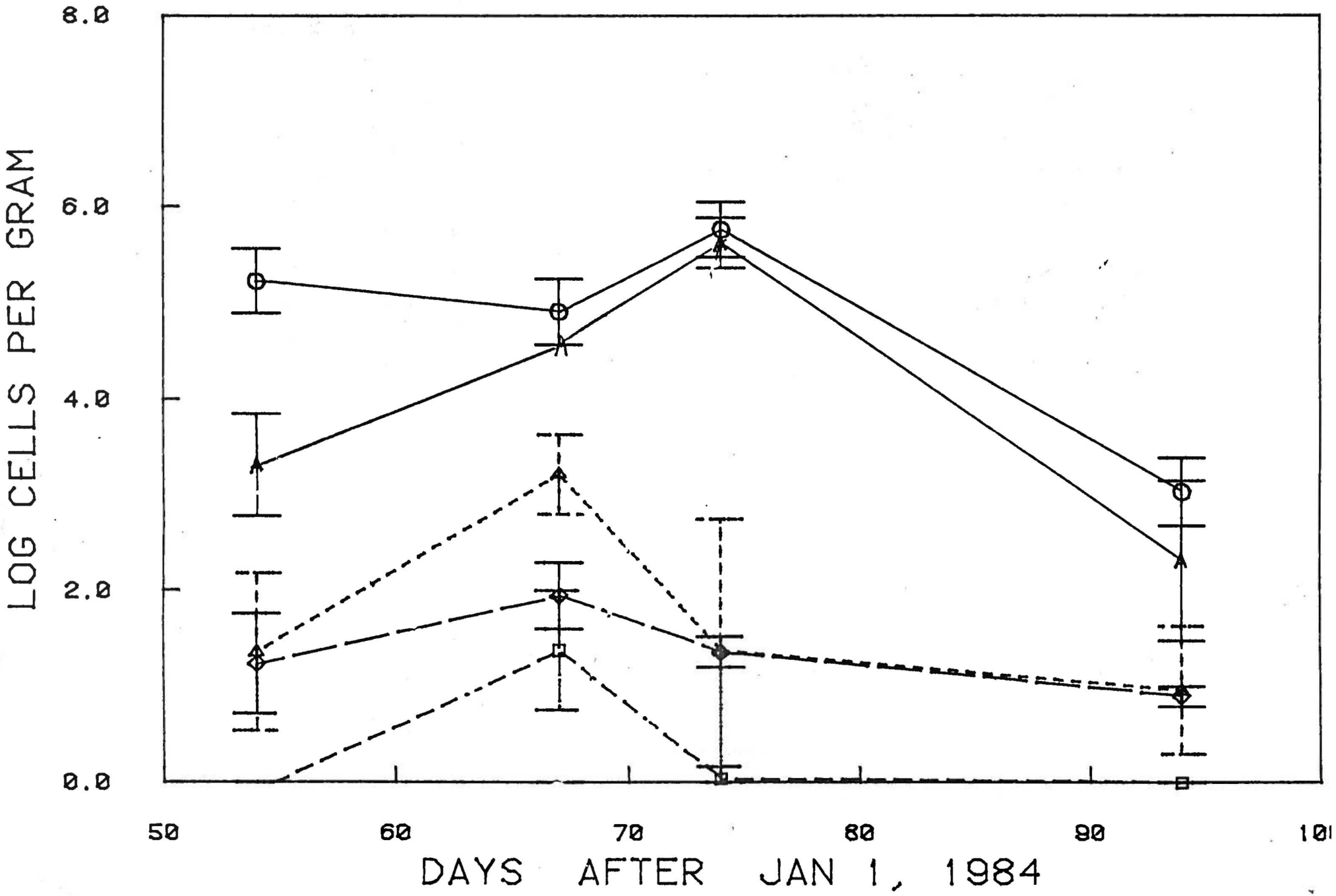
PLOT 66 -- TREATMENT streptterra

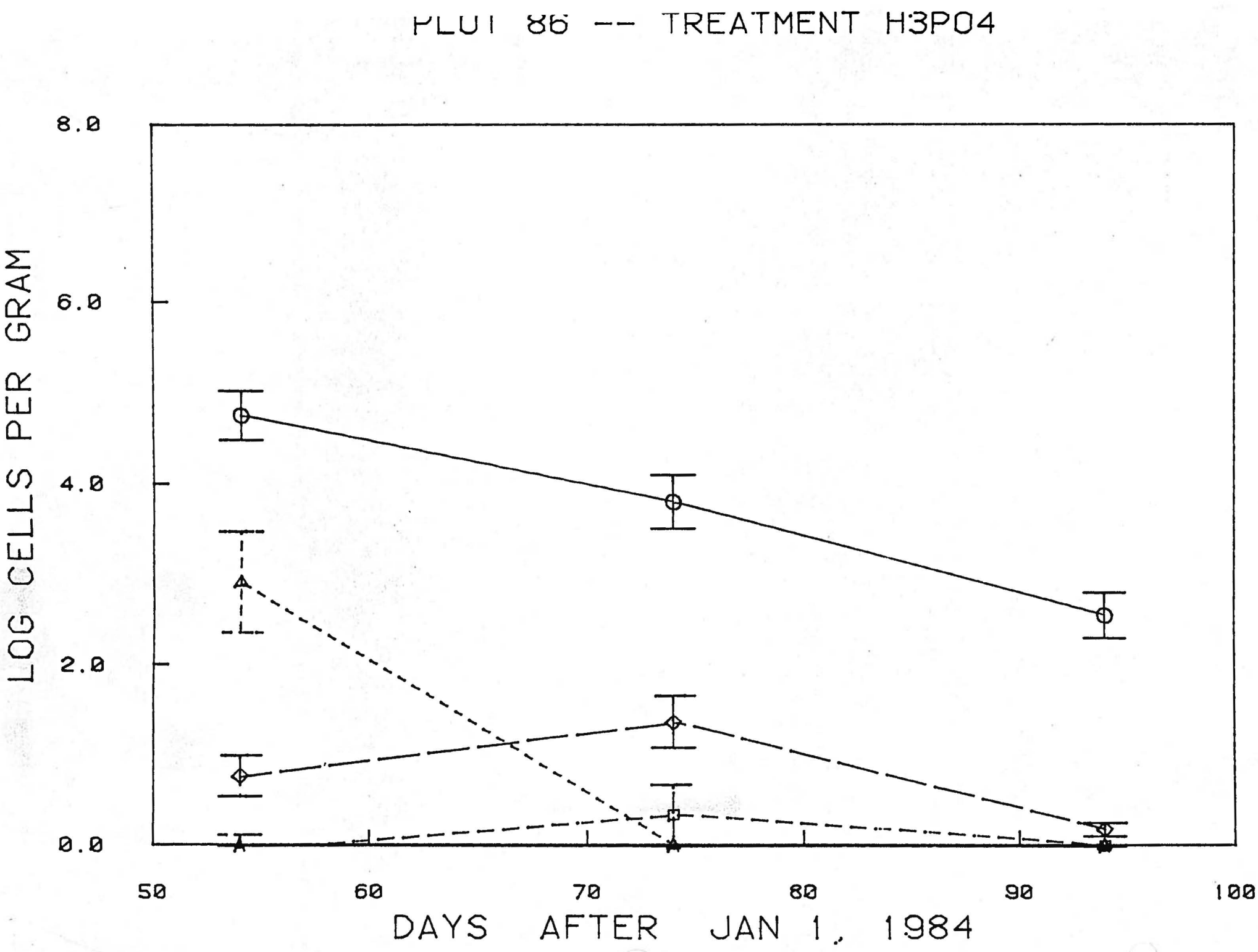


PLOT 66 -- TREATMENT KOC1DETMANEB



PLOT 66 -- TREATMENT A566+A526




PLOT 86 -- TREATMENT H3P04
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2.0
0.0
LOG CELLS PER GRAM
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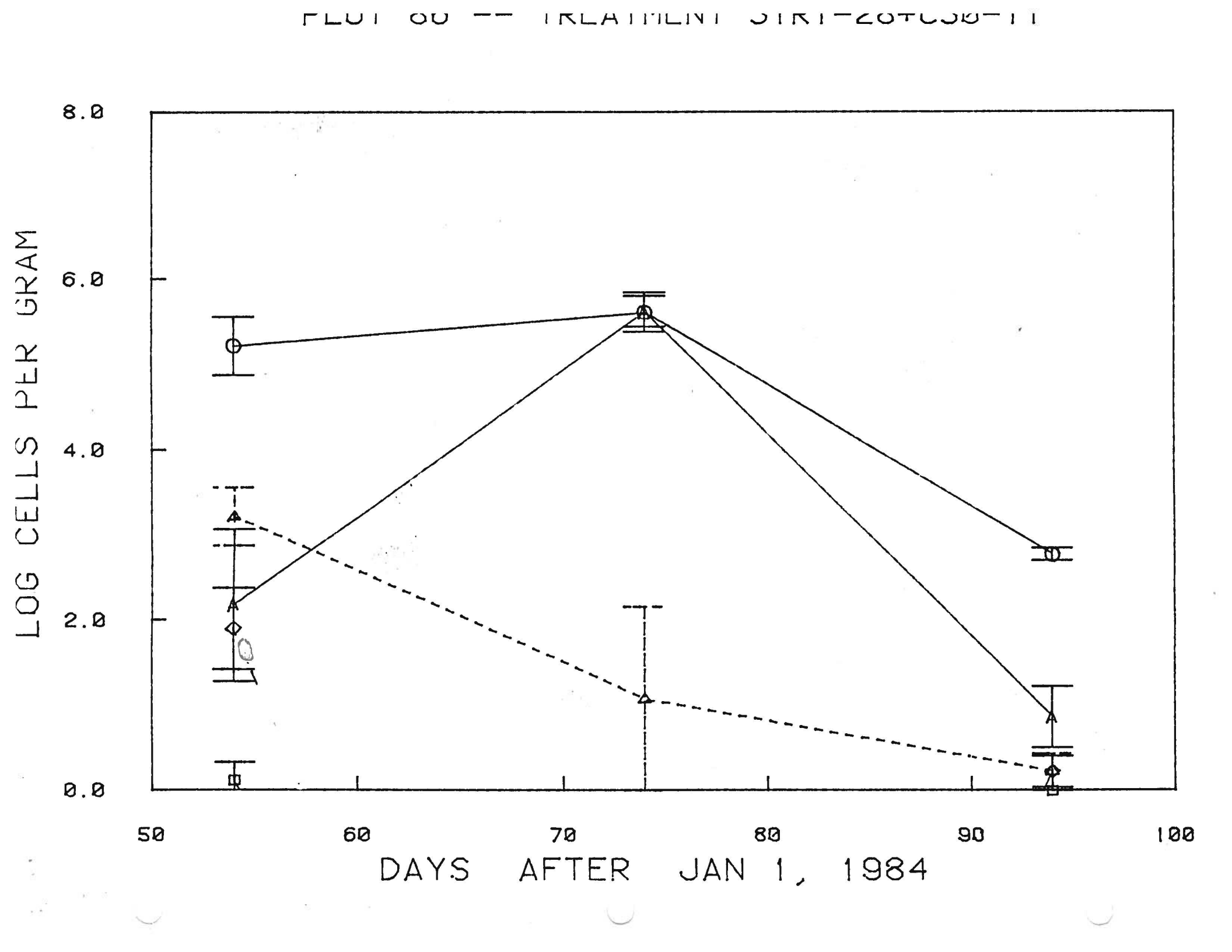
60
70
80
90
100
DAYS AFTER JAN 1, 1984


PLOT 80 -- TREATMENT STRT-28+C38-11
8.0
6.0
4.0
2.0
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LOG CELLS PER GRAM
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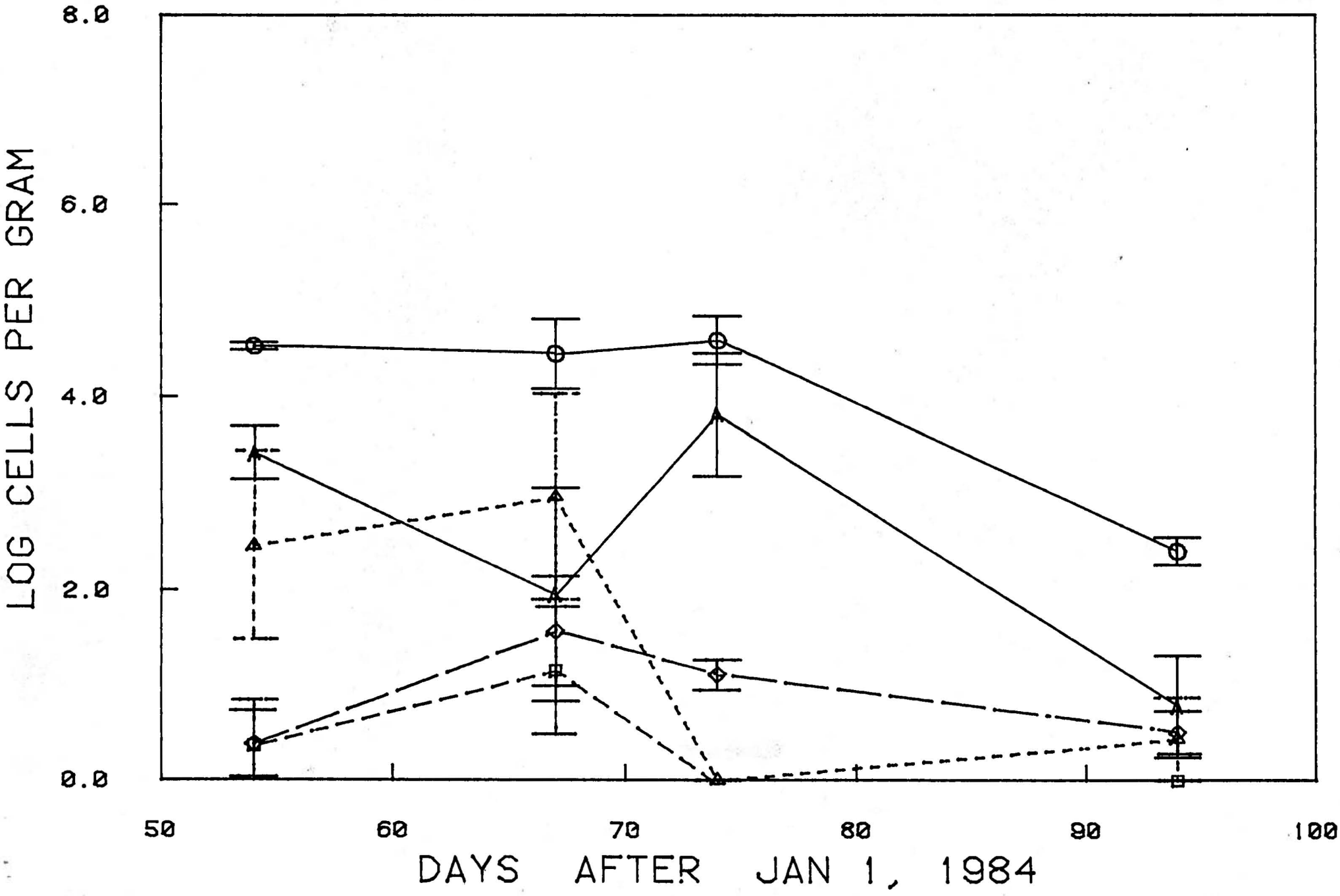
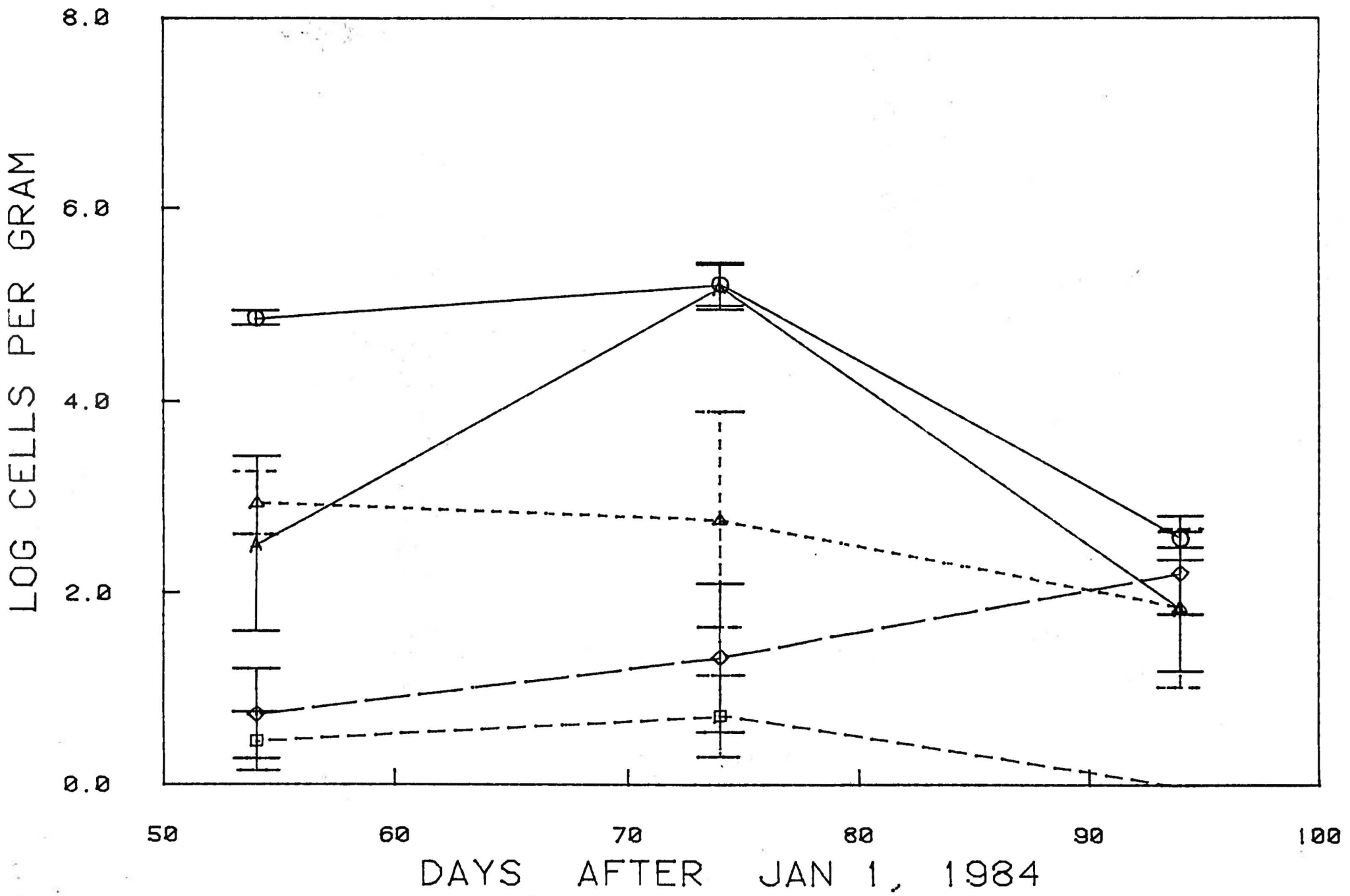
60
70
80
90
100
DAYS AFTER JAN 1, 1984

PLOT 66 -- TREATMENT CTT13-12




8.0
6.0
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LOG CELLS PER GRAM
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DAYS AFTER JAN 1, 1984