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THE EFFECTS OF ENVIRONMENTAL STRESSES ON GROWTH OF BLUE GRAMA (*BOUTELOUA GRACILIS*) IN ENVIRONMENTAL CONTROL GROWTH CHAMBERS

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ABSTRACT

Blue grama plants from Pawnee ESA (Environmental Stress Area) were placed in individual pots and subjected to water and temperature stresses in NREL growth chambers from October to December 1971. Growth was estimated in terms of yield per pot dry weight over this period. Photosynthetic activity was determined on the basis of total available carbohydrates (TAC) after the experiment was completed.

Results indicate enhanced photosynthetic and other metabolic activities at high temperatures (29°/18°C, 12 hr:12 hr, day/night regimes) under irrigated, and fertilized and irrigated conditions. The other experimental types, untreated and fertilized plants, responded well in terms of growth, TAC, phosphorus and total nitrogen at 13°/7°C, day/night temperatures for 65 days. A decline in these constituents was noted at higher temperature level conditions at the end of the growing season. The dilution, or translocation effect, was more pronounced in untreated and fertilized plants compared to irrigated and fertilized and irrigated plants, especially at higher temperatures.

INTRODUCTION

Plants growing under natural conditions are subject to diurnal and seasonal changes in their environment variations which are impossible to control in the field and difficult to duplicate in the laboratory. Thus the effects of these factors on plant growth and metabolic processes cannot be easily and accurately measured. But certain defined environmental conditions can, to a certain degree, be determined rather easily under controlled environmental growth chambers. The literature, in fact, is filled with this kind of information; however, the fundamental growth phenomenon of various grass species still remains to be identified. For this reason we set up a preliminary experiment to examine specific features of grass growth in chambers at NREL.

OBJECTIVES

This experiment was designed to study the response of plant growth to various environmental factors created in environmental control growth chambers. Attempts were made to control photoperiod, temperature, humidity, and soil water simultaneously. Laboratory conditions for the most part were selected in an attempt to duplicate or parallel those existing during the growing season on Pawnee Site. Specifically, answers to the following questions were explored in this experiment:

1. How is a combination of environmental factors for best growth of blue grama plants under growth chamber conditions approximated or achieved?

2. What is the effect of various temperature stresses on the growth, total available carbohydrates (TAC), phosphorus and total nitrogen of blue grama plants under growth chambers conditions?
3. What is the effect of nitrogen fertilizer, under irrigated and non-irrigated conditions, on the quantity and quality of blue grama plants at various temperature conditions?

METHODS AND MATERIALS

Three environmental chambers used for this experiment were obtained in 1970 and installed in NREL. These chambers are 66 inches wide by 30 inches deep by 52 inches high and are ideal for animal respiration and plant studies. Blue grama plants were brought from ESA plots at Pawnee to NREL. Plants were selected from the following treatment plots:

1. Irrigated (I)
2. Fertilized (F)
3. Non-irrigated (control) (C)
4. Fertilized + irrigated (FI)

To avoid a mixture of other species, the ESA plots were surveyed with the help of C. Dickinson, and specific sites were located in each treatment area from which the experimental plants were obtained. Plants from each treatment area were removed with a soil corer. The core size fit no. 10 cans. At the time of soil coring, unwanted species were removed by hand sorting from each treatment area. Soil samples were taken from the same location and stored temporarily in plastic bags for a later soil water determination.

At the time of collection, the plants from the two fertilized treatments (F and FI) had received watering continuously to maintain 0 to 0.8 bar moisture tension.

Plants were taken immediately to NREL and transferred to one of the growth chambers. These plants remained in the growth chambers for 2 weeks at 13°/7°C day/night temperature, 60% RH and 2000 to 5000 footcandles for preconditioning prior to the experiment. Before transferring plants to each experimental chamber, we had ascertained that the prescribed temperature settings had stabilized during the week preceding transferring. The potted plants were weighed before and after the 2 weeks preconditioning period and water was adjusted to bring the moisture content to uniform level for all replicates.

At the end of 2 weeks preconditioning period, the plants were divided into three groups, each group comprising eight pots from each of four treatments, two pots from each treatment were harvested at the beginning of the experiment to establish initial conditions. Subsequently, two pots from the remaining six pots were removed at 45-, 65-, and 95-day intervals during the experiment for sampling chemical constituents.

Before transferring plants to each chamber from each treatment, all plants were clipped close to the crown and weighed. One set of plants (F) was given ammonium nitrate fertilizer treatment at the rate of 150 kg N/ha when transferred to the individual chambers. Plants from the water stress (I, FI treatments) plots were irrigated daily to maintain field capacity of soil water. Untreated (C) and fertilized (F) plants were given water at 4- to 5-day intervals to prevent them from extreme water stress.

A summary of the treatment schedule is as follows:

1. Water.
   a. Irrigated (I) and fertilized plus irrigated (FI) pots to field capacity by watering daily.
   b. Untreated (C) and fertilized (F) pots at 4- to 5-day intervals.
2. Fertilization.
   a. 150 kg N/ha of ammonium nitrate or 2.4 g N/pot. (Area of pot = 0.1621 m^2)

The following environmental conditions were maintained in each growth chamber which received eight no. 10 cans from each of the four treatments.

Growth chamber no. 1 - 13°/7°C day/night temperature
1900 ± 10 footcandles light intensity
43% RH

Growth chamber no. 2 - 24°/13°C day/night temperature
1900 ± 10 footcandles light intensity
58% RH

Growth chamber no. 3 - 29°/18°C day/night temperature
1900 ± 10 footcandles light intensity
32% RH

Photoperiod was maintained at 12 hr of light and 12 hr of dark in all three chambers throughout the 95-day growing season. Samples harvested were stored in paper bags, dried at 70°C for 2 days, and weighed to record the dry weight (yield) per pot. These were then ground in a small Wiley mill to pass 20-mesh screen. Ground samples were thoroughly mixed before taking an

aliquot for the determination of chemical constituents. Total available carbohydrates (nonstructural carbohydrates) were determined by a modified method of Smith (1969) outlined in Appendix I. Phosphorus was determined by the molybdo-vanad O phosphoric acid procedure, slightly modified by the NREL chemical laboratory. Total nitrogen was determined by the micro-Kjeldahl procedure as outlined in NREL standard procedure files.

RESULTS

Results are presented in Tables 1, 2, 3, and 4. For convenience, data from each treatment are tabulated separately.

Table 1 shows the data from untreated plants (C) exposed to various temperature regimes during the 95-day growing season. These results indicate an increase in dry matter production, TAC, P, and total N during the initial 45-day growth period. This increase continues for another 20 days under the 13°/7°C and 24°/13°C day/night temperatures. At the 29°/18°C condition the amounts of TAC, P, and total N are declining at the end of the 65-day growth period and continue to drop through the remaining 30-day growing period. It is also obvious from Table 1 that TAC, P, and N show declines at 13°/7°C and 24°/13°C day/night temperature at the end of the 95-day growing period. The decline in the contents of the three chemical constituents at the end of the growing period is 40 to 50% of the peak measured at 65 days. Similarly the increase in these constituents at 65 days is almost 50% higher than the levels at 45 days. There is also a rapid increase in dry weight, TAC, P, and total N at the end of the 45-day growing period. Both the increase and decrease in P and total N contents of these untreated plants appear to be at the same rate while the TAC increase and decrease is at a slower rate.

Table 1. The effects of various temperatures on total yield, TAC, total nitrogen, and phosphorus contents of untreated blue grama plants. Results are averages of two replicates.

Analyses Type	Days When Plants Harvested and Analyses Performed											
	13°/7°C				24°/13°C				29°/18°C			
	0[a/]	45	65	95	0[a/]	45	65	95	0[a/]	45	65	95
Yield (g/pot)	3.500	5.300	6.500	8.500	3.800	5.400	6.800	7.400	3.200	5.400	6.200	5.800
%TAC	4.500	7.500	11.600	8.700	4.300	8.600	13.700	7.300	4.000	8.700	11.500	5.800
% Total N	0.520	1.750	2.430	1.850	0.500	1.870	2.000	1.125	0.513	1.700	1.867	0.875
%P	0.080	0.125	0.185	0.155	0.075	0.153	0.202	0.123	0.008	0.110	0.175	0.110

[a/] 0 day refers to the time when plants were transferred to the growth chambers.

Table 2. The effects of various temperatures on total yield, TAC, total nitrogen, and phosphorus contents of fertilized blue grama plants. Results are averages of two replicates.

Analyses Type	Days When Plants Harvested and Analyses Performed											
	13°/7°C				24°/13°C				29°/18°C			
	0[a]	45	65	95	0[a]	45	65	95	0[a]	45	65	95
Yield (g/pot)	3.600	5.500	7.200	8.200	3.500	6.500	7.500	7.200	3.600	6.800	7.000	5.200
%TAC	5.200	7.800	11.800	7.500	5.500	7.800	14.800	6.300	5.300	8.300	12.500	6.500
% Total N	0.630	1.875	2.300	1.980	0.675	1.750	1.950	1.350	0.666	1.735	1.800	1.115
%P	0.075	0.120	0.190	0.145	0.080	0.165	0.185	0.137	0.080	0.115	0.165	0.125

[a] 0 day refers to the time when plants were transferred to the growth chambers.

Table 3. The effects of various temperatures on total yield, TAC, total nitrogen, and phosphorus contents of irrigated blue grama plants. Results are averages of two replicates.

Analyses Type	Days When Plants Harvested and Analyses Performed											
	13°/7°C				24°/13°C				29°/18°C			
	0[a]	45	65	95	0[a]	45	65	95	0[a]	45	65	95
Yield (g/pot)	3.500	7.200	8.500	10.200	3.700	7.500	8.700	9.500	3.500	7.500	8.500	8.700
%TAC	6.200	10.700	15.500	16.500	6.500	12.500	17.800	15.200	6.400	13.600	19.700	12.800
% Total N	0.812	1.850	2.800	1.950	0.800	1.900	3.120	1.900	0.818	2.150	3.225	1.850
%P	0.180	0.210	0.285	0.198	0.185	0.235	0.300	0.175	0.178	0.268	0.312	0.155

[a] 0 day refers to the time when plants were transferred to the growth chambers.

Table 4. The effects of various temperatures on total yield, TAC, total nitrogen, and phosphorus contents of irrigated and fertilized blue grama plants. Results are averages of two replicates.

Analyses Type	Days When Plants Harvested and Analyses Performed											
	13°/7°C				24°/13°C				29°/18°C			
	0[a]	45	65	95	0[a]	45	65	95	0[a]	45	65	95
Yield (g/pot)	3.600	8.400	9.700	12.800	3.700	8.800	10.200	11.700	3.400	8.600	11.300	11.500
%TAC	7.800	11.700	18.500	17.300	7.200	14.500	20.700	18.500	7.500	15.300	22.600	20.300
% Total N	1.320	2.210	3.825	2.550	1.300	2.780	4.780	2.950	1.287	3.120	5.212	2.855
%P	0.185	0.275	0.355	0.300	0.190	0.280	0.380	0.320	0.195	0.312	0.455	0.375

[a] 0 day refers to the time when plants were transferred to the growth chambers.

The results of the TAC, P, and total N from fertilized (F) plants are given in Table 2. Generally, fertilized plants grow much faster during the 45- to 65-day period when compared to untreated plants. These latter plants indicate rapid decline in TAC, P, and total N at the end of the 65-day growing period and continue to decline until the end of the growing period.

The TAC, P, and total N levels at 29°/18°C day/night temperature are lower when compared to plants grown under 13°/7°C or 24°/13°C day/night temperature. This tendency is more pronounced for N.

Table 3 shows the results of the irrigated plants (I) grown at various temperature regimes. As is evident from the table, the total yield, TAC, P, and total N contents of these plants are higher than the untreated (C) and fertilized (F) plants. While irrigated plants generally grow well at 13°/7°C and 24°/13°C temperatures for the whole 95-day period, a decline in the three chemical constituents is evident at 29°/18°C day/night temperatures. This decline is evident at the two lower temperature regimes but not to the same extent. The contents of TAC, P, and total N of the irrigated plants (I) at the three temperature regimes appear to be 30 to 40% greater than the untreated (C) and fertilized (F) plants growing under the same temperature range at the end of the 65-day growing period.

The decline of these three labile chemical constituents is quite apparent in contrast to the picture for the rest of the plant. Measures of total dry matter yield stay comparatively higher. In this latter component are both non-labile and labile components (those labile materials that were not translocated by the plants). The dry weight yield/pot from treated and fertilized

plants shows the greatest decline at 29°/18°C day/night temperature at the end of the 95-day growing period. There is also a slight reduction in yield at 45 days at the 29°/18°C temperatures as compared to the two lower temperature regimes. The fertilized and irrigated (FI) plants (Table 4) and the irrigated only (I) plants (Table 3) indicate insignificant reduction in yield even at the extreme temperature (29°/18°C) at the end of the 95-day period, compared to the reduction in yield from untreated and fertilized (F) plants during the same period of time. Fertilized and irrigated (FI) plants appeared to be greener and more vigorous throughout the experiment, in addition to having a greater yield, when compared to the plants from the other three treatments.

The results from the fertilized and irrigated (FI) plants are given in Table 4. It is quite obvious that these plants produced greater levels of total yield, TAC, P, and total N than plants from the other treatments. TAC, P, and N contents of the fertilized and irrigated (FI) plants remained almost constant at the three temperature regimes during the final 30-day growing period with only slight reduction in N content at 29°/18°C at the end of the 95-day growing period.

The results presented in Tables 1, 2, 3, and 4 suggest that growth of blue grama at higher temperatures under fertilized and irrigated (FI) conditions is not limited by CO_2 or light intensity when grown under controlled environmental conditions. The untreated plants (C) grown under growth chamber conditions which simulate environmental conditions at Pawnee, could not respond well to the water and temperature stresses imposed in this experiment.

The fertilized (F) plant response was poor and plants appeared unhealthy and withered at the end of the 95-day growing period. This indicates that blue grama plants may lack resistance or tolerance to higher salt or fertilizer application. The low nitrogen content, in the plants or perhaps more specifically, the protein content at higher temperature under water stressed conditions in untreated and fertilized plants, indicates that either the nitrogen fertilization impairs the uptake phenomenon of the root membrane by physical means or that a concentrated salty medium around the root zone per se causes plasmolysis at higher temperatures. Plants under fertilized and irrigated (FI) conditions appear not to be suffering from the above phenomenon. Generally plants under all treatments responded well to 13°/7°C day/night temperature throughout the growing period. Tables 5 through 8 show data of Tables 1 through 4, rearranged for the analyses of variance, the results of which follow in Tables 9 through 12. From these tables it appears that, except for the nitrogen-temperature effect, all main effects and interactions of the four treatments are highly significant ($\alpha = 0.01$). Based on the significance of the three-way interactions (time × treatment × temperature) shown in Tables 9 through 12, a series of regressions were run. The results of the polynomial regression, up to a cubic degree, are plotted in Fig. 1 through 21, in which y = yield and x = time. A comparison between the linear and quadric equations is given in Table 13. Results of this study will be discussed in view of the cubic polynomial which appears to provide the best fit. The numbers (1 through 4) in each figure refer to the individual treatment, i.e., fertilized, control, irrigated, and fertilized plus irrigated, respectively.

Table 5. The effects of various treatments under various temperatures on total yield (g/pot dry wt) of blue grama plants. Results are averages of two replicates.

Days When Harvested	Temperature Regimes											
	13°/7°C				24°/13°C				29°/18°C			
	C	F	I	FI	C	F	I	FI	C	F	I	FI
0[a/]	3.500	3.600	3.500	3.600	3.800	3.500	3.700	3.700	3.200	3.600	3.500	3.400
45	5.300	5.500	7.200	8.400	5.400	6.500	7.500	8.800	5.400	6.800	7.500	8.600
65	6.500	7.200	8.500	9.700	6.800	7.500	8.700	10.200	6.200	7.000	8.500	11.300
95	8.500	8.200	10.200	12.800	7.400	7.200	9.500	11.700	5.800	5.200	8.700	11.500

[a/] 0 day refers to the time when plants were transferred to the growth chambers.

Table 6. The effects of various treatments under various temperatures of total available carbohydrates (%) of blue grama plants. Results are averages of two replicates.

Days When Harvested	Temperature Regimes											
	13°/7°C				24°/13°C				29°/18°C			
	C	F	I	FI	C	F	I	FI	C	F	I	FI
0[a/]	4.500	5.200	6.200	7.800	4.300	5.500	6.500	7.200	4.000	5.300	6.400	7.500
45	7.500	7.800	10.700	11.700	8.600	7.800	12.500	14.500	8.700	8.300	13.600	15.300
65	11.600	11.800	15.500	18.500	13.700	14.800	17.800	20.700	11.500	12.500	19.700	22.600
95	8.700	7.500	16.500	17.300	7.300	6.300	15.200	18.500	5.800	6.500	12.800	20.300

[a/] 0 day refers to the time when plants were transferred to the growth chambers.

Table 7. The effects of various treatments under various temperatures of total nitrogen (%) of blue grama plants. Results are averages of two replicates.

Days When Harvested	Temperature Regimes											
	13°/7°C				24°/13°C				29°/18°C			
	C	F	I	FI	C	F	I	FI	C	F	I	FI
0[a/]	0.520	0.630	0.812	1.320	0.500	0.675	0.800	1.300	0.513	0.666	0.818	1.287
45	1.750	1.875	1.850	2.210	1.870	1.750	1.900	2.780	1.700	1.735	2.150	3.120
65	2.430	2.300	2.800	3.825	2.000	1.950	3.120	4.780	1.867	1.800	3.225	5.212
95	1.850	1.980	1.950	2.550	1.125	1.350	1.900	2.950	0.875	1.115	1.850	2.855

[a/] 0 day refers to the time when plants were transferred to the growth chambers.

Table 8. The effects of various treatments under various temperatures of phosphorus (%) of blue grama plants. Results are averages of two replicates.

Days When Harvested	Temperature Regimes											
	13°/7°C				24°/13°C				29°/18°C			
	C	F	I	FI	C	F	I	FI	C	F	I	FI
0[a/]	0.080	0.075	0.180	0.185	0.075	0.080	0.185	0.190	0.085	0.080	0.178	0.195
45	0.125	0.120	0.210	0.275	0.153	0.165	0.235	0.280	0.110	0.115	0.268	0.312
65	0.185	0.190	0.285	0.355	0.202	0.185	0.300	0.380	0.175	0.165	0.312	0.455
95	0.155	0.145	0.198	0.300	0.123	0.137	0.175	0.320	0.110	0.125	0.155	0.375

[a/] 0 day refers to the time when plants were transferred to the growth chambers.

Table 9. Analysis of variance--total yield.

Source	df	SS	MS	F	
Treatment	3	140.820	46.940	1753.00	***
Temperature	2	2.490	1.243	46.00	***
Treatment × Temperature	6	2.560	0.427	16.00	***
Error Treatment	12	0.321	0.027		
Time	3	400.240	133.413	2739.00	***
Time × Treatment	9	54.320	6.036	124.00	***
Time × Temperature	6	17.240	2.874	59.00	***
Time × Treatment × Temperature	18	4.670	0.260	5.34	***
Error Time	36	1.754	0.049		
Total	95	624.420			

*** Significant for $\alpha = 0.01$.

Table 10. Analysis of variance--%TAC.

Source	df	SS	MS	F	
Treatment	3	886.70	295.565	11215	***
Temperature	2	12.23	6.117	232	***
Treatment × Temperature	6	23.48	3.914	149	***
Error Treatment	12	0.32	0.026		
Time	3	1223.07	407.689	9356	***
Time × Treatment	9	202.94	22.549	517	***
Time × Temperature	6	38.45	6.408	147	***
Time × Treatment × Temperature	18	31.36	1.742	40	***
Error Time	36	1.57	0.044		
Total	95	2420.12			

*** Significant for $\alpha = 0.01$.

Table 11. Analysis of variance--%N.

Source	df	SS	MS	F	
Treatment	3	31.320	10.4400	9335.00	***
Temperature	2	0.001	0.0007	0.63	NS
Treatment × Temperature	6	3.120	0.5200	465.00	***
Error Treatment	12	0.013	0.0011		
Time	3	54.670	18.2220	7952.00	***
Time × Treatment	9	8.370	0.9300	406.00	***
Time × Temperature	6	1.120	0.1870	82.00	***
Time × Treatment × Temperature	18	1.660	0.0920	40.00	***
Error Time	36	0.082	0.0023		
Total	95	100.360			

NS Nonsignificant for $\alpha = 0.10$.

*** Significant for $\alpha = 0.01$.

Table 12. Analysis of variance--%P.

Source	df	SS	MS	F	
Treatment	3	.48443	.16148	8808	***
Temperature	2	.00153	.00077	42	***
Treatment × Temperature	6	.01564	.00261	142	***
Error Treatment	12	.00022	.00002		
Time	3	.21541	.07180	1219	***
Time × Treatment	9	.04655	.00517	88	***
Time × Temperature	6	.00381	.00064	11	***
Time × Treatment × Temperature	18	.01296	.00072	12	***
Error Time	36	.00212	.00006		
Total	95	.78267			

*** Significant for $\alpha = 0.01$.

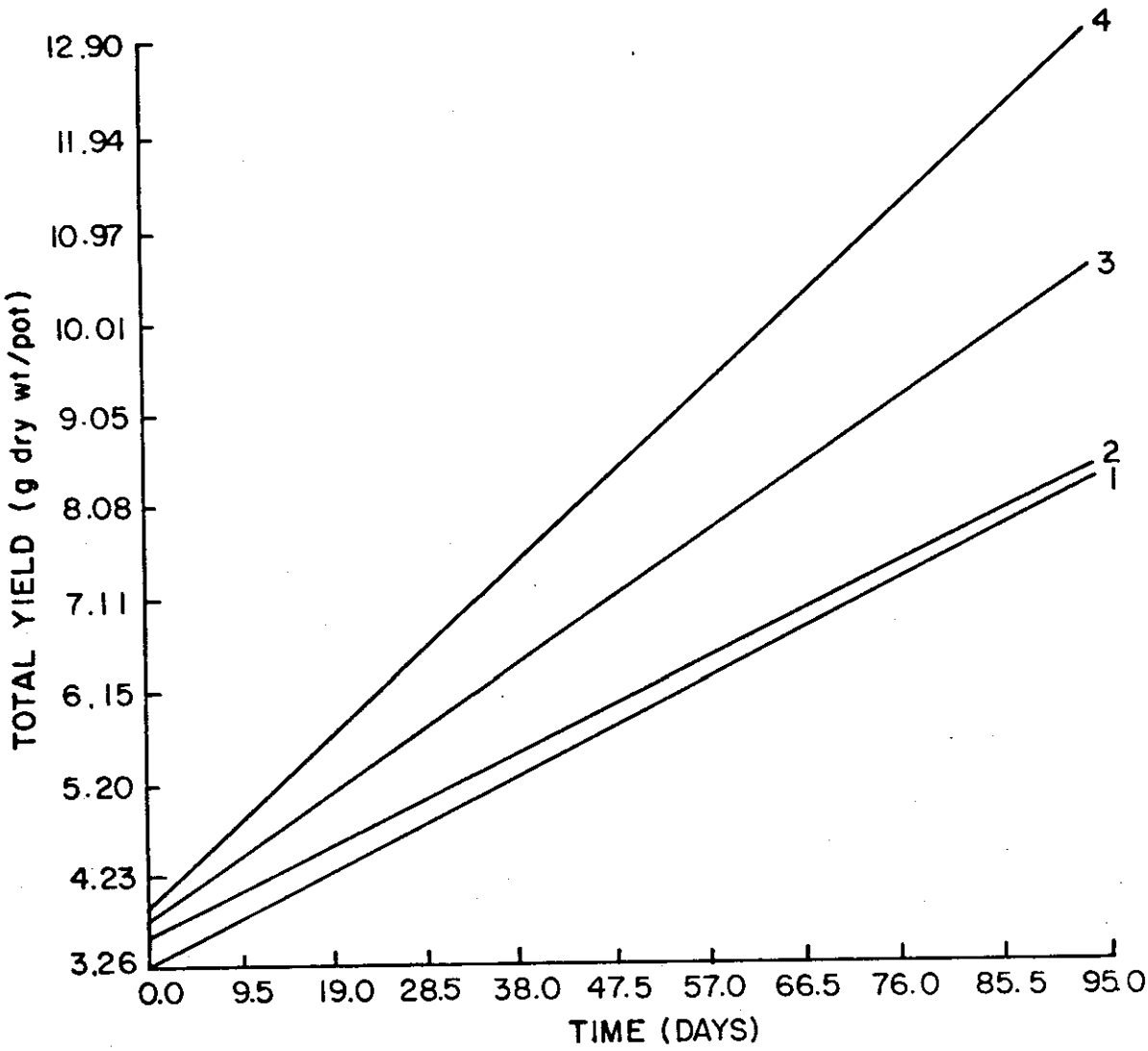

Fig. 1. Linear polynomial regressions for total yield vs. time at 13°/7°C.

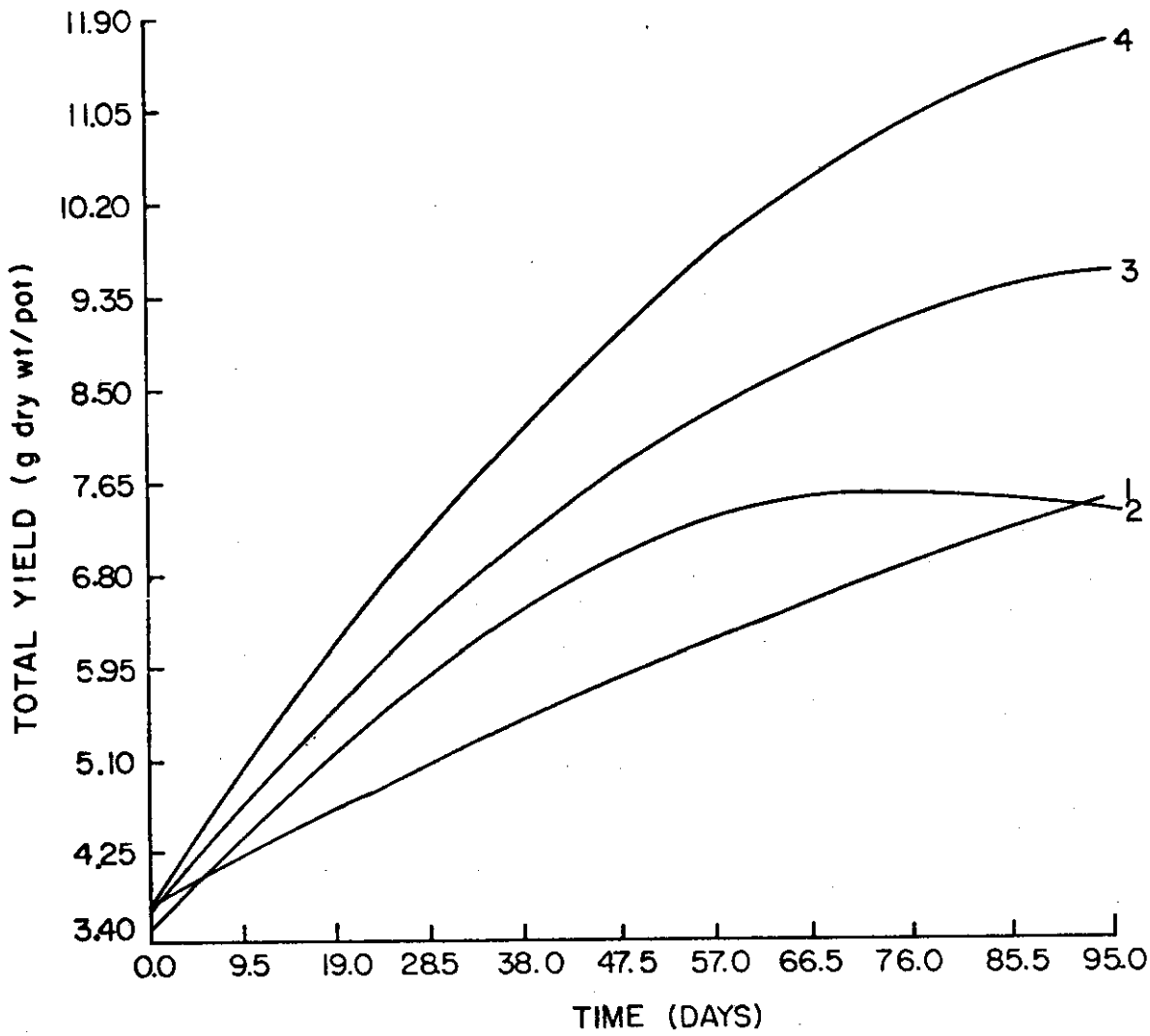

Fig. 2. Linear and quadratic polynomial regressions for total yield vs. time at 24°/13°C.

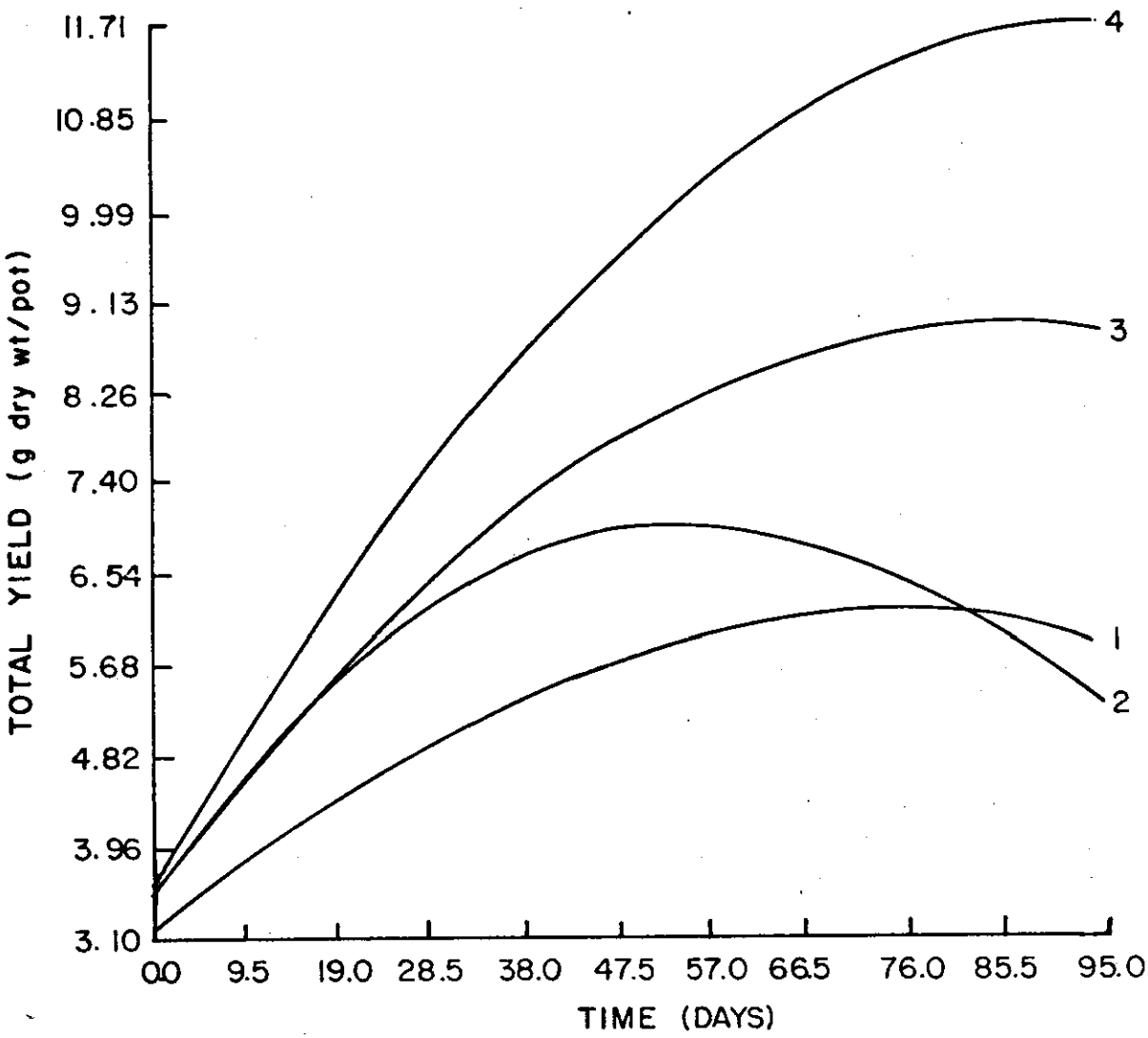

Fig. 3. Quadratic polynomial regressions for total yield vs. time at 29°/18°C.

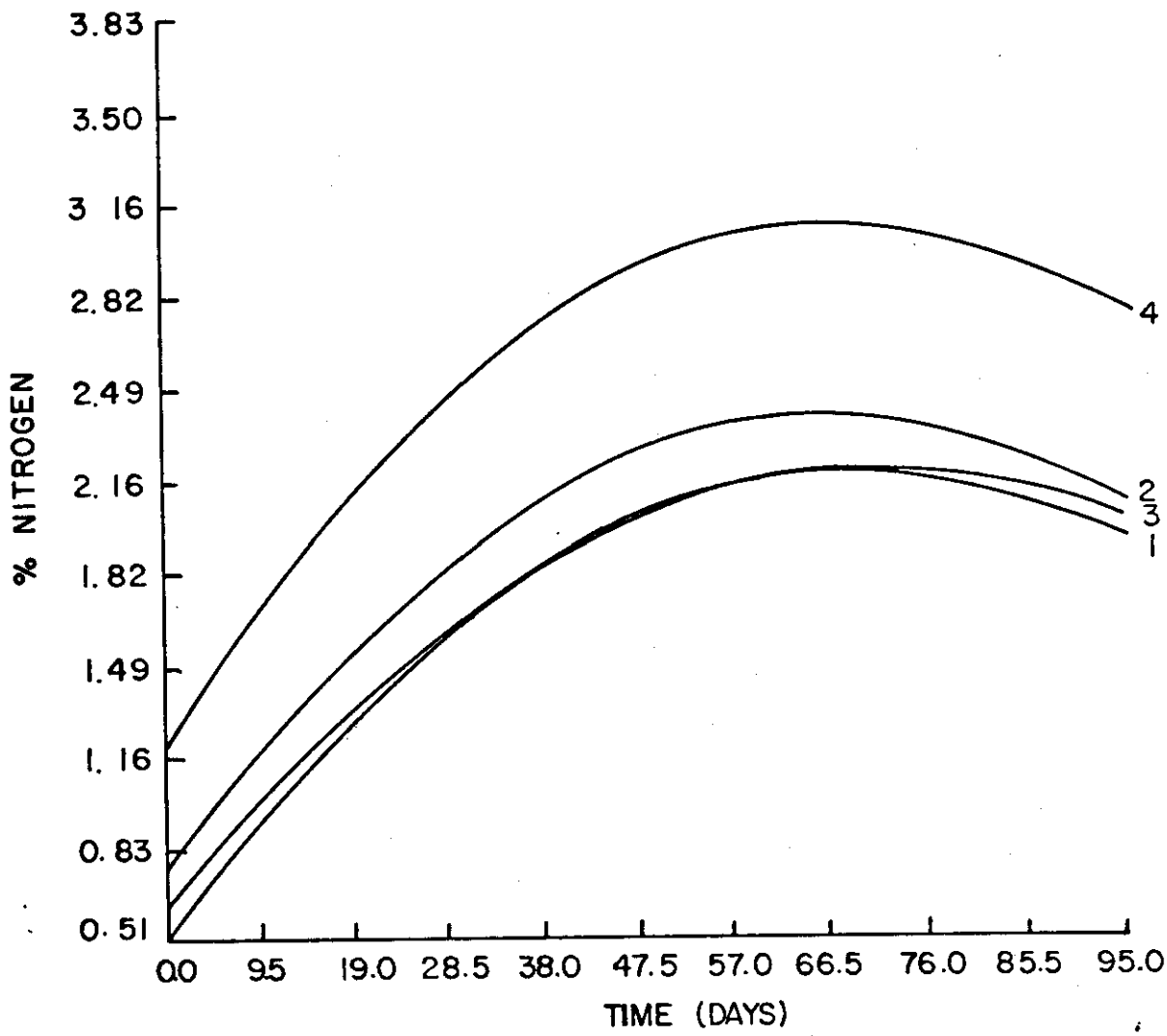

Fig. 4. Quadratic polynomial regressions for %N vs. time at 13°/7°C.

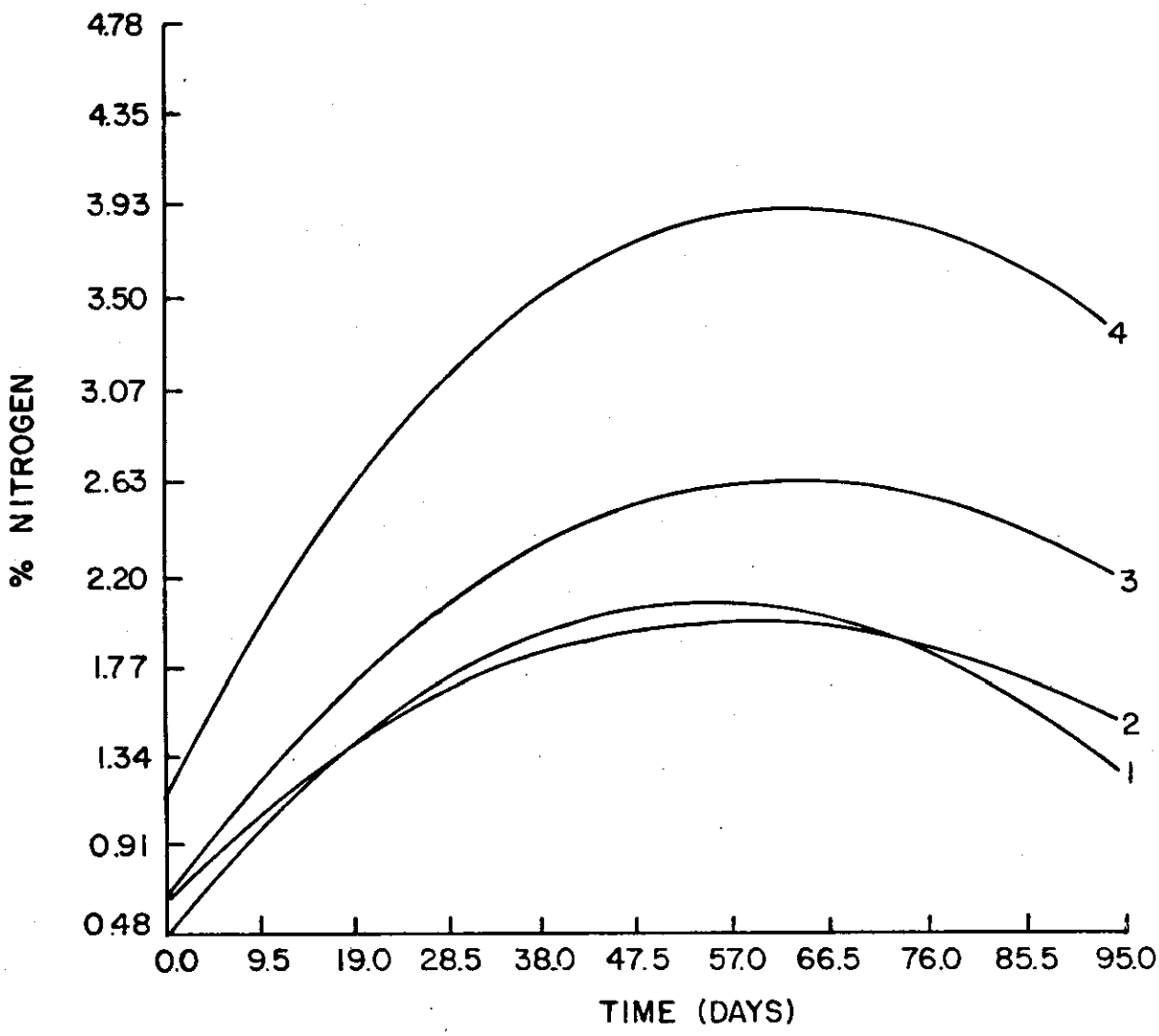

Fig. 5. Quadratic polynomial regressions for %N vs. time at 24°/13°C.

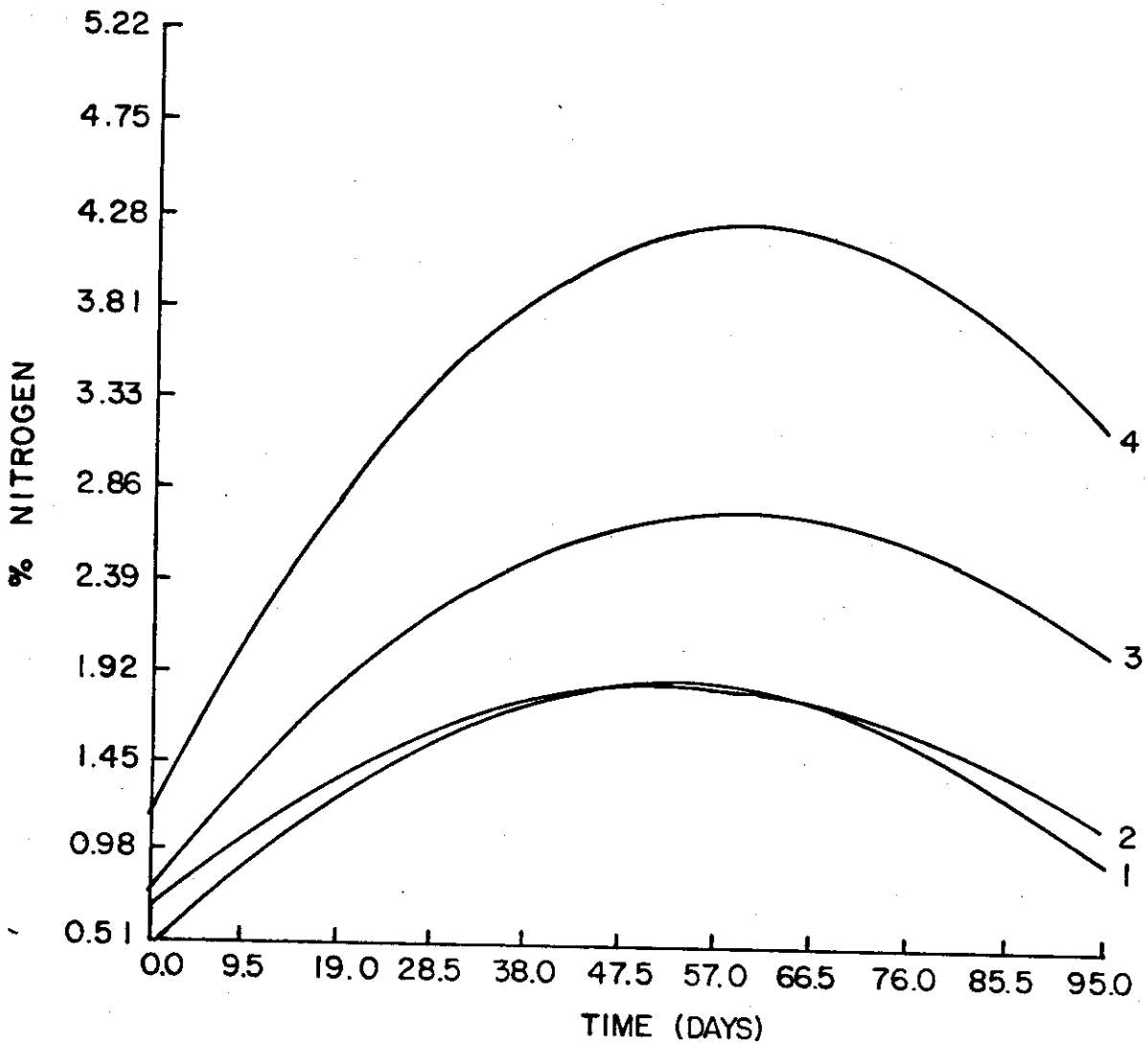

Fig. 6. Quadratic polynomial regressions for %N vs. time at 29°/18°C.

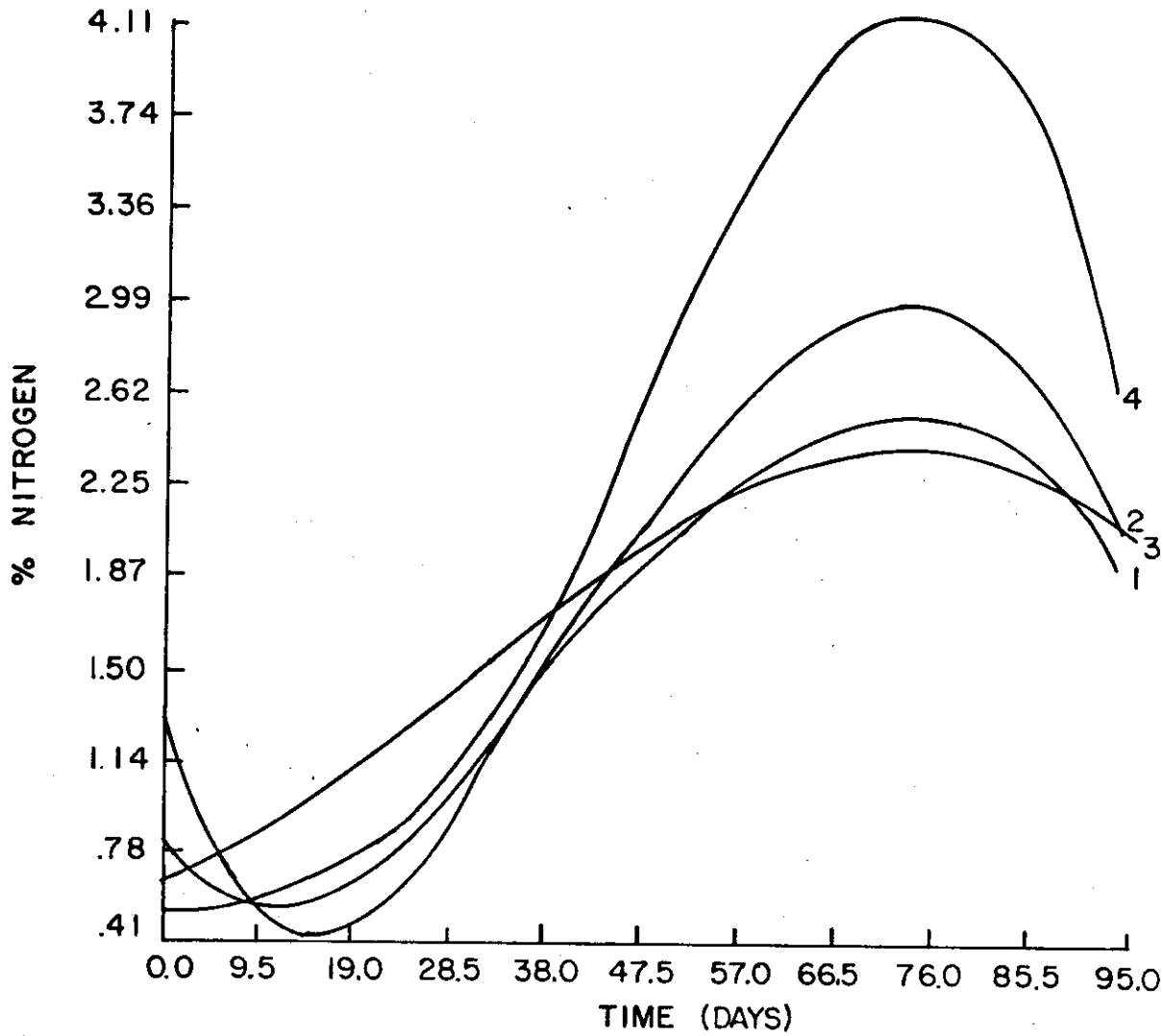

Fig. 7. Cubic polynomial regressions for %N vs. time at 13°/7°C.

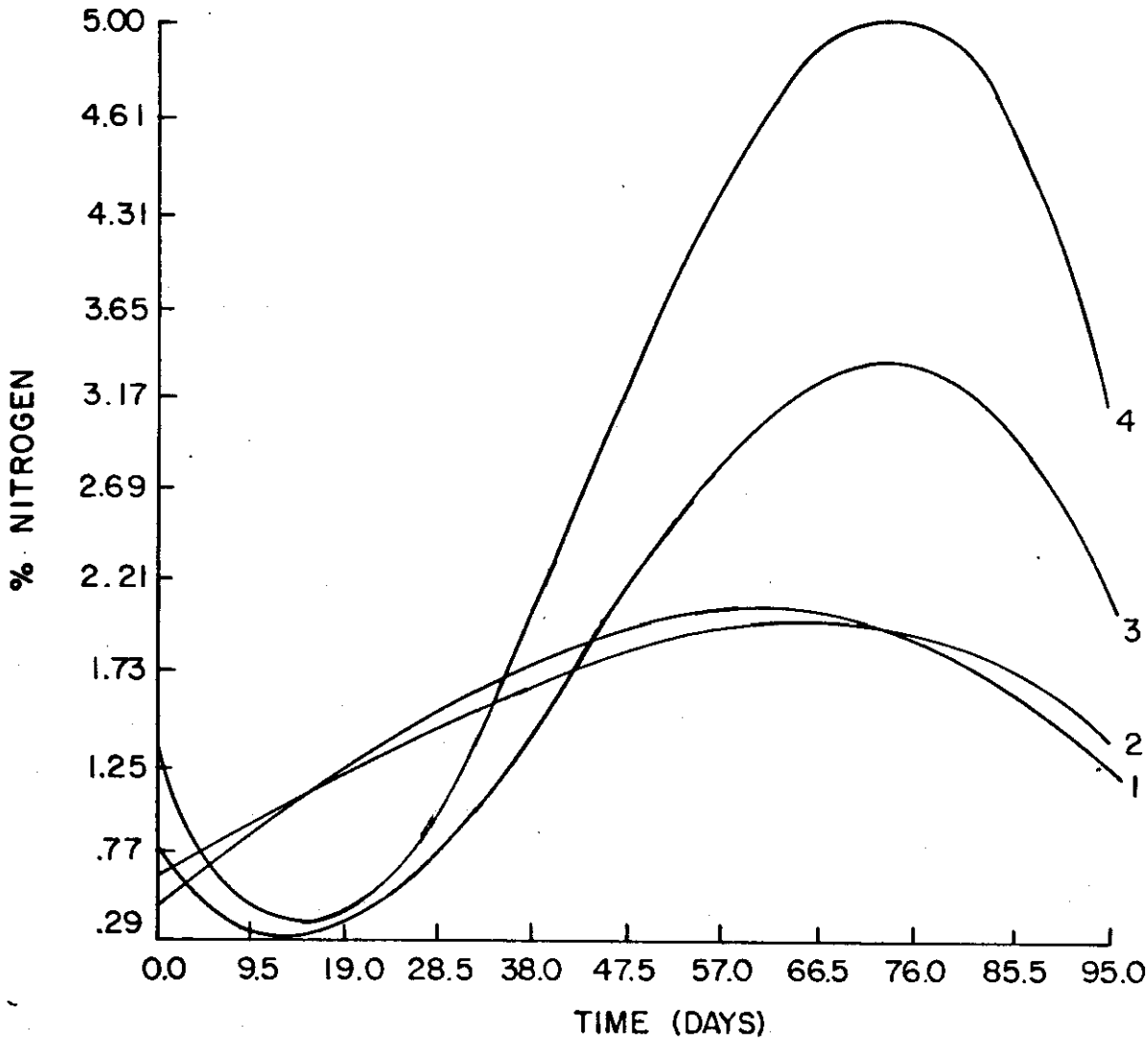

Fig. 8. Cubic polynomial regressions for %N vs. time at 24°/13°C.

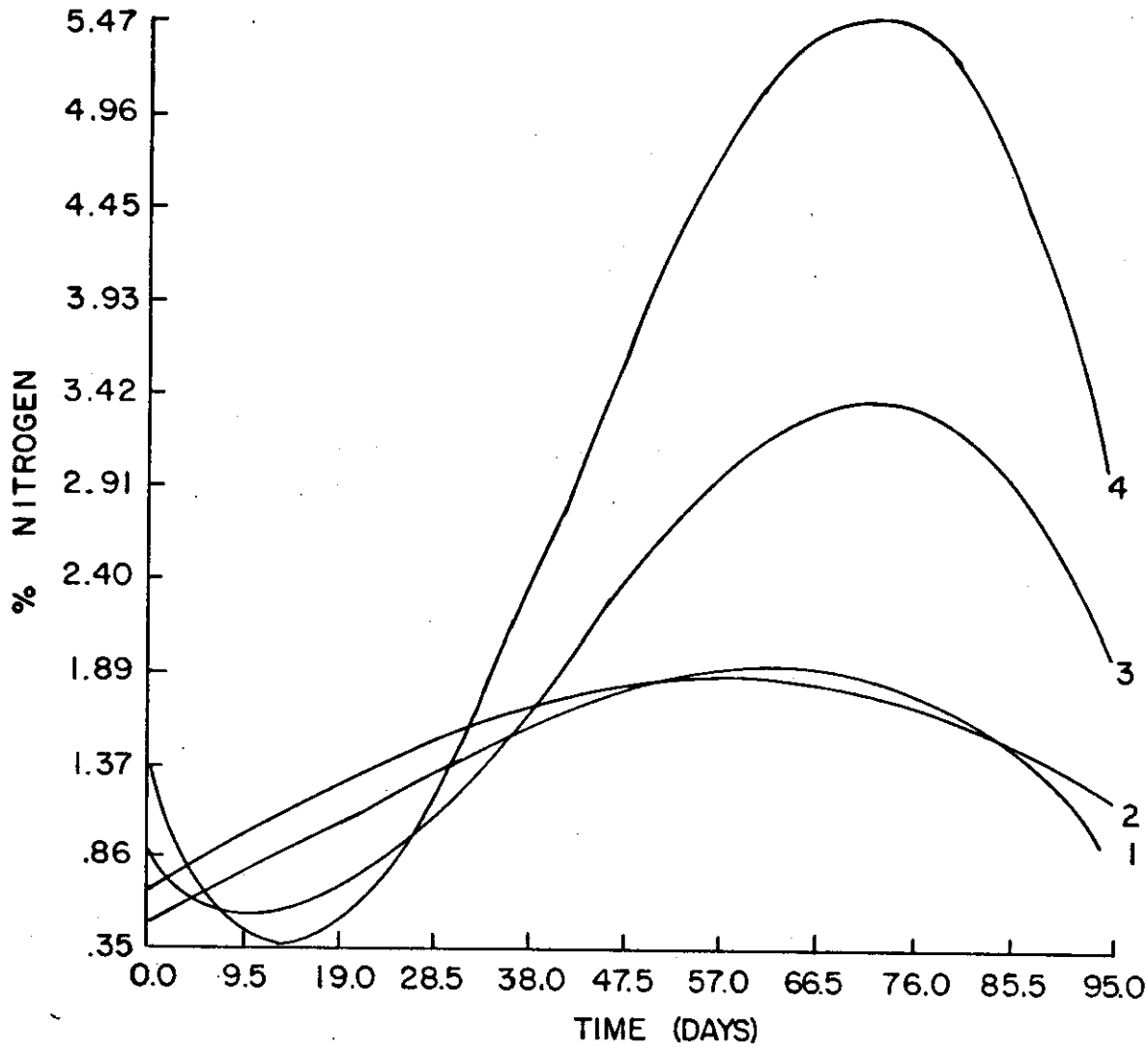

Fig. 9. Cubic polynomial regressions for %N vs. time at 29°/18°C.

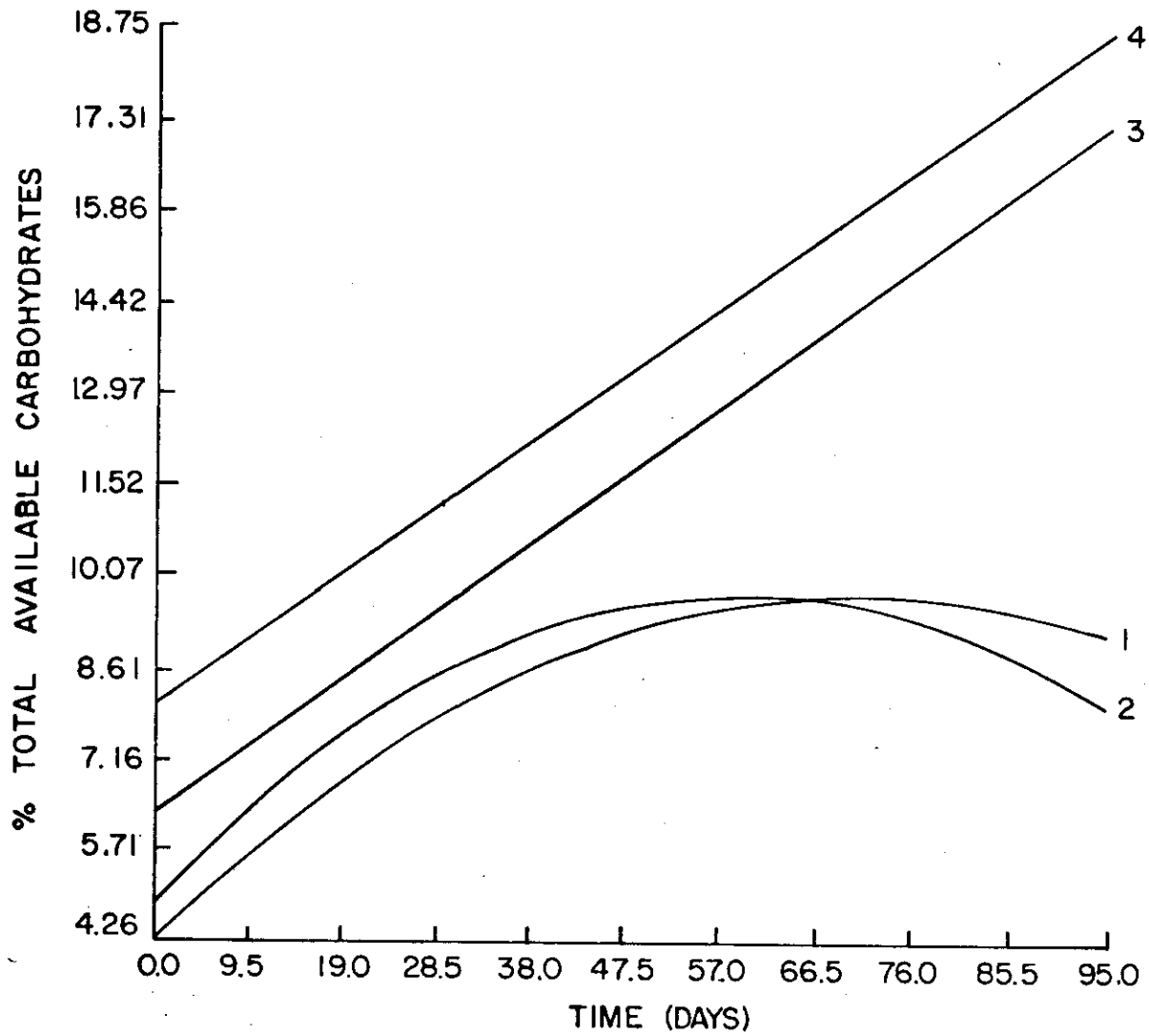

Fig. 10. Linear and quadratic polynomial regressions for %TAC vs. time at 13°/7°C.

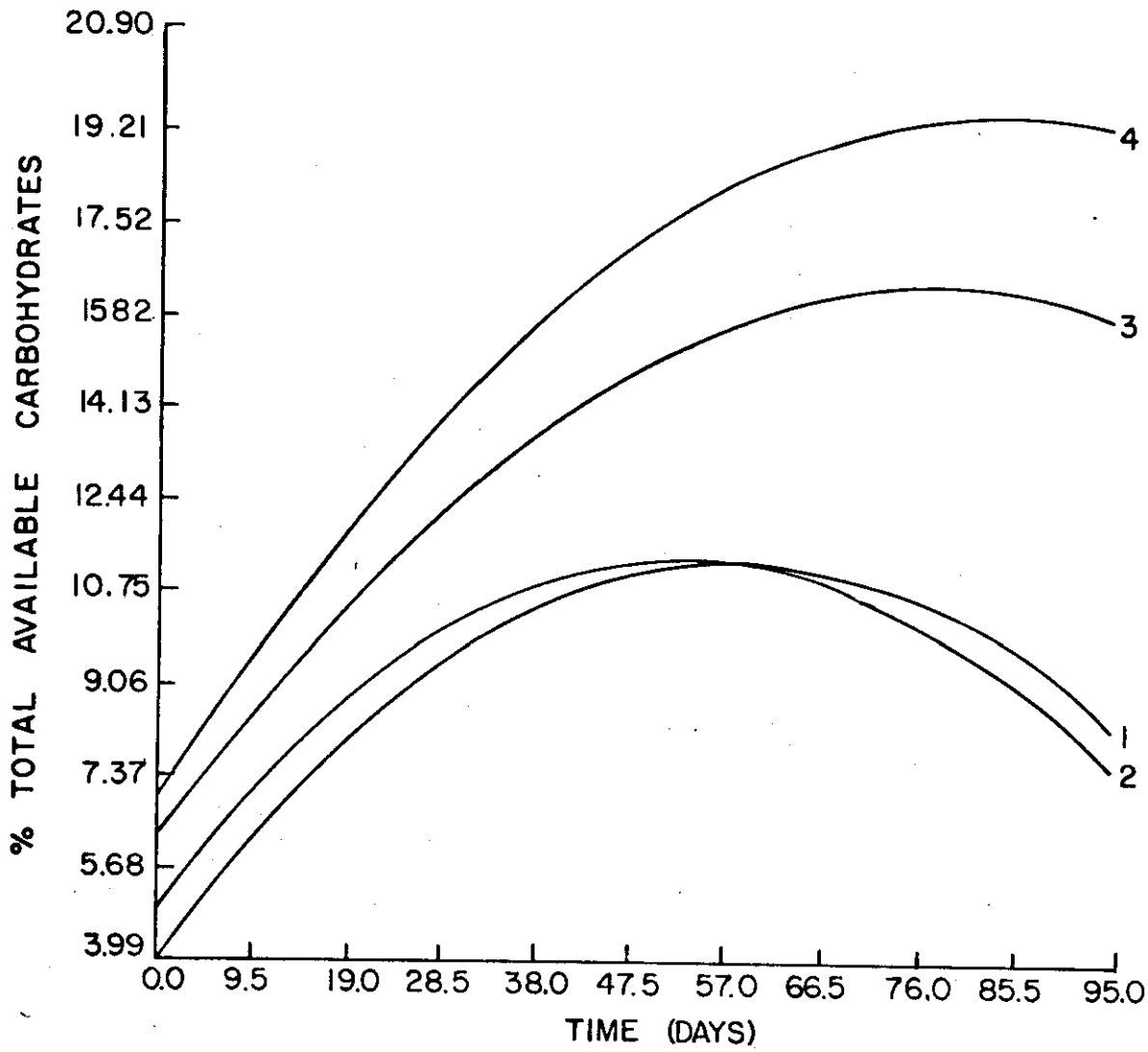

Fig. 11. Quadratic polynomial regressions for %TAC vs. time at 24°/13°C.

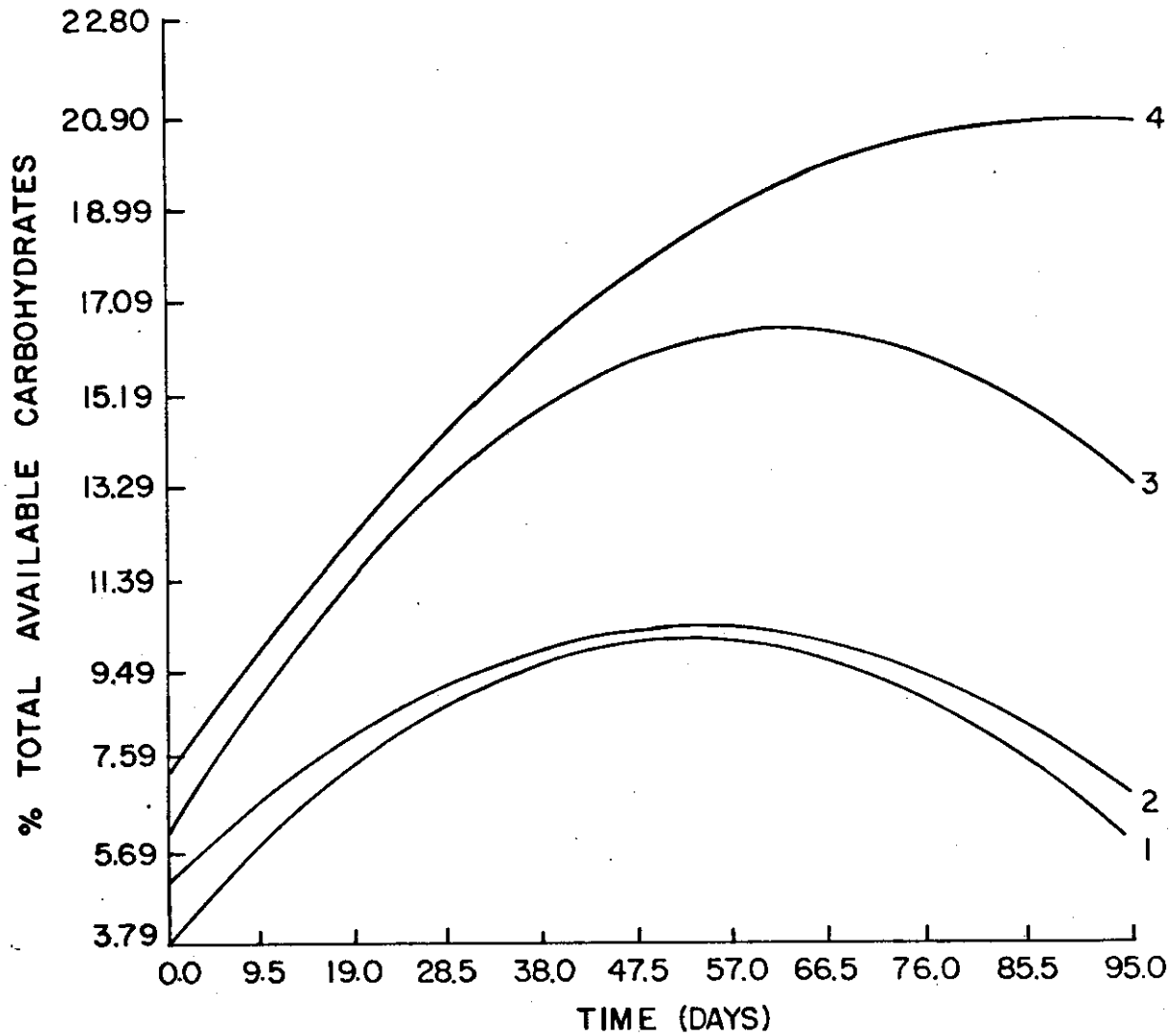

Fig. 12. Quadratic polynomial regressions for %TAC vs. time at 29°/18°C.

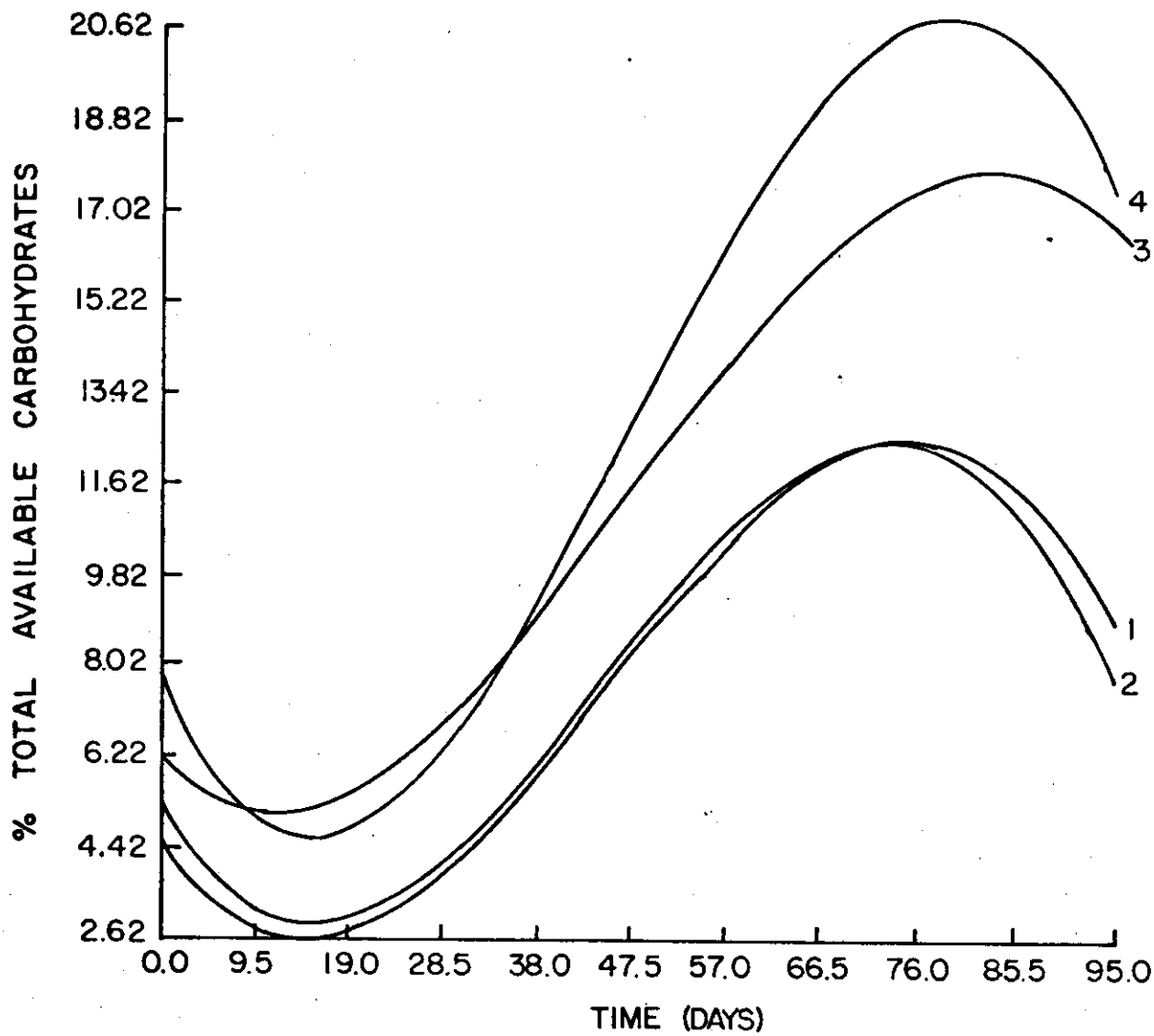

Fig. 13. Cubic polynomial regressions for %TAC vs. time at 13°/7°C.

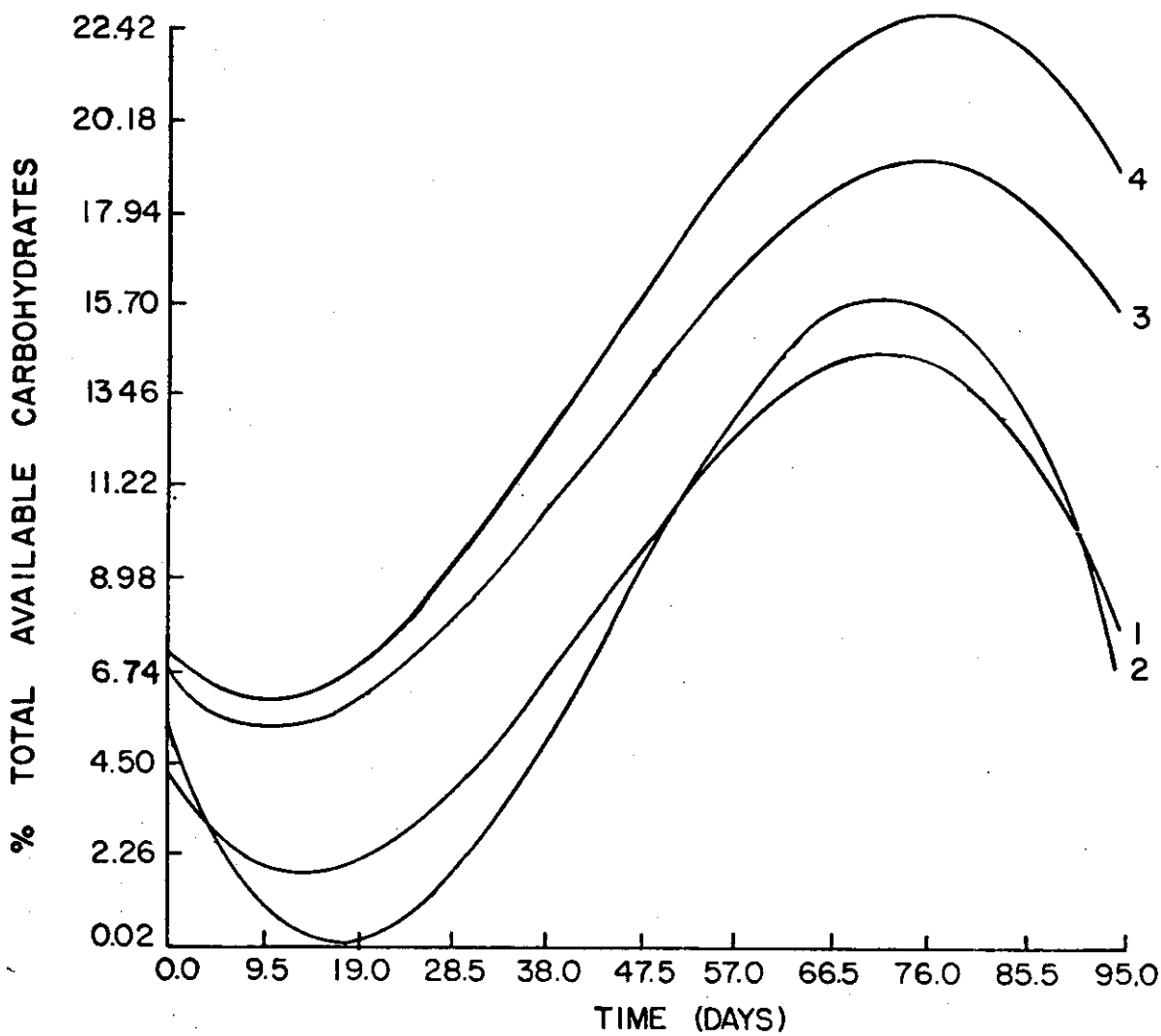

Fig. 14. Cubic polynomial regressions for %TAC vs. time at 24°/13°C.

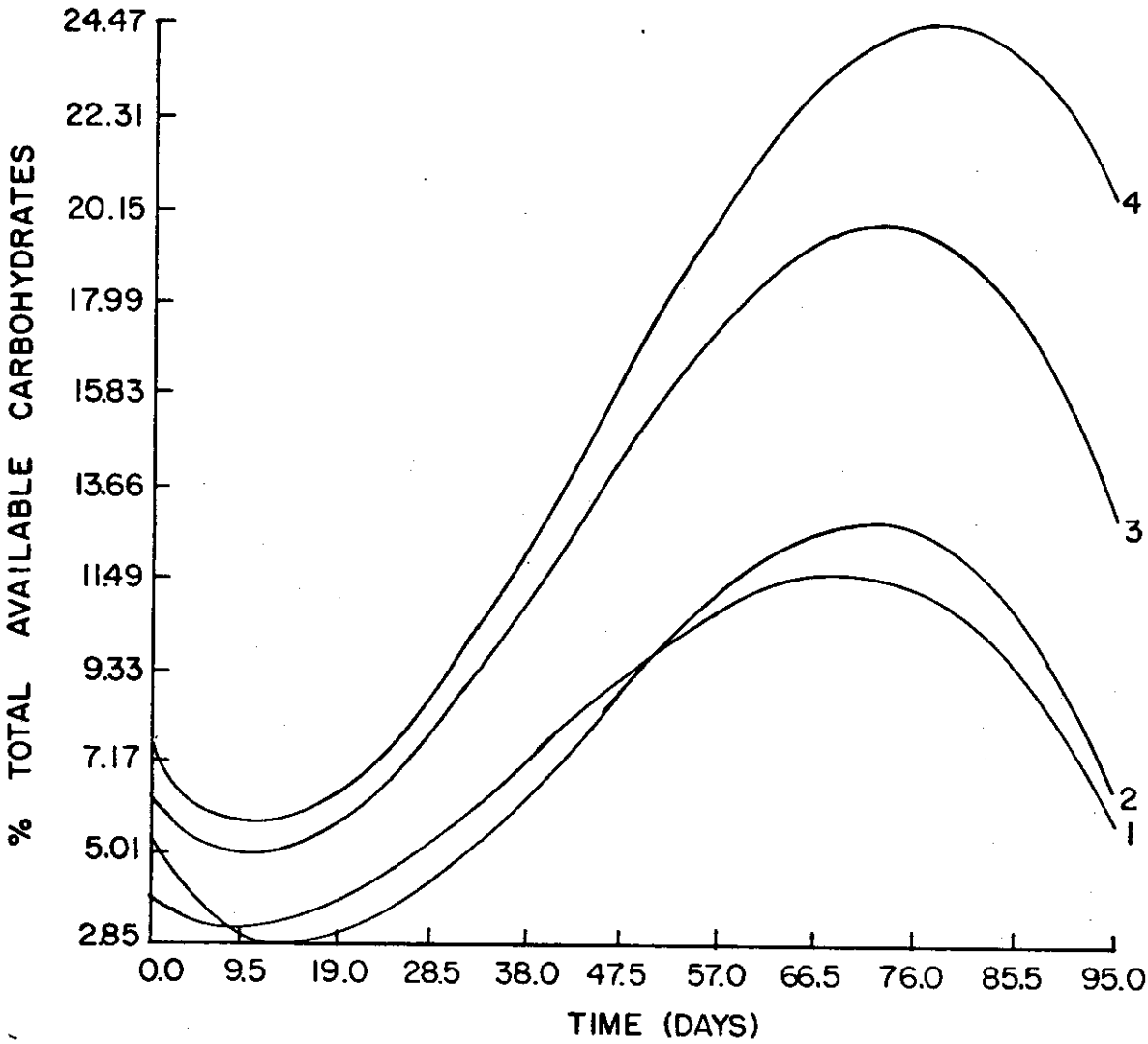

Fig. 15. Cubic polynomial regressions for %TAC vs. time at 29°/18°C.

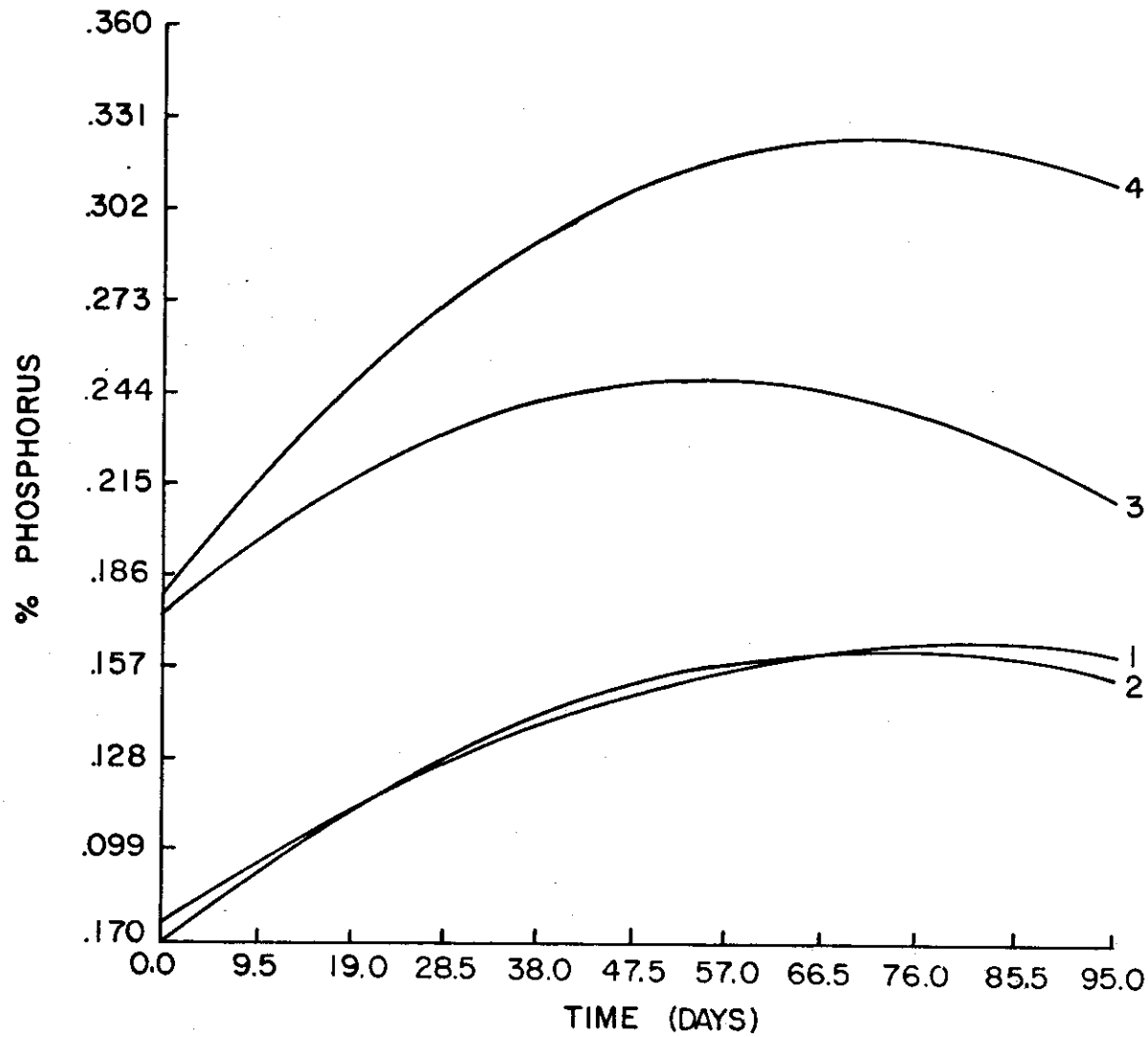

Fig. 16. Quadratic polynomial regressions for %P vs. time at 13°/7°C.

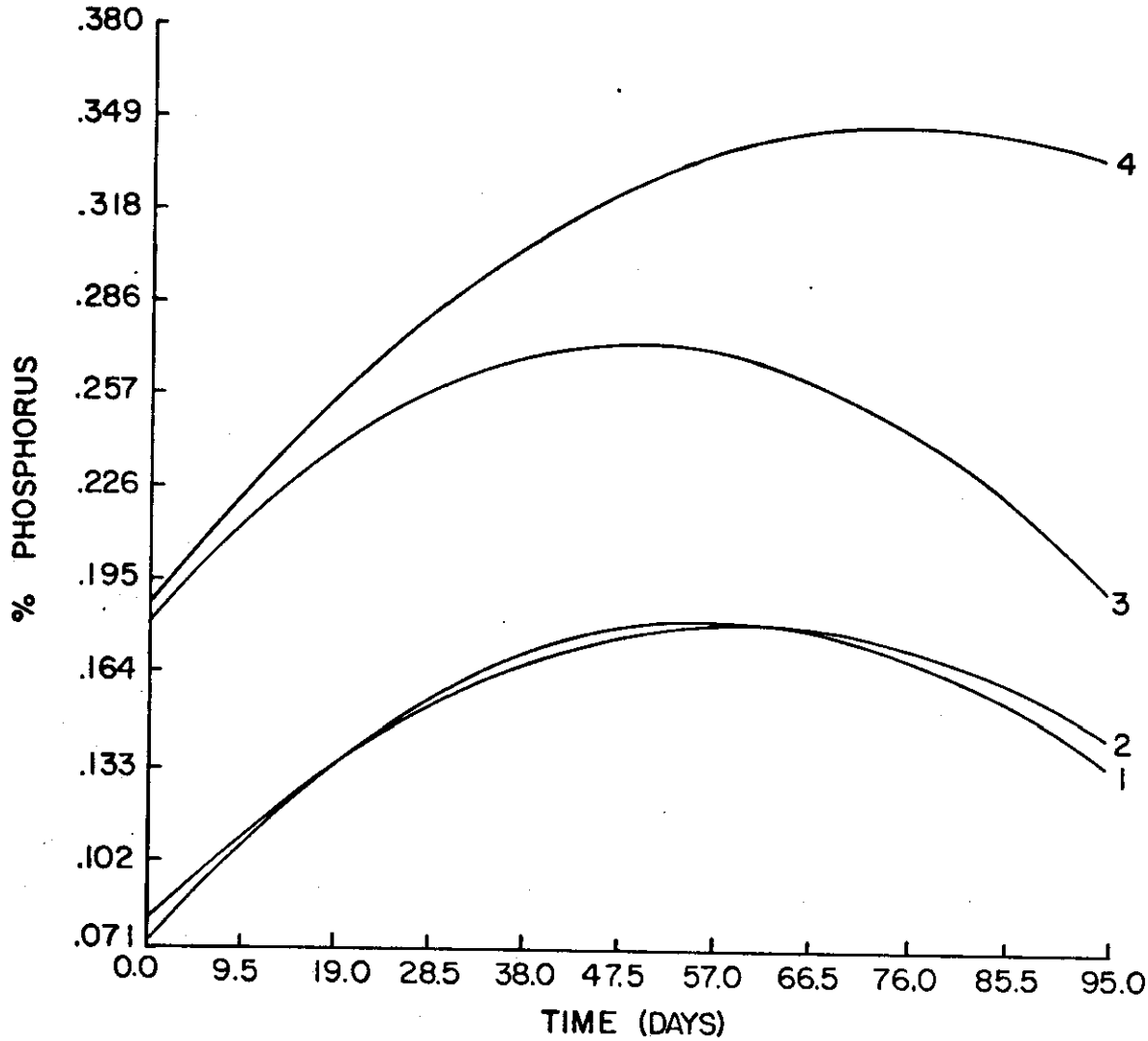

Fig. 17. Quadratic polynomial regressions for %P vs. time at 24°/13°C.

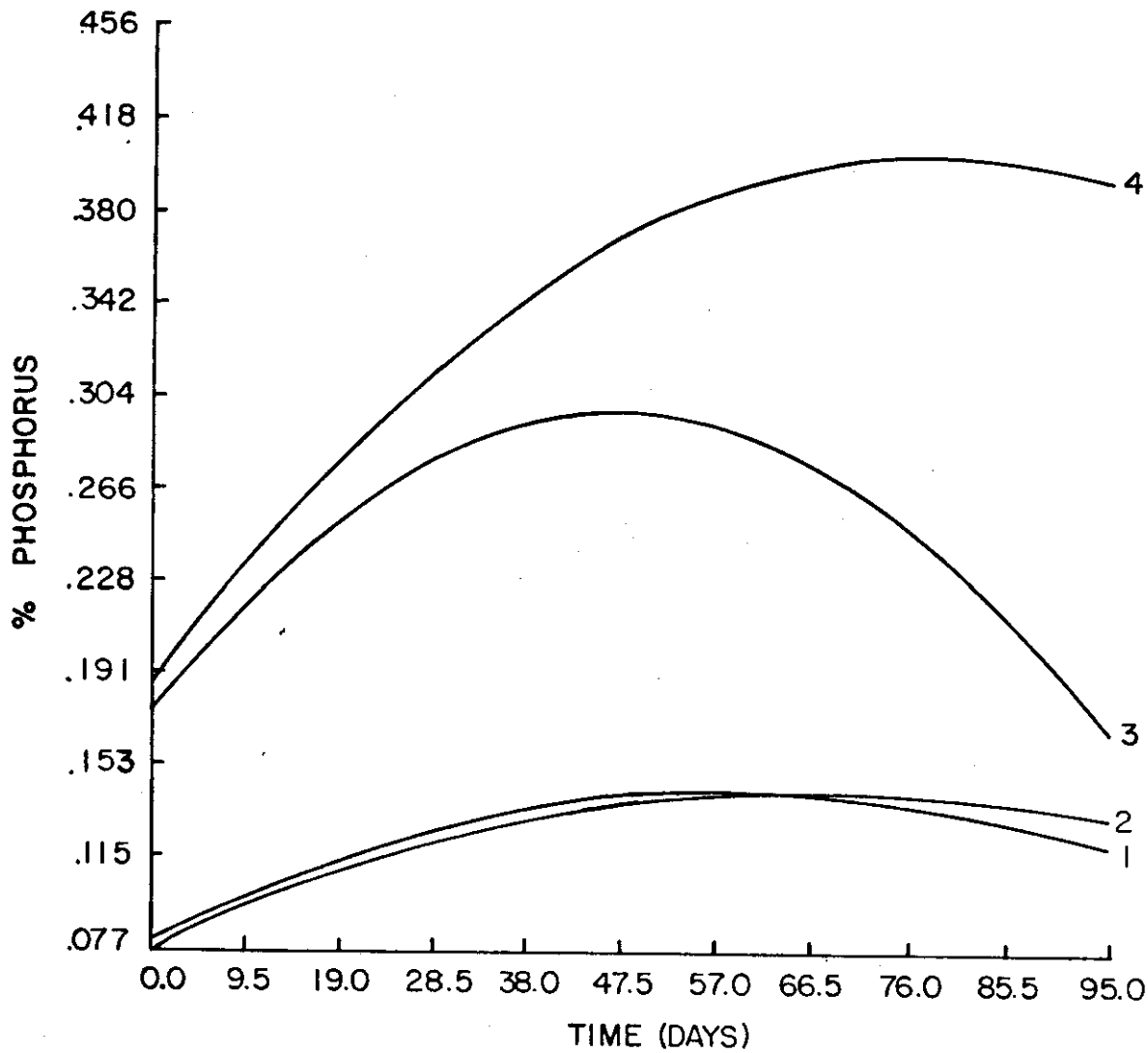

Fig. 18. Quadratic polynomial regressions for %P vs. time at 29°/18°C.

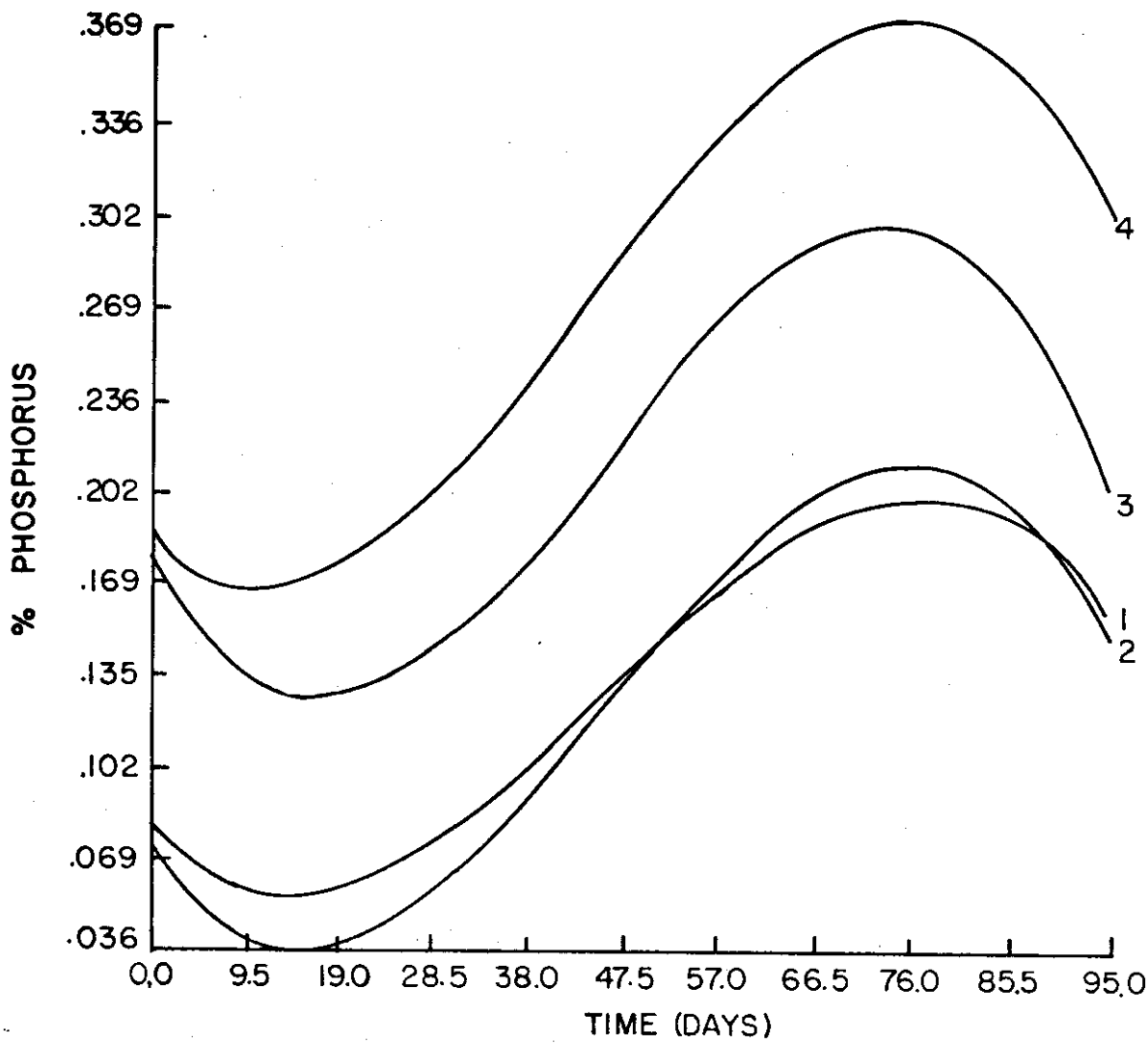

Fig. 19. Cubic polynomial regressions for %P vs. time at 13°/7°C.

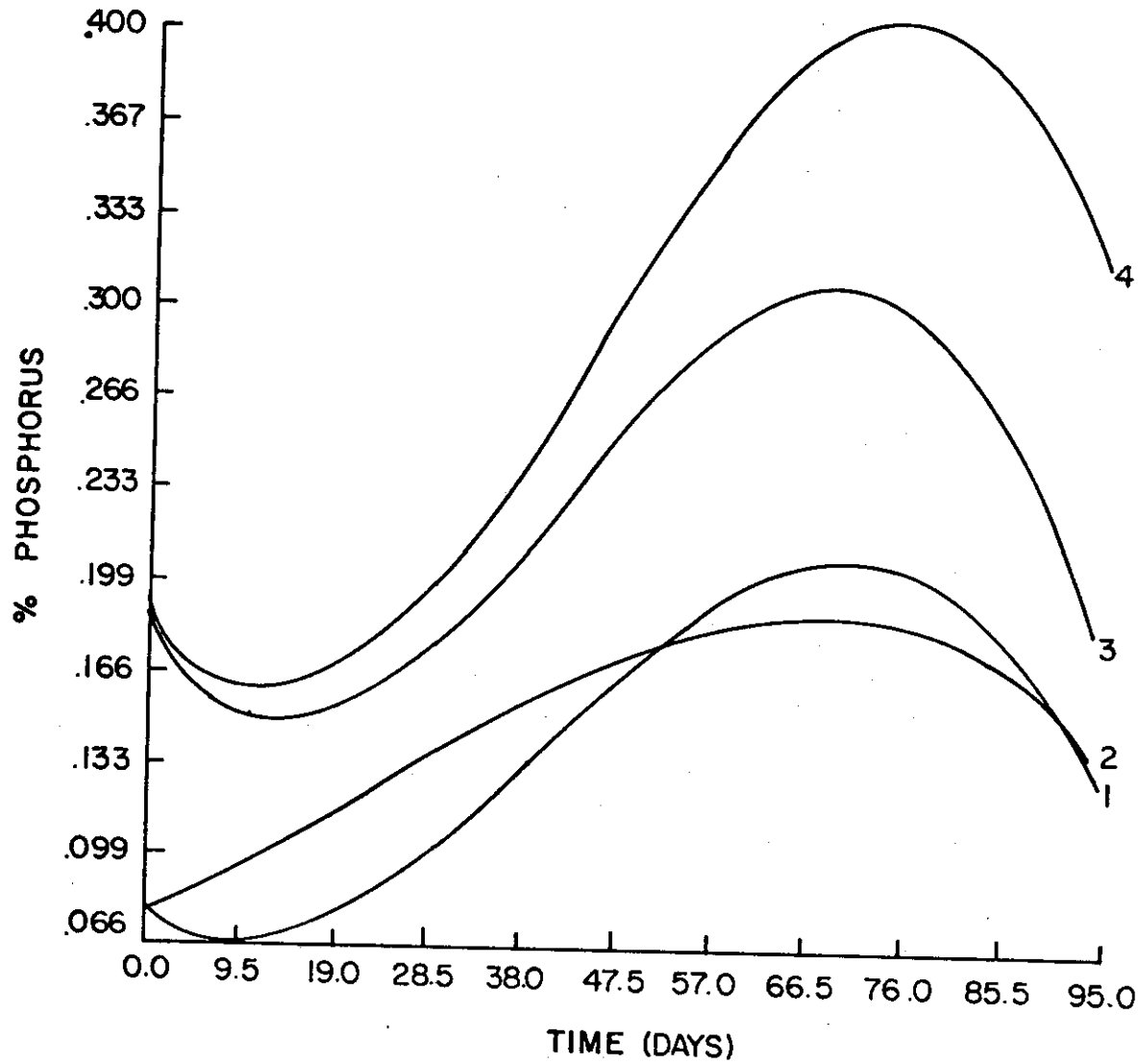

Fig. 20. Cubic polynomial regressions for %P vs. time at 24°/13°C.

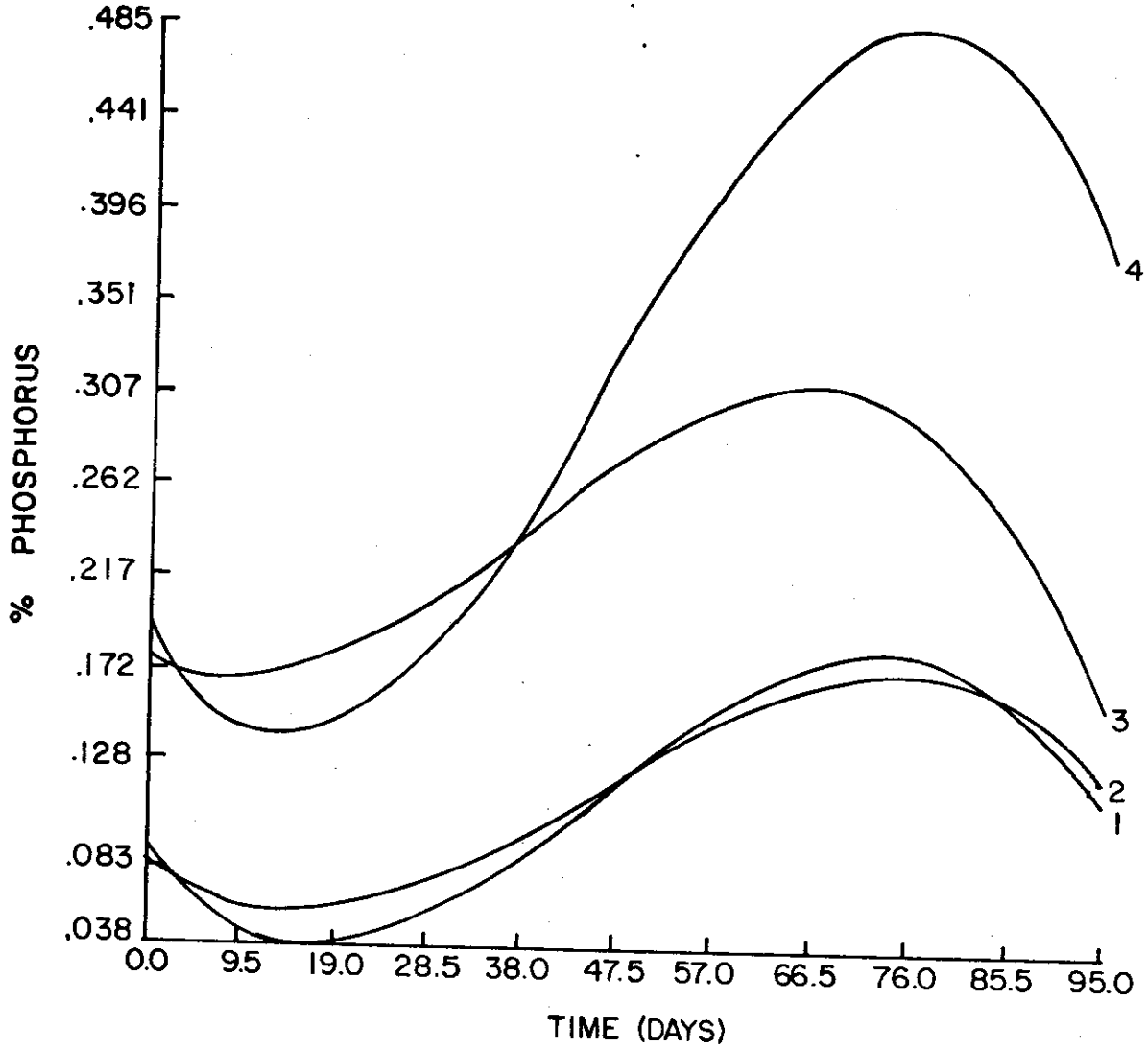

Fig. 21. Cubic polynomial regressions for %P vs. time at 85/65°F.

Table 13. A comparison of the linear and quadratic polynomial regression equations.

Temp. (°C)	Treatment	r^2 Linear	r^2 Quadratic	F_{prob} Linear	F_{prob} Quadratic	Best One
			Total yield			
13°/7°C	1	.963	.991	.00002	.00001	Quadratic
	2	.969	.969	.00001	.00017	Linear
	3	.990	.997	.00000	.00000	Linear
	4	.987	.989	.00000	.00001	Linear
24°/13°C	1	.956	.960	.00003	.00031	Linear
	2	.798	.980	.00278	.00006	Quadratic
	3	.943	.997	.00006	.00000	Quadratic
	4	.955	.997	.00003	.00000	Quadratic
29°/18°C	1	.782	.975	.00356	.00010	Quadratic
	2	.257	.986	.19985	.00002	Quadratic
	3	.872	.996	.00069	.00000	Quadratic
	4	.906	.981	.00027	.00005	Quadratic
			%TAC			
13°/7°C	1	.552	.757	.03481	.02913	Quadratic
	2	.285	.667	.17308	.06395	Quadratic
	3	.937	.944	.00008	.00074	Linear
	4	.810	.836	.00232	.01093	Linear

Table 13. (Continued).

Temp. (°C)	Treatment	r^2 Linear	r^2 Quadratic	F_{prob} Linear	F_{prob} Quadratic	Best One
			%TAC (continued)			
24°/13°C	1	.244	.719	.21293	.04192	Quadratic
	2	.086	.495	.48200	.18102	Quadratic
	3	.738	.893	.00625	.00370	Quadratic
	4	.792	.910	.00306	.00240	Quadratic
29°/18°C	1	.149	.852	.34522	.00848	Quadratic
	2	.124	.666	.39304	.06451	Quadratic
	3	.434	.848	.07567	.00908	Quadratic
	4	.810	.905	.00231	.00279	Linear
			%N			
13°/7°C	1	.653	.937	.01521	.00098	Quadratic
	2	.712	.977	.00848	.00008	Quadratic
	3	.525	.835	.04192	.01112	Quadratic
	4	.440	.668	.07272	.06342	Quadratic
24°/13°C	1	.222	.992	.23812	.00001	Quadratic
	2	.359	.983	.11663	.00004	Quadratic
	3	.416	.771	.08417	.02508	Quadratic
	4	.430	.731	.07748	.03751	Quadratic

Table 13. (Continued).

Temp. (°C)	Treatment	r^2 Linear	r^2 Quadratic	F_{prob} Linear	F_{prob} Quadratic	Best One
			%N (continued)			
29°/18°C	1	.121	.977	.39862	.00008	Quadratic
	2	.186	.996	.28540	.00000	Quadratic
	3	.348	.826	.12360	.01255	Quadratic
	4	.331	.747	.13532	.03207	Quadratic
			%P			
13°/7°C	1	.680	.804	.01183	.01704	Linear
	2	.540	.714	.03782	.04361	Linear
	3	.133	.522	.37363	.15794	Quadratic
	4	.641	.864	.01690	.00682	Quadratic
24°/13°C	1	.272	.852	.18461	.00840	Quadratic
	2	.388	.974	.09911	.00011	Quadratic
	3	.014	.685	.78360	.05565	Quadratic
	4	.653	.828	.01522	.01234	Quadratic
29°/18°C	1	.216	.522	.24636	.15794	Quadratic
	2	.484	.730	.05543	.03776	Quadratic
	3	.001	.869	.94280	.00616	Quadratic
	4	.664	.817	.01380	.01427	Linear

DISCUSSION

The energy that plants receive in the photosynthetic process is utilized several ways in plant growth. Part of this energy is needed for cell expansion and multiplication and other metabolic processes. The surplus energy accumulates as starch in warm-season grasses and most legumes, and as water soluble carbohydrates in cool-season grasses. Under conditions of low leaf area, low light intensity, or temperature above optimum for growth, the energy balance is likely to be negative, resulting in utilization of the reserve or surplus carbohydrates. At this point, it seems appropriate to discuss the importance of reserve carbohydrates, the exact role of which will be explored in subsequent studies.

Work on carbohydrate reserves is not recent, but a number of important questions relating to translocation of carbohydrates from top to roots, reserves' accumulation in the root during maturity, and finally their utilization for regrowth of root and tops, is not fully understood in grasses. The term reserve has been defined by Graber et al. (1927) as "those carbohydrates and nitrogen compounds elaborated, stored and utilized by the plant itself as food for maintenance and for the development of future top and root growth." The word reserve, according to Bernatowicz (1958), "connotes provision for the future--purposeful accumulations and signifies teleological thinking." A better choice of a term in his view is "accumulation" since it is noncommittal concerning purpose or intent.

The accumulation and ultimate utilization of reserve carbohydrates plays an important role in both pure and applied plant physiology. The determination of TAC is of greater significance in applied plant physiology

than that of individual carbohydrates or groups of carbohydrates. According to Weinmann (1948) the term "total available carbohydrates" is defined as including all those carbohydrates which can be used in the plant body as a source of energy or a building material, either directly or indirectly after having been broken down by enzymes.

The principal carbohydrate reserves in grasses are sugar fructosans, and starch. Grasses native to cool, temperate climates accumulate fructosans, while warm-climate adapted species accumulate sugars and starch as reserve carbohydrates. In higher green plants the bulk of available carbohydrate is composed of sugars, fructosans, dextrin, and starch. Cellulose and pentosan, not considered reserves, are mainly structural materials, do not exhibit frequent fluctuation in concentration, and cannot be further utilized in the same way as reserve carbohydrates. The precise role of hemicellulose is not known, but it is considered to play an intermediate role between storage organs.

Seasonal fluctuations of reserve carbohydrates in grasses are influenced by various environmental factors, e.g., nutrients, water content, and temperature. The exact role of nutrients affecting reserve carbohydrates cannot be ascertained without taking into consideration the availability of nutrients in soil and their mutual interactions, and the water potential of the soil and the roots immediately in contact with soil. Temperature and water responses are believed to be mutually synergistic. The effects of defoliation or grazing on reserve carbohydrates have been studied by many workers and can

be found in the review by May (1960). The reserve carbohydrate level in roots and tops depends upon the time and frequency of cutting, the species, and the environmental conditions.

Adequate watering and nitrogen both have stimulating effects on plant growth. Nitrogen for synthesis of protein (as enzymes, membranes) in developing plants must be supplied through the soil. If the nitrogen supply is limited, new tissue formation and growth rate is retarded. Water in the plant cell helps the structuring of protein with other chemical constituents in the cell membrane. The primary effect of soil water stress on plant growth is not well understood. Water-stressed conditions may close stomata to CO_2 entry and thus result in reduced photosynthetic activities.

In this experiment low soil water under fertilized and control conditions appears to have no appreciable effect on photosynthesis. Direct estimation of photosynthesis cannot be made from the results reported here because CO_2 uptake or release was not measured. However, some conclusions concerning metabolic energy balance in blue grama can be drawn from the growth rate and TAC data.

The increased TAC content of irrigated and fertilized plants under higher temperature is indicative of the increased photosynthetic efficiency of blue grama plants. The fact that the increase in yield or dry matter production at the end of the 95-day growing period had no dilution effects on other chemical constituents, indicates that irrigated and fertilized plants remain photosynthetically active throughout the growing period.

Untreated and fertilized plants reached senescence earlier than the irrigated or the fertilized and irrigated plants. This is evident from the decline in TAC and total nitrogen at the end of the growing season.

Protein degradation or translocation of TAC towards the maturity of plants appears to be following the expected trend usually observed under field conditions.

Fig. 1 to 3 indicate that blue grama plants do well under all temperatures to which the plants were exposed during the 95-day growing period. However, the response of plants to 13°/7°C day/night temperature is consistently linear under the four treatments, indicating that optimal growth of blue grama can be obtained at lower temperatures. Plants that received nitrogen plus watering as well as those that received only watering, showed higher yield than those of fertilized and control plants.

It is apparent that under the conditions of this experiment, water and nutrients are limiting factors for growth under natural conditions. This fact can be verified by the growth of control plants which resulted in lower yield as compared to the fertilized plus irrigated or the irrigated plants, especially under the low temperature. At the next two higher temperature regimes (24°/13°C, 29°/18°C), growth in terms of yield drops toward the end of this experiment. Added nitrogen appears to be increasing not only the aboveground biomass but also other related chemical constituents (Fig. 1 to 21).

The TAC, nitrogen, and phosphorus contents of plants given fertilizer and water treatment as well as those given only water treatment follow the same trend as that of yield (growth rate) obtained at various developmental stages. The slow response, in terms of TAC, nitrogen, and phosphorus, of plants during the early growth period appears to be a result of preconditioning under the changed environmental conditons in the growth chambers.

Plants subjected to growth chamber conditions had higher contents of TAC, total nitrogen, and phosphorus at the start of the experiment. This may be a result of carry-over effect from the field where similar treatment had been given to these plants. At low temperatures, there is a steady increase in TAC, total nitrogen, and phosphorus contents of all the plants, while at higher temperatures a decreasing tendency develops toward the end of the growing season. This may be attributed to either rapid translocation of photosynthates to the roots or degradation or dilution effect. It will be interesting to investigate the turnover rate of these growth-related chemical constituents in the top as well as in the roots at various developmental stages.

Light intensity and CO_2 does not seem to be limiting under all temperature regimes. Under low temperature, the added nitrogen and water seems to impart higher resistance to these temperature conditions and at the same time to increase the photosynthetic activities of plants. The linear response of plants under low temperature in terms of TAC contents suggests that an adequate supply of nitrogen and water maintains a steady rate of photosynthesis, and thus may be prolonging the growing season of these plants.

The effect of added nitrogen to the soil on the rate of photosynthesis appears to be a result of new growth (greater leaf area).

Water-stressed plants under fertilized and control conditions (Fig. 1 to 21) responded poorly under all temperature regimes; however, these plants were able to maintain a steady rate of growth throughout the growing season.

Under low temperature, the rate of translocation of photosynthates from the source (leaves) to the immediate sink (phloem cells in the leaves) may

be fast; however, the export rate to the remote sink (root) may be slow. Under high temperature and low light intensity, most of the reserve carbohydrates are utilized to provide energy for the various growth processes. In this experiment, under high temperature (29°/18°C), the light intensity does not seem to limit needed energy for growth. There seems to be an inverse relationship between growth and reserve carbohydrates (Fig. 1 to 21), which in perennial grasses reflects the metabolic energy balance. These grasses may be storing enough surplus energy (in the form of reserve carbohydrates) for new growth after defoliation when plants have limited leaf area for photosynthesis. Limitation of new growth and subsequent growth is dependent upon the balance between the energy needs of plants for growth and the supply through photosynthesis.

Under low temperature, growth and TAC both increase (Fig. 1 to 21), indicating higher photosynthetic and other metabolic activity, which provides energy for growth. In this experiment, it appears that adequate energy through respiration is provided for growth (protein synthesis, new tissue formation) under all temperature regimes. These results suggest that at the end of the growing season or when the plants are close to senescence, there may be transformation or restructuring of different quality of protein in lesser or greater proportions, which might provide an altogether different effect on consumers compared to the influence exerted earlier in the growing season. This supposition can be evaluated by fractionating different types of protein by means of gel electrophoresis at different stages of growth. The flow diagram (Fig. 22) shows the flow of energy (in terms of

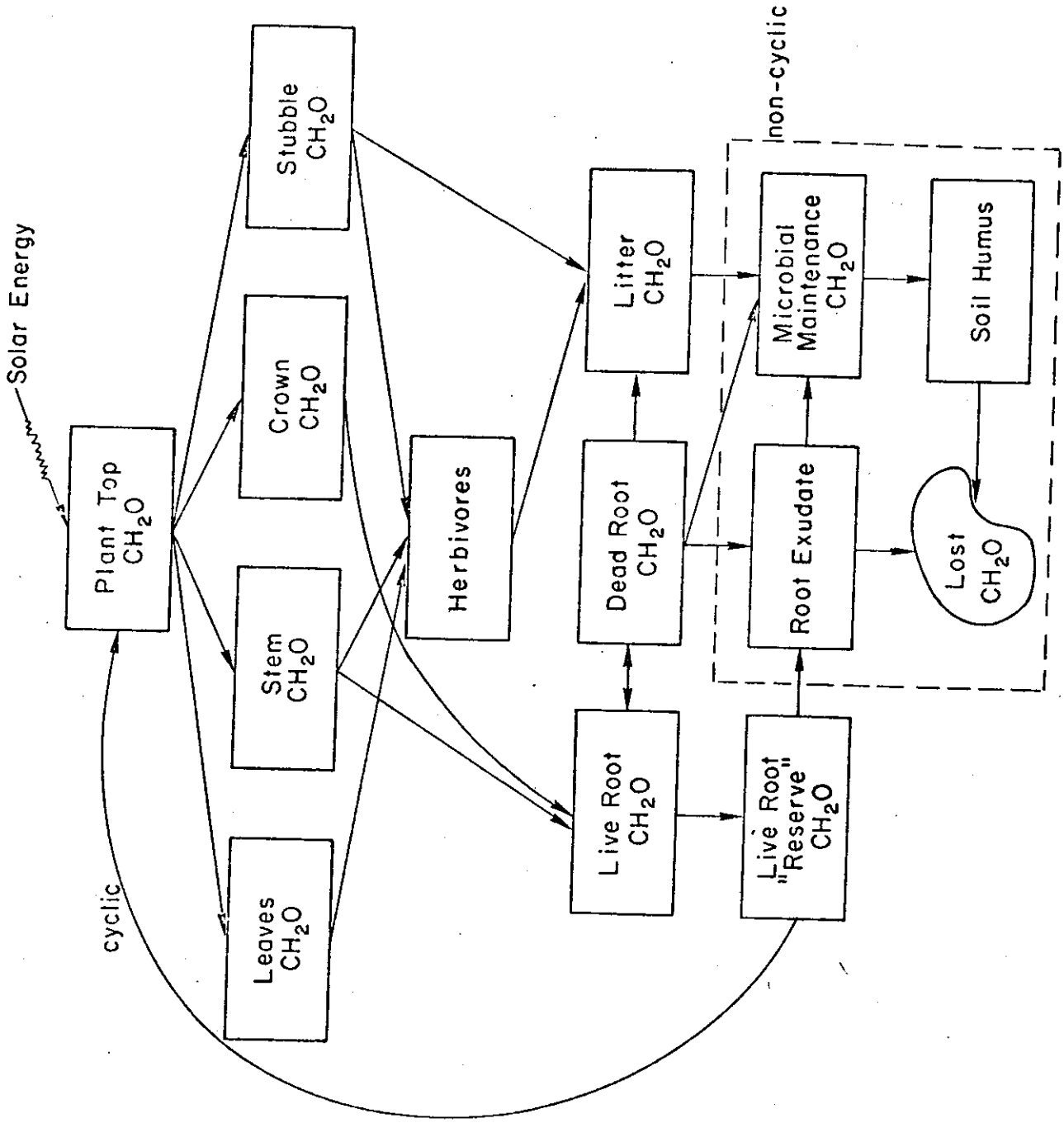

Fig. 22. Flow diagram of plant growth relationships for blue grama grassland ecosystem.

carbon or energy equivalents) which will be discussed in detail during later studies. At this point, the data available are not adequate to cover the whole model. Studies currently undertaken are aimed at assessing the flow of energy from tops to roots and to other consumers. This will be done by first estimating the solar energy (or its equivalent) harvested by the green plants, the portion of this energy utilized for internal maintenance of plants, and the remaining translocated to roots in the form of reserve carbohydrates. That portion of energy harvested by herbivores will be determined by clipping intensity and frequency of clipping. Generally energy flow is noncyclic in any ecosystem; however, part of the energy stored in roots as reserve carbohydrates can be recalled during spring for new top and root growth. This can be termed the "cyclic flow of energy" because all of this energy is not lost to the system in an ecological sense.

These preliminary results suggest several possibilities of exploring further questions:

1. How rapidly do plants respond to irrigation and fertilization in terms of photosynthate turnover rate in time?
2. Do various temperature regimes in presence of adequate water affect the rate of photosynthesis, such as CO_2 assimilation rate through stomata, or stomata opening, or both?
3. How do these plants accumulate "reserve carbohydrates" under temperature and water-stressed conditions?
4. What is the effect of these temperatures on translocation of photosynthate into the roots?

5. What is the effect of water-stressed conditions on the basic plant building materials, such as RNA and protein synthesis, at various developmental stages?

Work in progress is aimed at the effect of various temperature regimes on translocation of carbohydrates and on the changes in carbohydrate reserves throughout the growing season, under various water stresses and various soil temperature conditions.

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APPENDIX I

MODIFIED METHOD FOR TAC DETERMINATION

Extraction of total available carbohydrates (TAC) from plant materials.

1. Dry plant tissue at 100°C for 1 hr, complete drying at 70°C to constant weight.
2. Grind tissue to pass 20-mesh sieve.
3. Place 0.500 g samples in 200-ml round bottom flask. Add 50 ml of 0.2 N H_2SO_4 and attach the flask to a reflux condenser, and reflux for 1 hr in a boiling water bath, during which time the carbohydrates are hydrolyzed.
4. Filter the hot solution through Whatman's no. 42 filter paper.
5. Cool the filtrate, add phenolpthalein, and add 25% NaOH until the indicator end point is reached.
6. Remove the indicator color by making the solution slightly acid with a few drops of 5% HCl.
7. Dilute the filtrate to 250 ml or 100 ml with distilled water.
8. Take an aliquot for glucose analysis using Schaffer-Somogyi method as described by Heinze and Murneek (1940).

Analysis for reducing power.

1. Add 10 ml of Reagent "50" to an aliquot of the unknown solution (up to 15 ml containing 1.0 to 3.0 mg sugar) to a 25 × 200 mm test tube. Mix thoroughly and cover with a marble.
2. Test also Reagent "50" blank, and a monosaccharide standard solution blank.

3. Heat tubes for 15 min in a boiling water bath with test tubes held firmly to avoid undue agitation.
4. Cool test tubes in a cool water bath to less than 30°C.
5. Add 2 ml potassium iodide-potassium oxalate solution to each tube, followed with 10 ml 1.0 N H_2SO_4.
6. Rotate tubes to dissolve the cuprous oxide.
7. Titrate with 0.02 N sodium thiosulfate using about 0.25 ml gelatinized starch solution as the indicator.

Solutions for reducing power test.

1. Reagent "50"--Dissolve 25 g anhydrous sodium carbonate and 25 g sodium potassium tartrate (Rochelle Salt) in about 600 ml of distilled water. Add 75 ml of a 10% copper sulfate ($CuSO_4$ 0.5 H_2O) solution with a pipette extended below the surface of the liquid. Next add 20 g sodium bicarbonate, 1 g potassium iodide, and 200 ml potassium iodate solution containing 3.567 g (exactly) of pure KIO_3 per liter. Mix thoroughly, rinse into a liter volumetric flask, and bring to 1 liter volume with distilled water.
   Reagent "50" should be carefully prepared with high quality chemicals. The solution will remain unchanged for months if kept in a stoppered, colored Pyrex bottle and protected from strong light. The reagent's iodate content may be varied according to the amount of sugar to be determined. If reagents are made as directed, any quantity less than 4.40 mg of glucose or fructose per 10 ml of solution may be determined.

2. Potassium iodide-potassium oxalate solution--Dissolve 2.5 g of each together in 100 ml of distilled water. Deterioration products may be avoided by making a new solution each week and storing it in a colored bottle in a refrigerator. The presence of free iodine will cause considerable error in carbohydrate determinations.
3. 1.0 N sulfuric acid--Pour 27 ml concentrated H_2SO_4 into a quantity of distilled water and dilute to 1 liter volume. The resulting solution is normal.
4. Starch indicator--Stir ca. 1 g of soluble starch into 10 to 15 ml cold distilled water. Heat 100 ml distilled water to boiling and add 1 g boric acid crystals. Add starch solution, allow to boil for about 1 min, and cool slowly. Store in a refrigerator.
5. 0.02 N sodium thiosulfate--Prepare and approximate 0.1 N stock solution by dissolving 25 g pure sodium thiosulfate and 1 g NaOH in distilled water and diluting to 1 liter. Allow this stock solution to stand several hours before making the 0.02 N solution. Store stock solution in a stoppered colored bottle protected from strong light.

   Make the 0.02 N titration solution by diluting 100 ml stock solution to 500 ml. Make the diluted solution (ca. 0.02 N) every few days because it does not maintain its stability as long as the stronger stock solution.

   The 0.1 N thiosulfate stock solution can be readily standardized with 0.1 N $K_2Cr_2O_7$. To prepare the latter solution, dry pure $K_2Cr_2O_7$ at 110°C: dissolve 4.9033 g in distilled water and dilute

to 1 liter. Transfer 25 ml standard dichromate to a large beaker containing 3 g potassium iodide and dilute to 500 to 600 ml with distilled water. Add 10 ml of concentrated HCl and titrate immediately with the thiosulfate solution. Add the starch indicator near the end of the titration. The solution turns from blue to light green at the end point. If the thiosulfate used was pure, the titration will be a little less than 25 ml. The thiosulfate stock solution can be adjusted to 0.1 N by diluting with water. If the titration was 24.7 ml of thiosulfate, add 0.3 ml water to every 24.7 ml of thiosulfate or add $975.3/24.7 \times 0.3 = 11.8$ ml of water to 975.3 ml of the remaining thiosulfate solution. The resulting solution should be 0.1 N and can be readily checked by titrating against 0.1 N dichromate.

6. Sugar standard (1 mg/2 ml)--Dry ASC-grade glucose or fructose in a petri dish over P_2O_5 in a desiccator. Carefully weigh 1 g of the sugar and transfer to a clean 1-liter volumetric flask. Fill the flask to volume with a saturated solution of fenzoic acid. Store in a refrigerator and remake at least every 6 months.

Calculation:

$$\text{mg glucose in sample} = \frac{\text{standard glucose (mg)} \times \text{blank - sample titration (ml)}}{\text{blank (ml) - (glucose standard (ml))}}$$

$$\%\text{TAC} = \frac{\text{mg glucose in sample} \times \text{dilution factor} \times 100}{\text{sample weight}}$$