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RESEARCH PROGRAM
United States-Egypt-Israel

FODDER PRODUCTION AND ITS UTILIZATION BY SMALL RUMINANTS IN ARID REGIONS (FOPAR)

Semi-Annual Report
September 1989-February 1990

A. DOVRAT, Coordinator

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TABLE OF CONTENTS

Page

Summary ..... *iii*

Examination of the Double-sampling Technique in the Estimation of Fodder Shrub Biomass. E.D. Ungar and A. Genizi. 1

The Edible Biomass and Regrowth of Three Species of Fodder Shrubs Following Complete Defoliation by Grazing. R.W. Benjamin, D. Barkai, Y. Hefetz and M. Forti 22

Effect of Inoculation with *Azospirillum brasilense* Strain CD and *Rhizobium* on the Root Morphology of Burr Medic (*Medicago polymorpha* L.). E. Yahalom, A. Dovrat, Y. Oron and H. Czosnek 62

SUMMARY

In this semi-annual report for the CALAR/FOPAR project, emphasis has been placed on evaluating the regrowth and recovery following complete defoliation by grazing of the fodder shrubs, *Atriplex nummularia*, *Atriplex canescens* and *Cassia sturtii*. These shrubs were planted at November 1984 at five planting densities, 10,000, 4,400, 2500, 1,100 and 625 shrubs per hectare, and left undisturbed until July 1987. From July 1987, to December 1989, the shrubs were grazed until complete defoliation in each year, between July and December. Following each grazing period, the shrubs were left to recover undisturbed. Measurements of the dimensions and estimates of the total standing biomass of the shrubs were made before and after each grazing period. In addition, the the stem and leaf biomass components of the total biomass were estimated.

Because the quantification of the recovery of the shrubs was based on the estimates of standing biomass before and after grazing, a statistical analyses were made of the accuracy of the double sampling technique used to estimate biomass parameters during this project. In all, 39 sets of calibration data made during the project were analysed and the results and preliminary conclusions are reported.

In addition, a final report is presented on the effect of inoculation with *Azopirillum* and *Rhizobium* and root morphology of *Medicago polymorpha* summarizing all the experimentation carried out during the project.

EXAMINATION OF THE DOUBLE-SAMPLING TECHNIQUE IN THE ESTIMATION OF FODDER SHRUB BIOMASS

E.D. Ungar and A. Genizi

ABSTRACT

The standing biomass of fodder shrubs have been estimated at the Migda Dryland Experimental by a double sampling technique during the eight years of the present project. During this time, over 40 sets of calibration data, have been made, 39 of which have been subjected to analyses to determine the most suitable form of the regression equation to be used to estimate the standing biomass of fodder shrubs. One conclusion was that height (H) and diameter (W), when expressed together as vertical profile (HW) or volume (HW^2), contribute strongly, but that subjective parameters (based on eye appraisal) contribute little to the correlation coefficient

Key words: *Atriplex canescens, Atriplex nummularia, Cassia sturtii, Atriplex linearis* and *Acacia victoriae* , planting density, double-sampling technique, height, diameter, vertical profile volume, correlation coefficient

INTRODUCTION

Standing biomass of fodder shrubs is estimated at Migda by a double-sampling technique. Various parameters which correlate with biomass (total, leaf or branch) are measured for a large number of plants ("survey plants"). A small subset of approximately 20 plants are cut, dried and weighed ("calibration plants"). The biomass measurements of the cut plants are regressed against the corresponding parameters measured in the field for the same plants. The regression equation is used to convert these parameters to biomass estimates for all the survey plants.

An earlier report on the estimation of the biomass of fodder shrubs by this method analysed 39 calibration data sets. One conclusion was that height (H) and diameter (W), when expressed together as vertical profile (HW) or volume (HW^2), contribute most strongly to the correlation coefficient.

Investigations into the form of regressions and the variables to be used were continued, and results are presented in this report. The question of whether a new calibration is necessary for each estimation process when previous data sets exist, is

addressed in detail. These analyses gave rise to a simple field investigation of certain aspects of the methodology. This experiment is also reported here.

THE COLLECTIVE ANALYSIS OF MANY CALIBRATION DATA SETS

Materials and Methods

Data sets from three different fields and five species were analyzed. Fields 5 and 7 were planted in November 1983. Each field was divided into two equal sub-plots for grazing in different seasons. In the area grazed in the winter (designated "Winter"), *Atriplex nummularia, Cassia sturtii* and *Acacia victoriae* were planted at the rate of 2000 seedlings ha^{-1} on 60, 30 and 10% of the area, respectively. In the area grazed in the summer (designated "Summer"), *Atriplex canescens, Atriplex nummularia, Cassia sturtii, Atriplex linearis* and *Acacia victoriae* were planted at the same density on 40, 20, 20, 10 and 10% of the area, respectively.

Field 14 was planted in November 1984. The field was divided into three equal sub-plots, one for each of the species *Atriplex canescens, Atriplex nummularia,* and *Cassia sturtii* . Each species was planted at five densities: 625, 1111, 2500, 4444, and 10000 shrubs ha^{-1}. Grazing history did not differ between sub-plots.

At each estimation a survey row of plants was randomly selected, and up to six variables were measured for each plant. Height (H) and diameter (W) were measured to the nearest 5 cm. The total DM index (I) is a visual estimation (0 to 5) of total biomass relative to a randomly selected reference shrub in the same row. The other estimates are total biomass density (Dt), leaf biomass density (Dl), and fruit biomass density (Df), each on a scale of 0 to 10. These three estimates are based on the fraction of the plant background obscured by the vertical profile of the plant.

Twenty shrubs from the surveyed row were harvested according to the relative frequency of non-zero I values in the row. The shrubs were dried, and total, leaf (plus fruit), and branch DM were each measured. These weights and the corresponding variables measured in the field for these plants make up a single calibration data set.

Results

<u>Form of regressions for individual calibration data sets</u>

The variance of shrub Total Dry Weight (V) or Leaf Dry Weight (L) is not stable but increases with any of the variables Diameter (W), Height (H), or HW^2. This may be overcome either by transforming V or by using weighted regressions. The latter was

used so that the overall V in a given field could be directly estimated. From a screening of all data it was estimated that the variance of V and L is proportional to HW, and all further regressions were weighted accordingly by weights of $(HW)^{-1}$.

Stepwise (weighted) regressions showed that for most experiments simple regressions of V on HW^2 and of L on DtHW (where Dt is Total Biomass Density Score) is sufficient with R^2~0.9. Regression coefficients for all the calibration data sets analysed are given in Table 1 for V and in Table 2 for L. No real improvement was achieved by adding further variables, except for *A. victoriae*, where regression of V on HW^2 and DtHW significantly increases R^2 from 0.88 to 0.95.

Analyses of variance showed that the slopes of separate regression lines, for V and L, of the different calibration sets in each species were significantly different, except for *A. linearis* in both regressions and *A. canescens* in V. These differences were usually small in magnitude. They could not be explained by grazing history alone.

Historical versus current calibration data

The purpose of this analysis was to estimate if a separate double sampling for each V (and L) determination is superior to using the regression equation obtained from all previous determinations in the species, and if so, by how much. In the latter case, only the non-destructive estimates on the survey plants would be required for each biomass determination.

The following procedure was used. The calibration sets of ~20 shrubs were divided into an 'estimation set' of 13 shrubs ($S_{k,13}$) and a 'validation set' of 7 shrubs ($S_{k,7}$). Division of the data sets was by the 'DUPLEX algorithm' method which ensures that each set will represent approximately the same range of HW^2. Regression equations were calculated from the estimation set $S_{k,13}$, and from the remainder of the data for the given species $S_{\neq k}$, i.e. excluding all of the k-th calibration set. The residuals of the predicted from the observed mean values of the validation set $S_{k,7}$ were calculated for these two regressions. Residuals were also calculated for these two regressions using the complete set S_k of 20 shrubs. The 'relative residuals' were also calculated, presenting each residual as a percentage of the observed mean. Using the regression based upon $S_{k,13}$ to estimate the mean for all shrubs in S_k is an analytic simulation of the double-sampling procedure used in the field by which a subsample of 20 shrubs is selected from a larger sample of shrubs for which V and L are not measured.

In almost all comparisons, the absolute or relative residual using the regression based upon $S_{\neq k}$ was considerably greater than the residual using the regression based upon

$S_{k,13}$. This result was obtained for residuals computed from the observed mean values of the validation set $S_{k,7}$ or from the complete set S_k of 20 shrubs. This indicates that the accuracy of estimation of shrub biomass would be significantly reduced by not using the double-sampling technique.

A DETAILED EXAMINATION OF THE DOUBLE -SAMPLING TECHNIQUE

Objectives

An experiment was conducted to investigate the following aspects of the double-sampling technique:

a) to compare survey data and calibration curves from different estimators;

b) to compare survey data and calibration curves for different rows in the same field;

c) to compare survey data for the same plants and estimator over a short time period;

d) to compare survey data and calibration curves from the winter and summer grazing sections of a field on the same date.

Materials and methods

Four sets of survey data were collected from Field 5 on 1 March, 1989. A calibration set was clipped from each survey set during the same month. All data was for *Atriplex nummularia*.

Set 1: Field 5, Winter sub-plot, Row 13 (76 live plants out of 82 plant positions). Surveyed by 4 estimators (Y, E, R, N) on 1.3.89. Calibration set of 19 plants cut on 7.3.89.

Set 2: Field 5, Winter sub-plot, Row 35 (81 live plants out of 82 plant positions). Surveyed by 3 estimators (Y, R, N) on 1.3.89. Calibration set of 20 plants cut on 12.3.89.

Set 3: Field 5, Summer sub-plot, Rows 11 & 12 (91 live plants out of 146 plant positions). Surveyed by one estimator (Y) on 1.3.89. Calibration set of 21 plants cut on 19.3.89.

Set 4: Field 5, Summer sub-plot, Rows 43 & 44 (123 live plants out of 148 plant positions). Surveyed by one estimator (Y1) on 1.3.89. Calibration set of 19 plants cut on 27.3.89. Survey of rows 43 & 44 repeated by same estimator (Y2) on 27.3.89.

The estimators represented the entire range of experience in gathering such data. Estimator Y was the most experienced estimator, and has conducted almost all shrub surveys carried out at Migda. Estimators R and N are well and moderately experienced estimators, respectively. Estimator E had never carried out such a survey before. Estimator Y explained and demonstrated to the other estimators exactly how each measurement was taken prior to the start of the experiment. This was of importance, since not even height and diameter are completely objective measurements according to the procedural definition in use since the start of the project.

Results and discussion

Table 3 shows the means of all relevant variables for the four survey and calibration data sets. Differences between estimators within sets 1 and 2 were very large for the most extreme comparisons. Mean shrub volume of estimators E and Y for set 1 was 73 and 40% greater than that for estimator R, respectively. Variation between estimators was smaller in set 2. The same estimator yielded a reduction of 6 cm in mean shrub diameter for set 4 when the same shrubs were re-surveyed after 27 days. Total and leaf biomass densities showed increases over the same period. Since only one calibration data set was clipped from this set of shrubs (on the latter date), it cannot be determined whether these changes were due to growth processes or inconsistencies of the estimator.

Differences of approximately 35% in mean dry weight per shrub were obtained between rows of the same sub-plot. A statistical analysis of these differences is not yet available.

Comparison of survey data

Mean shrub height and diameter for Set 1 ranged from 85 to 95 cm and 63 to 78 cm, respectively. The range in mean height and diameter for Set 2 was much less. This may be due to the fact that estimator E, who yielded extreme values for Set 1, did not participate in the survey of Set 2 plants. There was a large range in means for Total biomass density in Set 1, and for Leaf biomass density in Set 2. Relative to the mean shrub volume for estimator Y, estimates of 1.23, 0.71 and 0.97 were obtained for Set1, and 1.04 and 1.03 for Set 2.

The degree of subjectiveness in the definition of height and diameter is clear from the differences between estimators on an individual plant basis. The following results are based upon Set 1. Relative to estimator Y, measurements of height by estimator E

differed by -15 to +25 cm. For estimators R and N (who worked as a pair in the field) it was clear that the convention for measuring the height and diameter of shrubs growing at an unusual angle to the ground had not been explained. Thus a number of H and W measurements took opposite values to those for estimators Y and E. Excluding these exceptional deviations, measurements of height by estimators R and N differed by -25 to +15 cm and -25 to +30 cm, respectively, relative to estimator Y. On average, 50% of height estimates were within 5 cm of the value given by estimator Y.

For diameter, the range in deviation relative to estimator Y was -25 to +40 cm for estimator E, -40 to +10 cm for estimator R, and -30 to +15 cm for estimator N. On average, 47% of diameter estimates were within 5 cm of the value given by estimator Y.

Estimates of Total biomass density differed by -3 to +7 units between estimators E, R and N relative to estimator Y. On average, 38% of estimates were equal to the value given by estimator Y.

Estimates of Leaf biomass density differed by -3 to +6 units between estimators E, R and N relative to estimator Y. On average, 33% of estimates were equal to the value given by estimator Y.

Within each estimator, Total biomass density and Leaf biomass density were identical or very close for the majority of shrubs. Estimator Y gave the same value for 66% of shrubs, and 96% of deviations were between -1 and +1 units.

The same analysis for Set 2 in which measurements of estimators Y, R and N were compared, yielded qualitatively similar results.

Figures 1 and 2 show shrub volume (HW^2) for each estimator plotted against the corresponding value for estimator Y, for data sets 1 and 2. Estimator R yielded a consistent underestimation of shrub volume relative to estimator Y in set 1, but yielded a more diffuse scatter about the 45° line in set 2. Estimator N yielded a good degree of correspondence in both sets.

Comparison of calibration data

Table 4 shows the regression equations obtained using total biomass per shrub (V, grams) and leaf biomass per shrub (L, grams) as dependent variables, and the corresponding survey variables for the same shrubs as determined by each estimator to compute the independent variable. A weighting of 1/(HW) was used in all

regression analyses. These relationships are plotted together with the calibration data points in Figures 3 to 10. The correlation coefficients for the summer sub-plot were lower than those for the winter sub-plot.

Various tests for uniformity of slopes and intercepts were carried out for the above regression analyses, as summarised in Table 5. All comparisons carried out for leaf biomass as the dependent variable yield non-significant differences for both slopes and intercepts. Comparisons for total shrub biomass as dependent variable yielded less consistent results. Different estimators produced significantly different regression lines for set 1 but not for set 2. A comparison of regression lines for sets 1 and 2 for the same estimator yielded every possible outcome depending on the estimator.

Comparison of field means

The final step in the analysis is to apply a regression equation obtained from a calibration data set to a set of survey data and obtain the estimated mean biomass for the field. When simple linear regression is used, the regression equation can be applied directly to the mean value of the independent variable (HW^2 or HWDt) for the survey data set. Table 6 shows the estimated mean total and leaf biomass per shrub for all combinations of regression equation and survey data. A statistical analysis of these means is not yet available. Nevertheless, a number of points emerge from an examination of the means only:

1. Despite large differences in the estimation of inidividual plants, different estimators yielded similar means for both total and leaf biomass per shrub when comparing within sets and for corresponding survey and calibration data sets.

2. The ratio of mean total biomass per shrub in set 2 to that of set 1, for the same estimator, ranged from 1.18 to 1.41. The ratio of mean leaf biomass per shrub in set 2 to that of set 1, for the same estimator, ranged from 1.08 to 1.20. The ratio of total and leaf biomass per shrub in set 4 to that of set 3 was 1.27 and 1.50, respectively. These results seem to indicate a high degree of heterogeneity between rows of plants within the same sub-plot and of the same grazing history. If these differences prove to be statistically significant, appropriate changes in methodology should be considered.

3. The application of a regression equation from a different set (same or different estimator; i.e. comparing values across the rows in Table 6) can result in very different or almost identical means. Even where the same estimator, sub-plot and date of measurement are taken, and where the regression equations for the different rows within the sub-plot do not differ significantly, a difference of 24% can be obtained in the estimated mean total biomass per shrub. (Based on application of regression equations for estimator N from set 1 and set 2 to set 1 or set 2 survey data.)

Table 1. Results of weighted simple linear regression analyses for total biomass per shrub (V, grams) for all calibration sets. H=height (cm), W=diameter (cm).

Field	Species	Date	Data set ID	Mean V	Intercept	Slope
5 Summer	A. Canescens	Jan 85	26	106	9	0.001147
		May 85	23	553	28	0.001134
		Jun 85*	37	342	19	0.001189
		Oct 85	32	481	26	0.001078
		Jun 86	6	691	128	0.001304
		Aug 86	60	397	4	0.001601
	A. Linearis	Jan 85	25	98	-3	0.001109
		May 85	19	264	27	0.000924
		Jun 85*	38	106	8	0.001144
		Nov 85	30	465	25	0.001147
		Jun 86	8	1116	-4	0.001423
	A. Numm.	Jan 85	24	146	21	0.001239
		May 85	22	728	151	0.001334
		Jun 85*	18	172	7	0.001639
		Jun 86	5	918	244	0.001492
		Sep 86	48	609	50	0.002440
	C. Sturtii	Jan 85	28	30	2	0.000799
		May 85	21	143	10	0.000771
		Jun 85*	39	57	-8	0.001375
		Jun 86	4	194	19	0.001822
		Sep 86	41	151	34	0.001800
	A. Victoriae	Jan 85	27	28	1	0.000769
		May 85	20	113	2	0.001225
		Jun 85*	36	67	1	0.001949
5 Winter	A. Numm.	Oct 85	33	798	118	0.001339
		Feb 86	29	824	105	0.001378
		Nov 86	49	1130	179	0.001856
		Feb 88	55	2516	306	0.001834

Field	Species	Date	Data set ID	Mean V	Intercept	Slope
	C. Sturtii	Oct 85	34	172	-1	0.000953
		Nov 86	42	57	5	0.003146
	A. Victoriae	Oct 85	31	138	12	0.001255
		Nov 86	46	118	0	0.001749
7 Summer	A. Canescens	Nov 85	15	1014	162	0.001027
		May 86	7	1378	31	0.001243
		Jun 87	61	680	-7	0.001818
	A. Linearis	Nov 85	13	1080	63	0.001096
		May 86	58	1116	-4	0.001423
		Jun 87	59	764	-4	0.001424
	A. Numm.	Nov 85	17	1454	75	0.001303
		May 86	10	2286	536	0.001413
		Jun 87	50	2232	116	0.002334
		Jun 88	56	1521	214	0.002082
	C. Sturtii	Nov 85	14	663	49	0.001120
		May 86	9	764	60	0.001203
		Jun 87	43	731	31	0.001923
	A. Victoriae	Nov 85	16	473	20	0.000706
7 Winter	A. Numm.	Dec 87	54	252	50	0.005546
	C. Sturtii	Feb 86	11	208	16	0.001538
	A. Victoriae	Feb 86	12	294	15	0.000481
9	A. Numm.	Jun 87	51	1994	174	0.002308
14	A. Canescens	Jun 86	3	1168	167	0.001129
		Jul 87	62	1129	-58	0.001988
		Jul 88	63	1284	162	0.002552
	A. Numm.	Jun 86	1	2695	131	0.002209
		Jul 87	53	4299	290	0.002617
		Dec 88	57	1753	14	0.004290
	C. Sturtii	Jun 86	2	943	70	0.001189
		Jul 87	44	954	9	0.001678
		Jun 88	45	1232	112	0.003513

* regrowth of previously cut shrubs

Table 2. Results of weighted simple linear regression analyses for leaf biomass per shrub (L) for all calibration sets. H=height (cm), W=diameter (cm), Dt=total biomass density (-).

Field	Species	Date	Data set ID	Mean L	Intercept	Slope
5 Summer	A. Canescens	Oct 85	32	53	5	0.002041
		Jun 86	6	173	36	0.005842
	A. Linearis	Nov 85	30	100	-8	0.005919
		Jun 86	8	320	-13	0.009235
	A. Numm.	Jun 86	5	290	-10	0.008858
	C. Sturtii	Jun 86	4	46	-2	0.007633
5 Winter	A. Numm.	Oct 85	33	293	6	0.006281
		Feb 86	29	288	-9	0.009007
		Nov 86	49	242	22	0.006602
		Feb 88	55	727	-32	0.013034
	C. Sturtii	Oct 85	34	11	8	0.000458
		Nov 86	42	0		
	A. Victoriae	Oct 85	31	19	1	0.002188
		Nov 86	46	15	0	0.003212
7 Summer	A. Canescens	Nov 85	15	428	43	0.008411
		May 86	7	454	-42	0.009167
		Jun 87	61	163	-14	0.007534
	A. Linearis	Nov 85	13	418	28	0.007688
		May 86	58	320	-13	0.009235
		Jun 87	59	154	-2	0.005518
	A. Numm.	Nov 85	17	486	-49	0.008310
		May 86	10	1026	-101	0.014868
		Jun 87	50	546	44	0.010023
		Jun 88	56	237	-11	0.008999
	C. Sturtii	Nov 85	14	301	2	0.007580
		May 86	9	257	14	0.006801
		Jun 87	43	132	10	0.005793
	A. Victoriae	Nov 85	16	123	11	0.003567

Field	Species	Date	Data set ID	Mean L	Intercept	Slope
7 Winter	A. Numm.	Dec 87	54	23	7	0.012356
	A. Victoriae	Feb 86	12	19	0	0.001827
9	A. Numm.	Jun 87	51	490	3	0.009848
14	A. Canescens	Jun 86	3	458	47	0.007871
		Jul 87	62	311	-31	0.010841
		Jul 88	63	249	-6	0.010795
	A. Numm.	Jun 86	1	1511	-259	0.024852
		Jul 87	53	1311	-17	0.016948
	C. Sturtii	Jun 86	2	353	-4	0.007108
		Jul 87	44	228	-5	0.008212
		Jun 88	45	152	-32	0.009580

Table 3. Means of variables in the survey and calibration data for the four sets of data collected in March 1989 in Field 5, for all estimators.

	Set 1				Set 2			Set 3	Set 4	
					Estimator				1.III	27.III
	Y	E	R	N	Y	R	N	Y	Y1	Y2
SURVEY PLANTS										
H *	87	95	85	90	99	105	105	70	80	81
W	72	78	63	70	73	72	73	50	54	48
Dt	6.4	6.2	7.1	6.7	5.6	5.7	6.0	2.0	2.4	3.5
Dl	6.6	6.8	6.8	6.3	6.0	6.9	5.2	2.4	2.7	4.1
$HW^2(x10^3)$	582	718	416	567	644	667	662	210	267	219
HWDt	46009	50964	41101	45564	44968	46462	48137	8182	11270	15535
CALIBRATION PLANTS										
H	84	92	86	91	101	107	108	69	81	84
W	72	80	63	73	76	79	76	50	56	48
Dt	6.4	6.2	6.8	6.6	5.6	5.6	5.9	2.1	2.4	3.6
Dl	6.7	6.2	6.8	5.6	6.0	6.9	5.2	2.5	2.6	4.1
$HW^2(x10^3)$	590	725	422	649	700	802	731	195	267	211
HWDt	44255	51109	40008	46841	47301	52026	50543	7439	11074	15826
Wet wt		3692				4728		811	1098	
V		1333				1802		380	517	
S		904				1269		316	406	
L		422				527		61	108	

* H=Height (cm); W=Diameter (cm); Dt=Total biomass density (-); Dl=Leaf biomass density (-); HW^2=Volume (cm^3); HWDt=Total density profile (cm^2); V=Total dry weight per shrub (g); S=Stem dry weight per shrub (g); L=Leaf dry weight per shrub (g)

Table 4. Results of weighted simple linear regression analyses for total biomass per shrub (V) and leaf biomass per shrub (L) for each calibration set and estimator. H=height (cm), W=diameter (cm), Dt=total biomass density (-).

Set	Estimator	Independent variable	Dependent variable	Intercept	Slope	r^2
1	Y	V	HW^2	151.6	0.001988	0.94
	E	V	HW^2	-6.4	0.001846	0.92
	R	V	HW^2	64.9	0.002976	0.86
	N	V	HW^2	181.8	0.001737	0.87
2	Y	V	HW^2	246.4	0.002214	0.91
	R	V	HW^2	361.3	0.001774	0.87
	N	V	HW^2	273.7	0.002075	0.78
3	Y	V	HW^2	76.2	0.001571	0.33
4	Y1	V	HW^2	41.0	0.001780	0.47
	Y2	V	HW^2	69.4	0.002129	0.52
1	Y	L	HWDt	-7.3	0.009613	0.94
	E	L	HWDt	-33.2	0.008778	0.96
	R	L	HWDt	-33.1	0.011227	0.84
	N	L	HWDt	-40.1	0.009795	0.87
2	Y	L	HWDt	-39.5	0.011968	0.86
	R	L	HWDt	-10.3	0.010203	0.90
	N	L	HWDt	-109.9	0.012399	0.73
3	Y	L	HWDt	-13.3	0.010477	0.73
4	Y1	L	HWDt	-9.8	0.010495	0.43
	Y2	L	HWDt	21.8	0.005502	0.35

Table 5. Tests for uniformity of slopes and intercepts of weighted simple linear regression equations $V=f(HW^2)$, $L=f(HWDt)$ for different estimators, rows and sub-plots. Analysis of variance F-test significance for the 0.05 level.

Comparison	Dependent variable	
	V	L
Same row, different estimators. Set 1: Y, E, R, N	different slopes	NS
Same row, different estimators. Set 2: Y, R, N	NS	NS
Same estimator, same sub-plot. Y: set 1, set 2	different intercepts	NS
Same estimator, same sub-plot. R: set 1, set 2	different slopes	NS
Same estimator, same sub-plot. N: set 1, set 2	NS	NS
Same estimator, same sub-plot. Y: set 3, set 4 (Y1)	NS	NS
Same estimator, different sub-plots.		
Y: set1, 2, 3, 4 (Y1)	different intercepts	NS
Same estimator, same row, different dates.		
Set 4: Y1, Y2	NS	NS

Table 6. Estimations of field mean total biomass per shrub and field mean leaf biomass per shrub for each data set and estimator, using the regression equation for the corresponding (shown in bold-face) and other calibration data sets.

Survey data to which equation is applied:		Calibration data from which regression equation is derived									
		Set 1				Set 2			Set 3	Set 4	
		Estimator									
Set	Estimator	Y	E	R	N	Y	R	N	Y	Y1	Y2
		Mean total biomass per shrub (g)									
1	Y	**1308**	1067	1796	1192	1534	1393	1481	990	1076	1308
1	E	1578	**1319**	2201	1428	1835	1635	1763	1204	1319	1597
1	R	979	761	**1303**	904	1167	1099	1137	730	781	955
1	N	1279	1040	1752	**1167**	1502	1367	1450	967	1050	1277
2	Y	1432	1183	1982	1301	**1672**	1504	1610	1088	1187	1441
2	R	1477	1225	2050	1340	1723	**1544**	1658	1124	1228	1489
2	N	1468	1216	2035	1332	1712	1536	**1648**	1116	1219	1479
3	Y	570	382	691	547	712	735	710	**407**	416	517
4	Y1	683	487	860	646	838	835	828	496	**517**	638
4	Y2	586	397	715	562	730	749	727	420	430	**535**
		Mean leaf biomass per shrub (g)									
1	Y	**435**	371	483	411	511	459	461	469	473	275
1	E	483	**414**	539	459	570	510	522	521	525	302
1	R	388	328	**428**	362	452	409	400	417	422	248
1	N	431	367	478	**406**	506	455	455	464	468	272
2	Y	425	362	472	400	**499**	449	448	458	462	269
2	R	439	375	489	415	517	**464**	466	473	478	277
2	N	455	389	507	431	537	481	**487**	491	495	287
3	Y	71	39	59	40	58	73	-8	**72**	76	67
4	Y1	101	66	93	70	95	105	30	105	**108**	84
4	Y2	142	103	141	112	146	148	83	149	153	**107**

Figure 1

Estimates of Shrub Volume for Each Estimator Plotted Against the Estimate of Estimator Y for Data Set 1

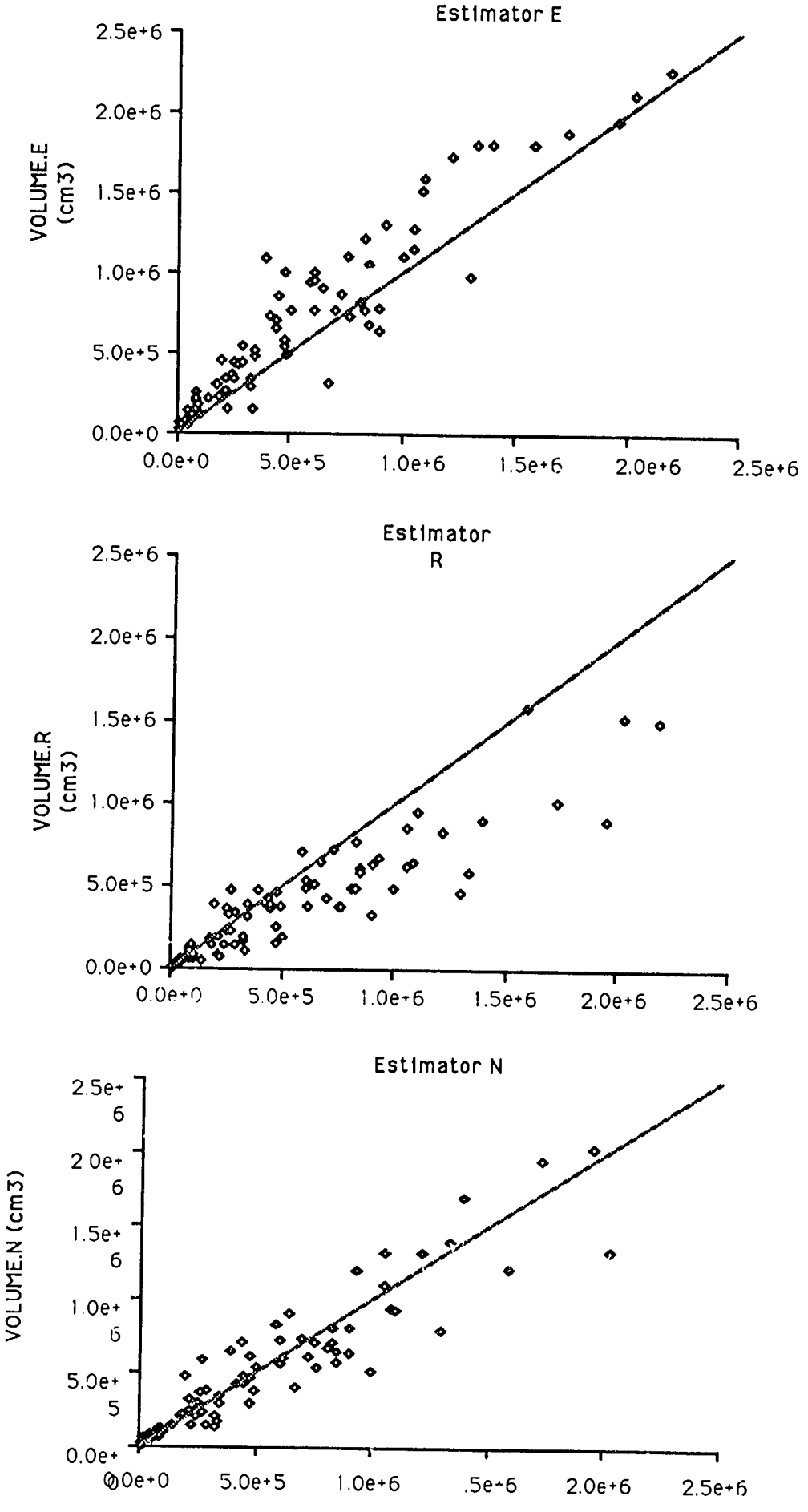


Figure 2

Estimates of Shrub Volume for Each Estmator Plotted Against the Estimate of Estimator Y for Data Set 2

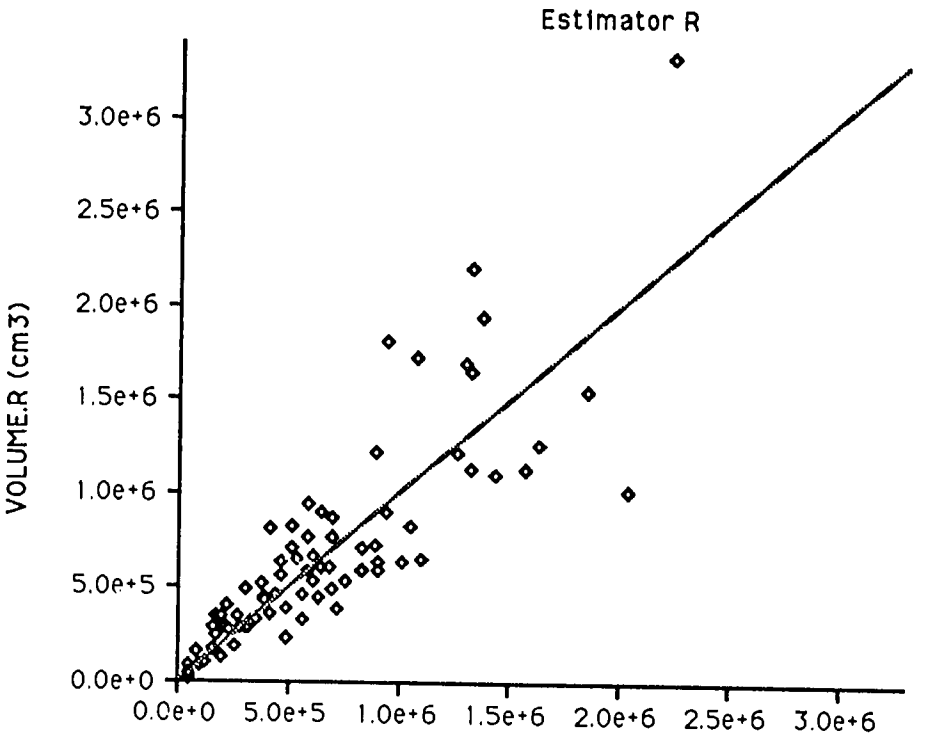


Figure 2b

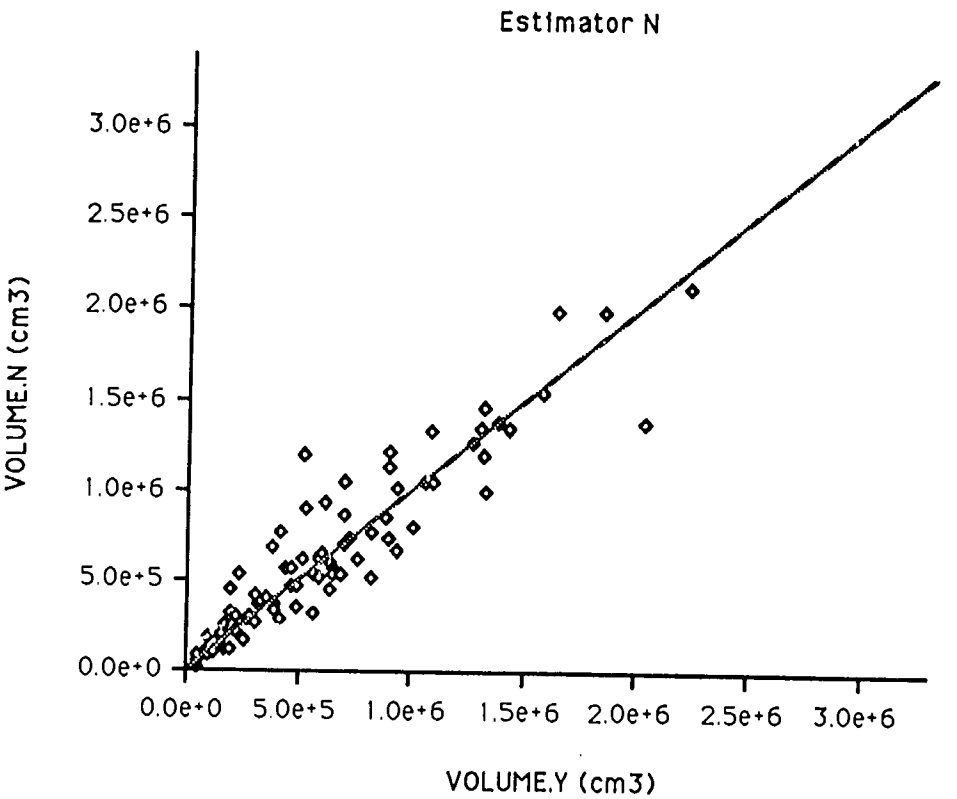
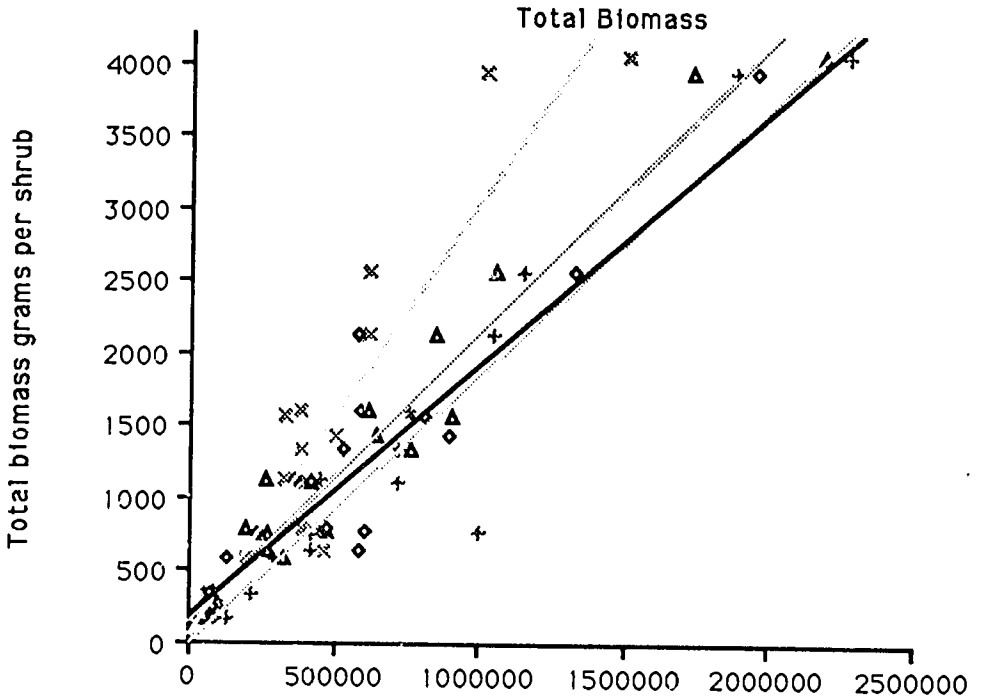


Figure 3

Relationship of Biomass per Shrub to Survey Variable Determined by Four Estimators Data Set 1



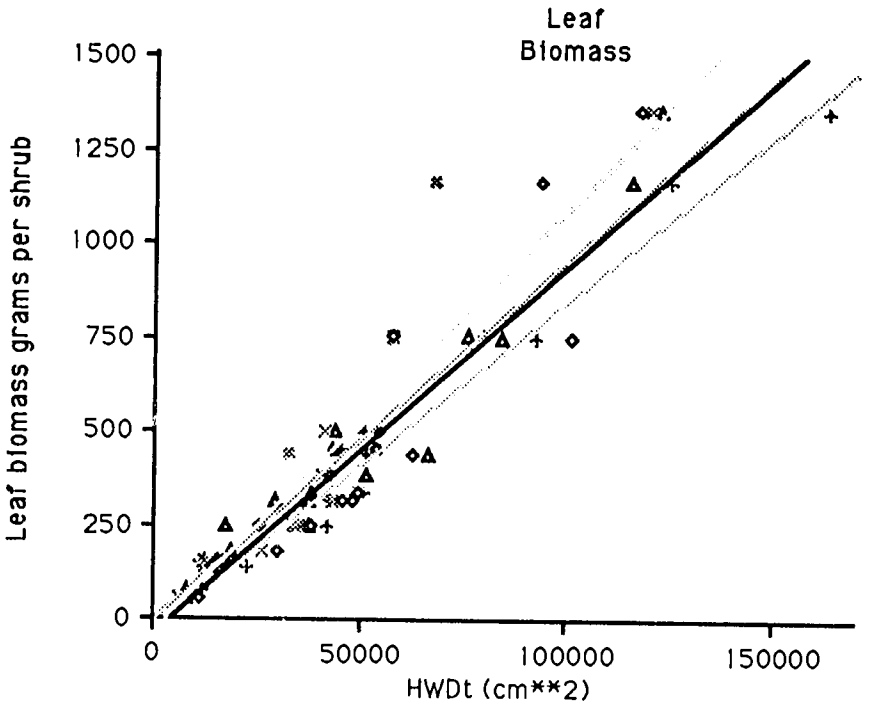


Figure 4

Relationship of Biomass per Shrub to the Survey Variable Determined by Three Estimators Data Set 2

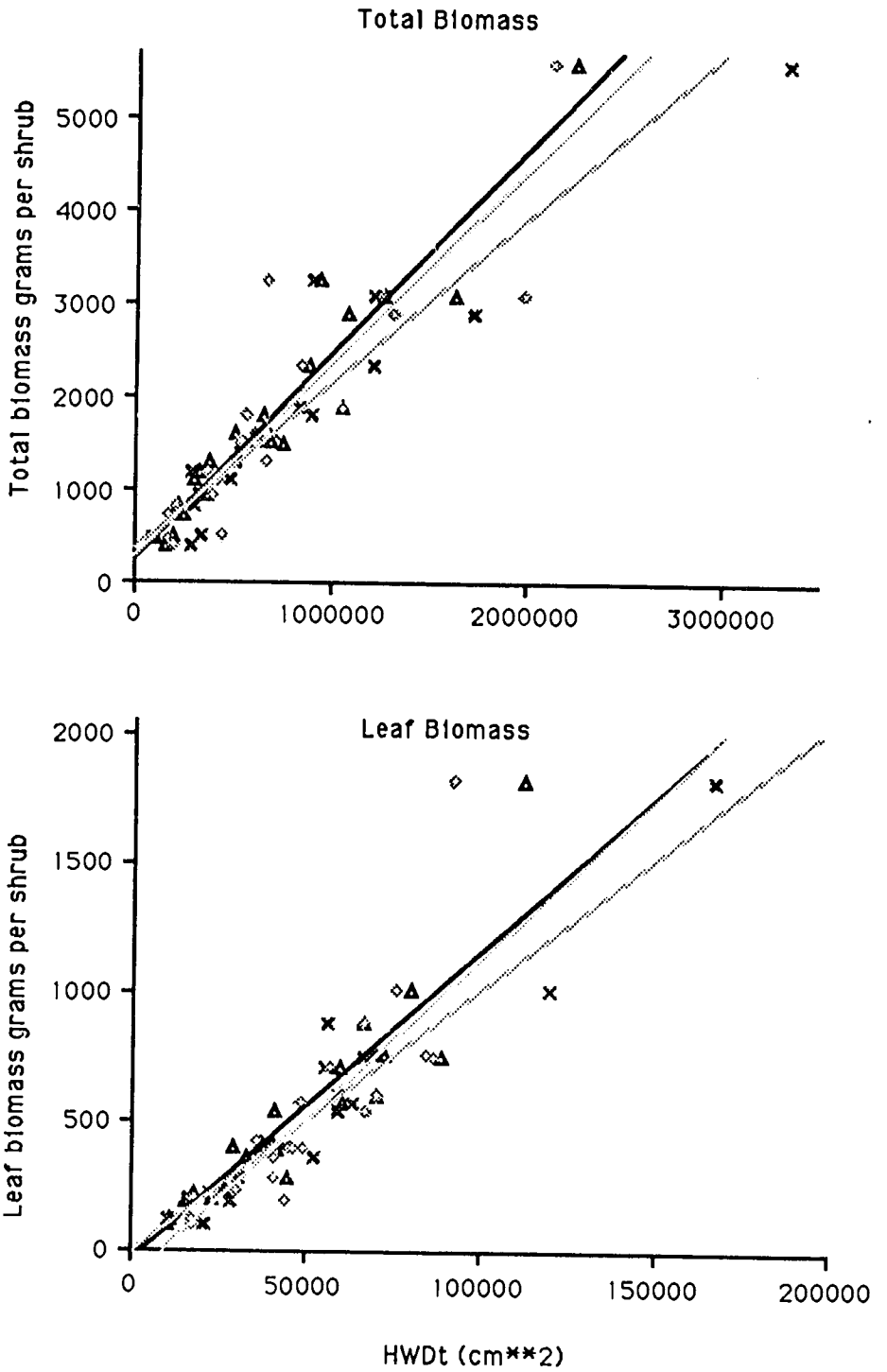
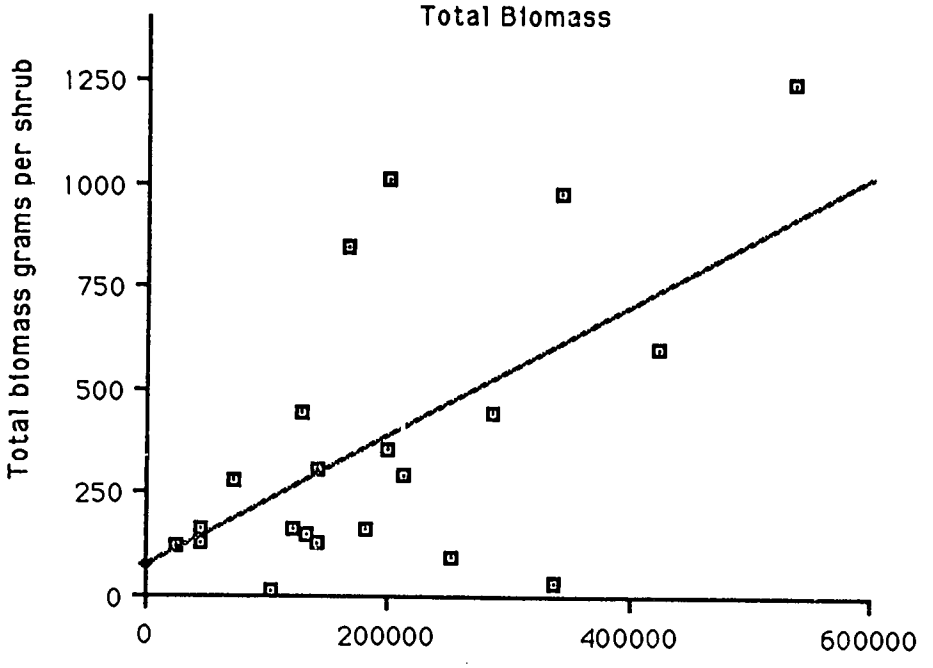


Figure 5

Relationship of Biomass per Shrub to the Survey Variable Determined by Estimator L Data Set 3



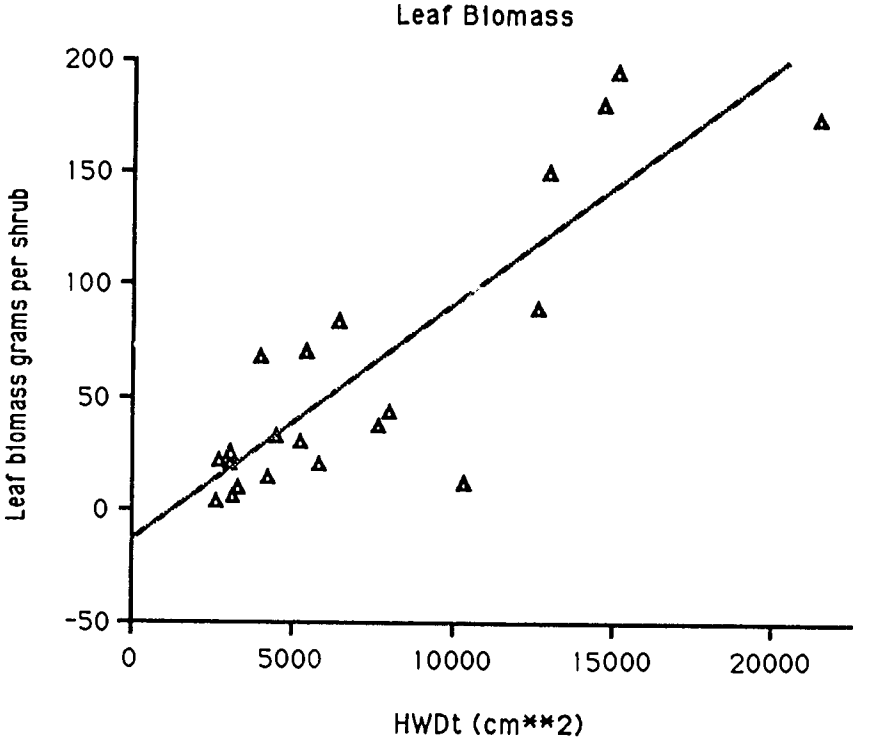
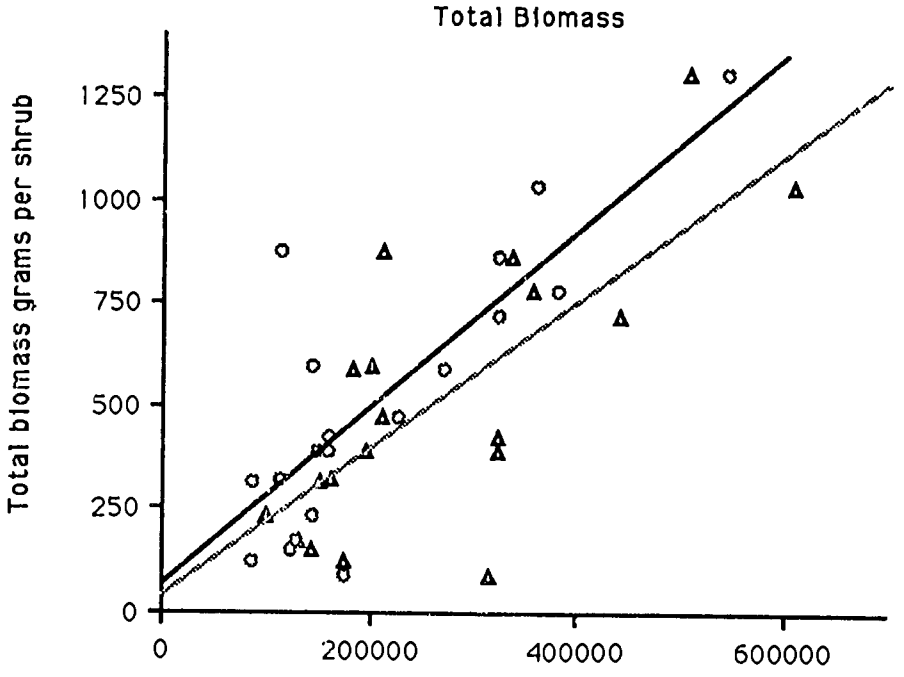
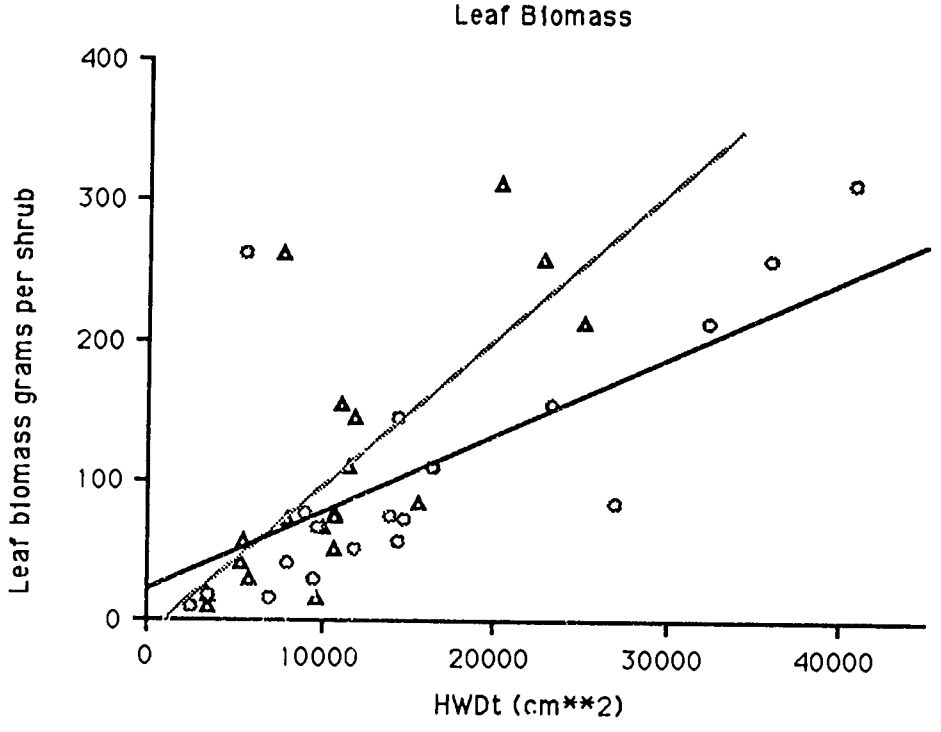


Figure 6

Relationship of Biomass per Shrub to the Survey Variable Determined by Two Estimators Data Set 4





THE EDIBLE BIOMASS AND REGROWTH OF THREE SPECIES OF FODDER SHRUBS FOLLOWING COMPLETE DEFOLIATION BY GRAZING

November 1988 to November 1989

R. W. Benjamin, D. Barkai, Y. Hefetz, M. Forti,and Y. Lavie

ABSTRACT

The fodder shrubs *Atriplex nummularia, Atriplex canescens* and *Cassia sturtii* were planted in a 4.8 ha field at the Migda Dryland Experimental Farm in November 1984 at five densities: 625, 1111, 2500, 4444 and 10,000 shrubs per ha. There were six replicates of each treatment; three were fertilized with NPK in November 1986 and in November 1987, and three were left unfertilized. Their undisturbed growth was studied for three years, after which shrub regrowth was studied during the summers of 1987 and 1988 and 1989, after being grazed each year until the plants were completely defoliated. Regrowth was estimated at the end of each subsequent growing season. In general, *Atriplex nummularia* was superior to the other two species in its value as a fodder shrub. It showed the highest biomass after three years of undisturbed growth and the best recovery after grazing. It also produced more leaves, especially at the highest planting densities.

Key words: *Atriplex canescens, Atriplex nummularia, Cassia sturtii,* defoliation, edible biomass, fodder, grazing, Migda, planting density, recovery from grazing

INTRODUCTION

The undisturbed growth of the fodder shrubs *Atriplex nummularia, Atriplex canescens* and *Cassia sturtii*, planted at five different densities in November 1984, was studied at the Migda Dryland Experimental Farm. Every year for three years the accumulated biomass of stems and leaves per shrub and per unit area was estimated in June-July.

However, since only the leaves and an undefined fraction of the stems (soft twigs) are edible by small ruminants , the main interest in forage production from shrubs is in the annual regrowth of edible biomass following grazing and its relation to browsing time, planting density, standing biomass and shrub structure. Only if the purpose of the forage shrubs is to maintain a large undisturbed standing biomass as a drought reserve, is accumulated standing biomass of major importance. Unfortunately, most of the biomass yields reported in the literature are for the total accumulated standing biomass of undisturbed shrubs and the planting density, age of the plantation, and

amounts of leaves and stems in the standing biomass are not always clearly stated.

To study regrowth, the above shrub plantation was grazed by sheep and goats, until the plants were completely defoliated, during the summers of 1987 and 1988 and by sheep in November 1989. Foliage removed was calculated after each grazing and the regrowth estimated the following season. Complete results for 1987 and the foliage removed in 1988 were reported by Benjamin *et al.*, (1989). The regrowth during 1989 and the foliage removed by grazing in November 1989 are reported in this study.

MATERIALS AND METHODS

The fodder shrubs *Atriplex nummularia*, *Atriplex canescens* and *Cassia sturtii* were planted in a 4.8 ha field in November 1984 at five spacings (4 * 4, 3 * 3, 2 * 2, 1.5 * 1.5, and 1 * 1 m), giving densities of 625, 1111, 2500, 4444 and 10,000 shrubs per ha. There were six replicates of each treatment, three of which were fertilized in November 1986 and in November 1987 with NPK, and three left unfertilized. The fertilizers applied by aerial topdressing were potassium nitrate and superphosphate at the rates of 60 and 40 kg of nitrogen and phosphate per ha, respectively.

The shrubs were not cut or grazed until July 1987. From the on, they were grazed to complete defoliation each year and shrub dimensions, accumulated standing biomass, and proportions of stems and leaves were determined each year immediately before and after grazing. A full description of the experiment and results for undisturbed growth was presented by Benjamin *et al.*, (1988) in the seventh annual report of this project.

The first grazing was from July to November 1987 by 37 sheep and 37 goats. In addition to shrub measurements, the standing biomass of the dry annual herbaceous species (annuals) present between the shrubs was estimated before and after grazing by the method described by Benjamin *et al.*, (1988). After November 1987 the plantation was left undisturbed, so as to recover during the 1987/88 growing season.

At the end of June 1988, the shrubs of each species in each planting density were measured and estimated for their dimensions and standing biomass, and the biomass of annuals present was also estimated. The field was then grazed a second time by 32 sheep and 32 goats until the shrubs were again completely defoliated. At the end of October the shrubs were measured and estimated once more for their dimensions and standing biomass, respectively, as was the remaining biomass of the annuals.

From October 19, 1989 the field was grazed a third time by 177 sheep to October 30, and there-after by 237 sheep until November 23, 1989, by which time the shrubs were completely defoliated and the herbaceous ground cover almost grazed out. Measurements and estimates of shrub dimensions and standing biomass, before and after grazing, were made as described above for 1987 and 1988.

RESULTS AND DISCUSSION

The total rainfall of 222 mm during 1988/89 (Table 1) was less than the mean annual rainfall of 250 mm for the region, and its monthly distribution in December, January and February, was relatively good for the growth of annual herbaceous species, eventhough the onset of effective rains was relatively late. However, the rainfall distribution may have been unfavorable for shrub growth, since there was only one really heavy downpour of 46 mm at February 12, 1989, which would have enabled penetration of water to a soil depth where the annuals could not compete with the shrubs for soil water.

As in previous years, there were no significant differences between the regrowth of fertilized and unfertilized shrubs in any of the planting density treatments. Probably after two seasons the fertilizer, last applied in November 1987, had been used up, mainly by the annual herbaceous species that grew between the shrubs in the following years

The results are discussed separately for each shrub species.

ATRIPLEX NUMMULARIA

Standing Biomass and biomass regrowth of (Table 2, Figures 1, 2, 3, 4 and 5)

The total, stem and leaf biomass regrowth of *Atriplex nummularia* per shrub at each planting density were estimated by calculating the difference between the standing biomass after grazing in November 1988 and before grazing in October 1989. Table 2 summarizes the regrowth during this 11 month period. These per-shrub yields and the per-0.1 ha yields derived from them are illustrated in Figures 1 for total standing biomass, in Figures 2 for stem and Figures 3 for leaf biomass. The relationships derived for biomass on planting density were curvilinear for per shrub parameters and linear for per 0.1 ha.

The shrub regrowth per shrub and per 0.1 ha are shown in Figures 4 and its composition into leaves and stems are presented in Figures 5. Best fit relationships of regrowth on planting density for both per shrub and per unit are curvilinear. In general, with the exception of the lowest planting density, the proportion of leaves in

the total shrub regrowth increased with decreasing plant density. However, on a per unit area basis there was significantly more leaf and stem regrowth at the highest planting density (1000 per 0.1 ha) than in any of the other densities.

The total biomass regrowth per shrub was positive at all planting densities compared with the mostly negative regrowth for the other two species studied (see below for *Atriplex canescens* and *Cassia sturtii*).

Biomass eaten at November 1989 (Table 3 and Figures 6)

Table 3 shows the measurements and composition of the standing biomass per shrub before and after grazing. The foliage removed and assumed to have been eaten is calculated by difference. The best fit relationship between eaten biomass per 0.1 ha and planting density, and the composition of the foliage eaten per 0.1 ha are illustrated in Figures 6. On a per unit area basis, more foliage was removed, and the proportion of leaves in that foliage was significantly higher, from the shrubs at the highest planting density. In fact significantly more stem biomass was removed by grazing than the regrowth measured during the previous 11 months. This was probably due to the mean high grazing pressure of 43.5 sheep per ha applied during 45 days of grazing.

The dimensions of the shrubs after grazing at December 1989 were not significantly different from those after grazing at November 1988, however, there was a significant lower standing stem biomass at December 1989 at all planting densities. It would appear that much more stem biomass was removed during the 1989 grazing than in the previous year, possibly because of the much higher grazing pressure (for a shorter time) applied in 1989.

ATRIPLEX CANESCENS

Standing Biomass and biomass regrowth (Table 4, Figures 7, 8, 9, 10 and 11)

These parameters were calculated as for *Atriplex nummularia*. Table 4 summarize the regrowth during this 11 month period. These per-shrub yields and the per-0.1 ha yields derived from them are illustrated in Figures 7 for total standing biomass, in Figures 8 for stem and Figures 9 for leaf biomass. As for *Atriplex nummularia*, the relationships derived for biomass on planting density were curvilinear for per shrub parameters and linear for per 0.1 ha.

The shrub regrowth per shrub and per 0.1 ha are shown in Figures 10 and its composition into leaves and stems are presented in Figures 11. Best fit relationship of regrowth on planting density for per shrub are curvilinear but for per unit area, linear. In general, with the exception of the lowest planting density, stem regrowth was

negative and most of the shrub regrowth was from leaves. In addition, on a per shrub basis, it appears that stem and leaf regrowth improved with decreasing plant density. However, on a per unit area basis there was significantly more leaf regrowth at the highest planting density (1000 per 0.1 ha) than in any of the other densities.

The total biomass regrowth per shrub was negative at the two highest planting densities and was negative for stem biomass at all but the lowest density (Table 4). The reason for the negative stem regrowth is not clear as the shrub regrowth dimensions at all planting densities were positive. If the calibration measurements of the 20 shrubs, sampled at both November 88 and October 1989, were accurate, then some branches must have died and broken off sometime after November 1988. However, a more likely explanation is that the stem biomass at the two dates were not significantly different.

Biomass eaten at November 1989 (Table 5 and Figures 12)

Table 5 shows the measurements and composition of the standing biomass per shrub before and after the 1989 grazing. The foliage removed and assumed to have been eaten is calculated by difference. The best fit relationship between eaten biomass per 0.1 ha and planting density, and the composition of the foliage eaten per 0.1 ha are illustrated in Figures 12. On a per unit area basis, more foliage was removed, and the proportion of leaves in that foliage was significantly higher, from the shrubs at the highest planting density.

The dimensions of the shrubs after grazing at December 1989 were not significantly different from those after grazing at November 1988, however, there was a significant lower standing stem biomass at December 1989 at all planting densities. It would appear that much more stem biomass was removed during the 1989 grazing than in the previous year, possibly because of the much higher grazing pressure (for a shorter time) applied during 1989.

CASSIA STURTII

Standing and regrowth biomass (Table 6 and Figures 13, 14, 15, 16 and 17)

These parameters were calculated as for the two previous species. Table 6 summarize the regrowth during this 11 month period. These per-shrub yields and the per-0.1 ha yields derived from them are illustrated in Figures 13 for total standing biomass, in Figures 14 for stem and Figures 15 for leaf biomass. As for the two Atriplex species, the relationships derived for biomass on planting density were curvilinear for per shrub parameters and linear for per 0.1 ha.

The shrub regrowth per shrub and per 0.1 ha are shown in Figures 16 and its composition into leaves and stems are presented in Figures 17. Best fit relationship of regrowth on planting density for per shrub and per unit area are curvilinear. Stem regrowth at all planting densities was negative and all of the shrub regrowth was from leaves. In addition, on a per shrub basis, it appears that stem and leaf regrowth improved with increasing plant density but the opposite was true for leaf regrowth. However, on a per unit area basis there was significantly more leaf regrowth at the highest planting density (1000 per 0.1 ha) than in any of the other densities.

The total biomass regrowth per shrub was negative at all but the highest planting density and was negative for stem biomass at all densities (Table 6). As for *Atriplex canescens*, the reason for the negative stem regrowth is not clear as the shrub regrowth dimensions at all planting densities were positive.

Biomass eaten at November 1989 (Table 7 and Figures 18)

Table 7 shows the measurements and composition of the standing biomass per shrub before and after the 1989 grazing. The foliage removed and assumed to have been eaten is calculated by difference. The best fit relationship between eaten biomass per 0.1 ha and planting density, and the composition of the foliage eaten per 0.1 ha are illustrated in Figures 18. On a per unit area basis, more foliage was removed, and the proportion of leaves in that foliage was significantly higher, from the shrubs at the highest planting density

The dimensions of the shrubs after grazing at December 1989 were not significantly different from those after grazing at November 1988, however, there was a significant lower standing stem biomass.at December 1989 at all planting densities. It would appear that much more stem biomass was removed during the 1989 grazing than in the previous year, possibly because of the much higher grazing pressure (for a shorter time) applied during 1989.

Death rate of the three shrub species in December 1990 (Table 8)

The higher death rates generally recorded at the highest planting density (4*4) were due to the destructive sampling made for the calibration of estimates of standing biomass from the dependant variables during the duration of the experiment. For this

reason the death rates for this treatment should not be compared with the other within species spacing treatments, however the between species comparisons can be made.

In general, *Atriplex nummularia* had lower death rates at all planting densities than *Atriplex canescens*, but was similar to *Cassia sturtii*. In all three species, higher death rates were recorded at the highest planting density (1*1), being exceptionaly high for *Atriplex canescens*,; the reason for this is not clear. Perhaps at this high planting density, the shrubs are more susceptable to the heavy grazing practiced because of their weaker stem structure. However, no explanation can be given for the heavier death rates recorded for *Atriplex canescens*,.

Summary of changes in the biomass and dimensions of the three species 1987 to 1989 (Figures 19-24)

The changes in the standing biomass and the dimensions of *Atriplex nummularia* , in each spacing treatment, before and after grazing from July 1987 to December 1989 are summarized in Figures 19 and 20, respectively; and for *Atriplex canescens* and *Cassia sturtii* in Figures 21, 22 and Figures 23 and 24, respectively.

None of the species in any spacing treatment recovered the standing biomass present at July 1987. The regrowth, after the first grazing, for both total and stem biomass was less than the biomass removed by grazing. This was caused primarily by the poor regrowth of leaves relative to the standing leaf biomass at July 1987, and the lower proportion of leaves in the total biomass. The regrowth of *Atriplex nummularia* after the second grazing was roughly equal to the biomass removed after the second grazing. The grazing pressure during both 1987 and 1988 were similar and possibly at this grazing pressure for this species the shrubs were able to recover the biomass removed. However, both *Atriplex canescens* and *Cassia sturtii* appeared, in general, to have a lower regrowth after the second grazing due to a loss of stem biomass as leaf regrowth did approximately equal the leaf biomass removed by the first grazing. The reason for the poor recovery of stem biomass is not clear and we have no explanation for this result, except that the estimated differences at the two dates (7/88 and 10/89) were not statistically significant.

In general, *Atriplex nummularia* seems to be superior to the other two species, in its value as a fodder shrub. It has the highest biomass and the best absolute recovery after grazing; the other two species have very low standing biomass at all dates before grazing relative to *Atriplex nummularia* However, *Atriplex nummularia* seems only

marginally better than the other two species in the proportions of leaves present just before both the grazing seasons. The greatest amounts of shrub leaves are found on *A. nummularia,i* especially at the highest planting densities. From previous reports, the amounts of annual herbaceous species present between the shrubs are not noticeably affected by the species or planting density of shrubs, although there appears to be somewhat more at lower shrub densities. Whether by necessity (small shrubs) or by choice, the animals seem to eat the highest relative amounts of stems from *Cassia sturtii* . When considering the edible biomass as a proportion of the total biomass, there does not appear to be any clear differences among the species. The proportion of stems in the edible biomass is somewhat higher in *Cassia sturtii* than in the other two species.

Table 1.
Rainfall during the winter of 1988-1989

Month	Date	Rain/day	Rain/month	% of total	Accumulated rainfall
October	30	2	2	0.9	2
November	19	13	13	5.9	15
December	13	4			19
	19	6			25
	24	23			48
	25	17			65
	26	3	53	23.9	68
January	3	23			91
	8	2			93
	11	5			98
	16	6			104
	21	26			130
	28	10	72	32.4	140
February	1	2			142
	12	46			188
	13	15			203
	15	2			205
	21	5		31.5	210
March	9	5			215
	14	7			222

Days of rain: 20

Table 2.
Dimensions and standing biomass of *Atriplex nummularia* shrubs after grazing in November 1988 and before grazing at October 1989

Spacing and date	Dimensions (cm) (mean ± SE) Height	Width	Standing biomass (g/shrub) (mean ± SE) Total	Stems	Leaves
1 * 1 m					
November 1988	66±2	38±2	647±23	647±23	0
October 1989	80±2	55±3	1028±62	833±53	195±8
Regrowth	26	14	381	186	195
1.5 * 1.5 m					
November 1988	71±2	50±2	889±53	889±79	0
October 1989	85±3	67±3	1337±90	1098±77	239±12
Regrowth	14	17	448	209	239
2 * 2 m					
November 1988	73±2	63±2	1180±77	1180±77	0
October 1989	91±2	84±3	1901±152	1583±131	318±21
Regrowth	18	21	721	531	318
3 * 3 m					
November 1988	77±2	71±5	1631±229	1631±229	0
October 1989	98±3	92±4	2948±280	2484±241	464±38
Regrowth	21	19	1317	853	464
4 * 4 m					
November 1988	79±2	85±5	2666±401	2666±401	0
October 1989	100±3	101±6	3398±476	2871±410	527±65
Regrowth	21	16	732	205	527

Table 3.

Difference in Dimensions and Biomass of Atriplex nummularia Before and After Grazing 1989

Spacing treatment and dates	Parameter	Height (cm)	Width (cm)	Biomass Shrub (g DM)	Biomass Stems (g DM)	Biomass Leaves (g DM)
1 * 1						
28/9/89	Mean±SE	80±1.95	54±2.54	1028±62	833±53	195
1/12/89	Mean±SE	63±1.8	41±1.91	545±12	545±12	0
	Difference	-17	-13	-483	-288	-195
1.5 * 1.5						
28/9/89	Mean±SE	85±2.65	67±2.65	1337±90	1098±77	239±12
1/12/89	Mean±SE	69±2.07	53±2.20	636±23	636±23	0
	Difference	-16	-14	-701	-462	-239
2 * 2						
28/9/89	Mean±SE	91±2.40	84±3.31	1901±152	1583±131	318±21
1/12/89	Mean±SE	76±1.83	68±2.53	793±35	793±35	0
	Difference	-15	-16	-1108	-790	-318
3 * 3						
28/9/89	Mean±SE	98±3.06	92±4.20	2948±280	2484±241	464±38
1/12/89	Mean±SE	79±2.30	74±3.51	916±64	916±64	0
4 * 4						
28/9/89	Mean±SE	100±2.96	101±5.51	3398±476	2871±410	527±65
1/12/89	Mean±SE	89±2.85	93±4.83	1278±137	1278±137	0
	Difference	-11	-8	-2120	-1593	-527

Table 4.
Dimensions and standing biomass of *Atriplex canescens* shrubs after grazing in November 1988 and before grazing at October 1989

Spacing and date	Dimensions (cm) (mean ± SE)		Standing biomass (g/shrub) (mean ± SE)		
	Height	Width	Total	Stems	Leaves
1 * 1 m					
November 1988	41±2	26±2	340±10	340±10	0
October 1989	52±3	37±3	321±26	237±19	84±7
Regrowth	11	11	-19	-103	84
1.5 * 1.5 m					
November 1988	48±2	41±2	391±14	391±14	0
October 1989	56±2	49±3	385±29	283±21	102±8
Regrowth	8	8	-6	-108	102
2 * 2 m					
November 1988	57±2	61±4	442±19	442±19	0
October 1989	65±3	64±4	583±65	427±47	156±18
Regrowth	8	3	141	-15	156
3 * 3 m					
November 1988	52±2	57±3	504±24	504±24	0
October 1989	68±2	70±3	653±59	478±43	175±16
Regrowth	16	13	149	-26	175
4 * 4 m					
November 1988	55±2	60±2	523±22	523±22	0
October 1989	69±2	75±3	761±60	557±44	204±16
Regrowth	14	15	238	34	204

Table 5.

Difference in Dimensions and Biomass of *Atriplex canescens* Before and After Grazing 1989

Spacing treatment and dates	Parameter	Height (cm)	Width (cm)	Biomass Shrub (g DM)	Biomass Stems (g DM)	Biomass Leaves (g DM)
1 * 1						
28/9/89	Mean±SE	52±2.78	37±3.25	321±26	235±19	86±7
1/12/89	Mean±SE	40±1.74	27±2.00	195±4	195±4	0
	Difference	-12	-10	-126	-40	-86
1.5 * 1.5						
28/9/89	Mean±SE	56±1.90	49±2.59	385±29	283±21	102±8
1/12/89	Mean±SE	45±1.62	37±1.89	211±6	211±6	0
	Difference	-11	-12	-174	-71	-103
2 * 2						
28/9/89	Mean±SE	65±2.68	64±3.53	583±65	427±47	156±18
1/12/89	Mean±SE	48±1.83	46±2.59	237±8	237±8	0
	Difference	-17	-18	-346	-190	-156
3 * 3						
28/9/89	Mean±SE	67±2.18	70±3.29	653±59	478±43	175±16
1/12/89	Mean±SE	52±2.16	55±2.83	275±13	275±13	0
	Difference	-15	-15	-378	-203	-175
4 * 4						
28/9/89	Mean±SE	69±2.20	75±2.91	761±60	557±44	204±16
1/12/89	Mean±SE	55±1.96	57±2.41	281±12	281±12	0
	Difference	-14	-18	-480	-276	-204

Table 6.

Dimensions and standing biomass of *Cassia sturtii* shrubs after grazing in November 1988 and before grazing at October 1989

Spacing and date	Dimensions and (mean ± SE)		Standing biomass (g/shrub) (mean ± SE)		
	Height (cm)	Width (cm)	Total	Stems	Leaves
1 * 1 m					
November 1988	31±2	26±1	225±22	225±22	0
October 1989	34±2	27±2	279±8	205±7	74±2
Regrowth	3	1	54	-20	74
1.5 * 1.5 m					
November 1988	43±2	41±2	409±24	409±24	0
October 1989	46±2	44±2	391±29	292±14	99±4
Regrowth	3	3	-18	-117	99
2 * 2 m					
November 1988	47±2	41±2	448±29	448±29	0
October 1989	50±2	49±2	433±22	325±17	108±5
Regrowth	3	8	-15	-123	108
3 * 3 m					
November 1988	63±2	58±2	923±70	923±70	0
October 1989	66±2	65±2	805±52	615±40	190±11
Regrowth	3	7	-118	-308	190
4 * 4 m					
November 1988	65±3	61±4	1205±102	1205±102	0
October 1989	70±3	71±3	1180±94	906±73	274±16
Regrowth	5	10	-25	-299	274

Table 7.

Difference in Dimensions and Biomass of Cassia sturtii Before and After Grazing 1989

Spacing treatment and dates	Parameter	Height (cm)	Width (cm)	Biomass Shrub (g DM)	Biomass Stems (g DM)	Biomass Leaves (g DM)
1 * 1						
28/9/89	Mean±SE	34±1.6	27±1.8	279±8	205±7	74±2
1/12/89	Mean±SE	29±1.4	25±1.4	184±3	184±3	0
	Difference	-5	-2	-95	-21	-74
1.5 * 1.5						
28/9/89	Mean±SE	46±1.7	44±2.3	391±18	292±14	99±4
1/12/89	Mean±SE	43±1.7	40±2.0	247±9	247±9	0
	Difference	-3	-4	-144	-45	-99
2 * 2						
28/9/89	Mean±SE	50±1.5	49±1.8	433±22	325±17	108±5
1/12/89	Mean±SE	45±1.4	41±1.7	251±9	251±9	0
	Difference	-15	-16	-182	-74	-108
3 * 3						
28/9/89	Mean±SE	66±1.4	65±2.2	805±52	614±40	191±1
1/12/89	Mean±SE	61±1.7	57±2.0	399±25	399±25	0
	Difference	-5	-8	-406	-215	-191
4 * 4						
28/9/89	Mean±SE	70±2.7	71±3.3	1180±9	907±73	273±2
1/12/89	Mean±SE	66±2.8	62±3.2	490±25	490±25	0
	Difference	-11	-8	-690	-417	-273

Table 8.

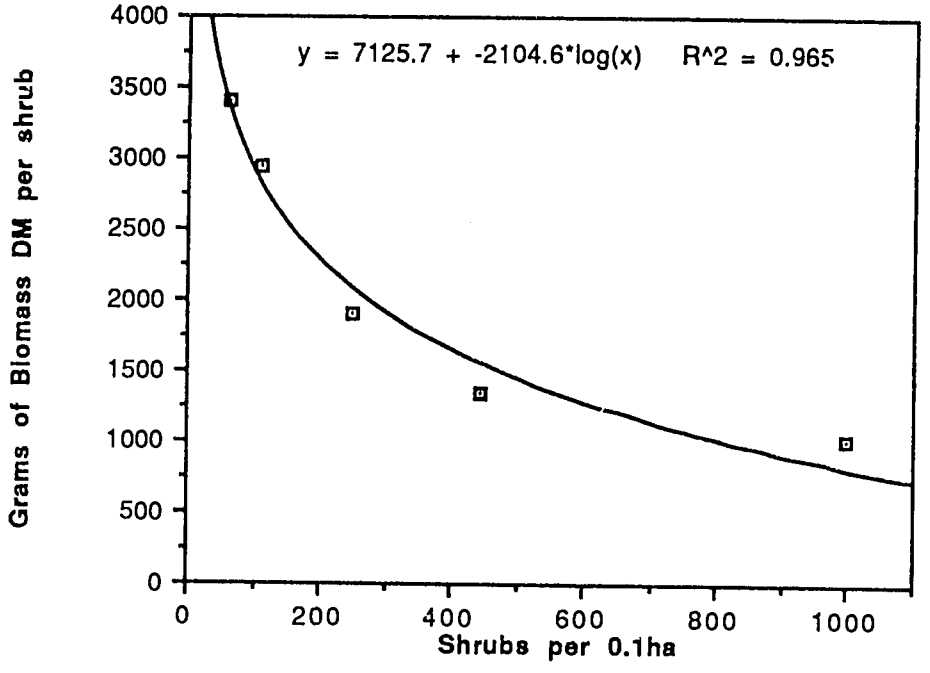
Death Rate of Three Species of Shrubs, Each Planted in 7 Rows of 42 Shrubs in November 1984 and Grazed from 1987 to 1989.

(Mean± S.D.)

Spacing treatment	Species	Number of dead shrubs/row	Percent dead shrubs/row
	Atriplex nummularia		
4 *4		12 ± 2	28 ± 2
3 * 3		5± 2	12 ± 2
2 * 2		4 ± 2	10 ± 5
1.5 * 1.5		4 ± 3	11 ± 6
1 *1		7 ± 4	17 ± 8
	Atriplex canescens		
4 *4		12 ± 2	30 ± 14
3 * 3		11± 5	27 ± 13
2 * 2		13 ± 4	31 ± 11
1.5 * 1.5		15 ± 2	35 ± 5
1 *1		19 ± 3	46 ± 8
	Cassia sturtii		
4 *4		10 ± 7	23 ± 16
3 * 3		7 ± 3	16 ± 7
2 * 2		7 ± 5	16 ± 13
1.5 * 1.5		7 ± 2	17 ± 4
1 *1		10 ± 3	24 ± 8

Figure 1

Shrub Biomass per Shrub of Atriplex nummularia at October 1989, 11 Months After Complete Defoliation



ihrub Biomass per 0.1ha of Atriplex nummularia at October 1989 11 Months After After Complete Defoliation

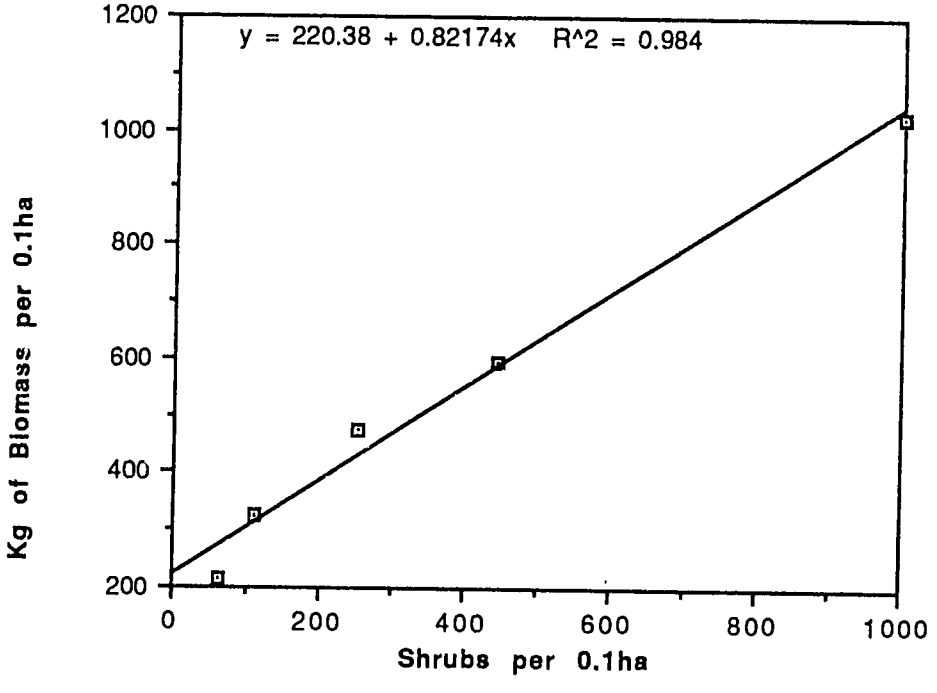
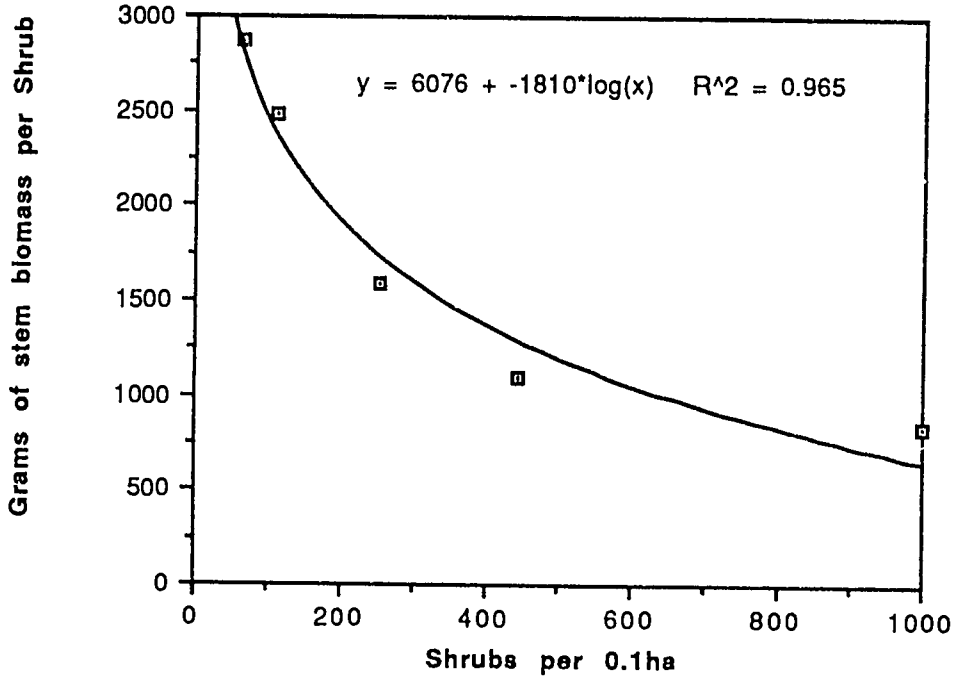


Figure 2

Stem Biomass per Shrub of Atriplex nummularia at October 1989, 11 Months After Complete Defoliation



Stem Biomass per 0.1ha of Atriplex nummularia at October 1989, 11 Months After Complete Defoliation

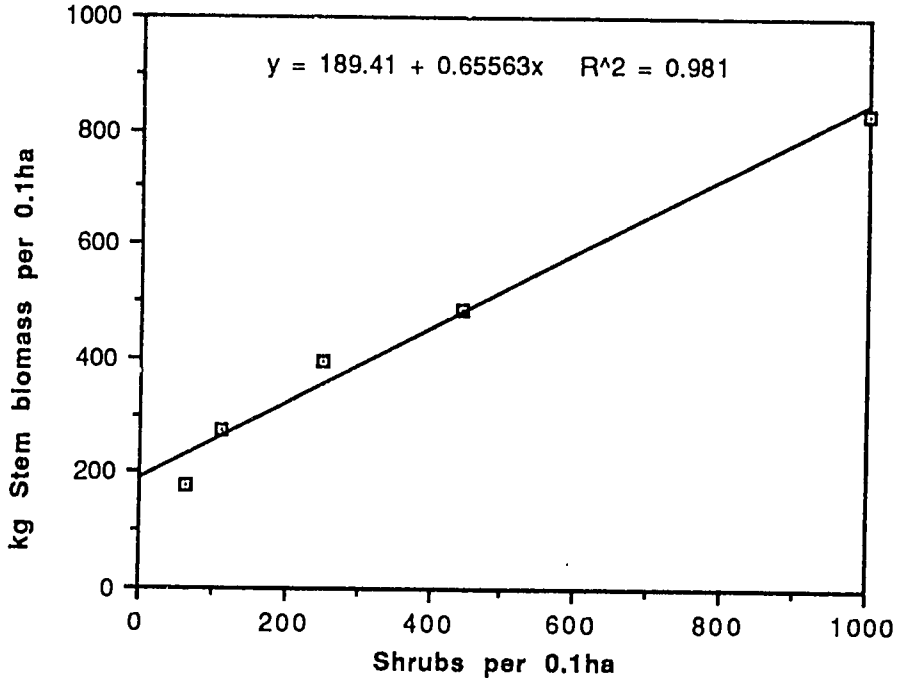
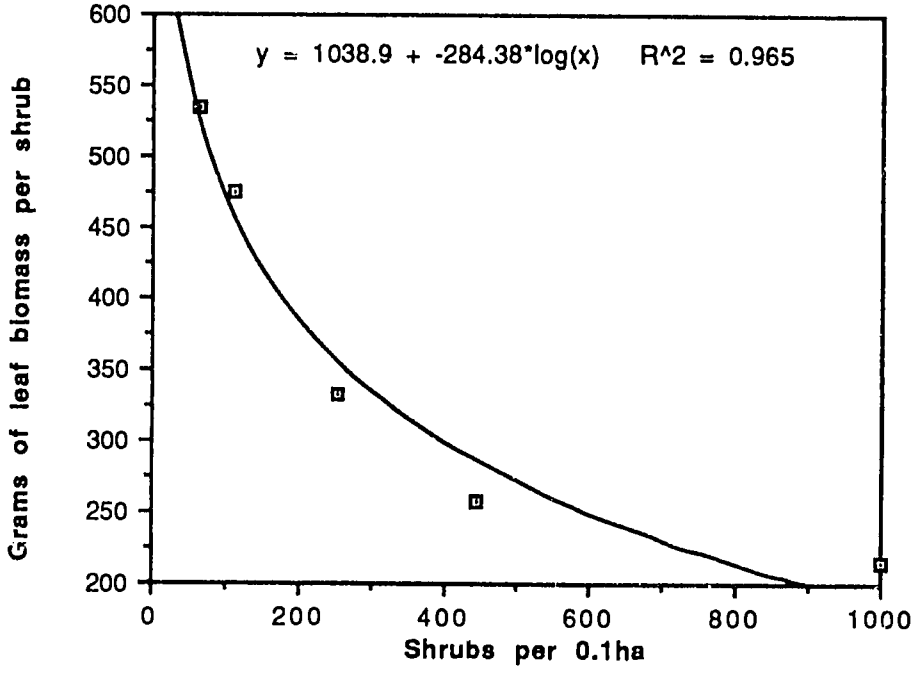


Figure 3

Leaf Biomass per Shrub of Atriplex nummularia at October 1989, 11 Months After Complete Defoliation



Leaf Biomass per 0.1ha of Atriplex nummularia at October 1989, 11 Months After Complete Defoliation

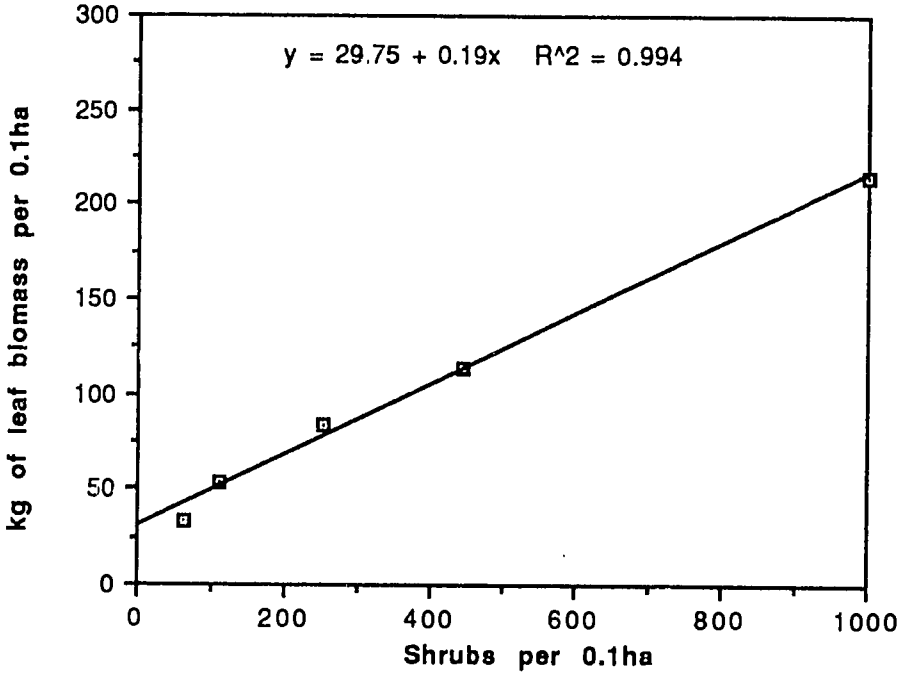
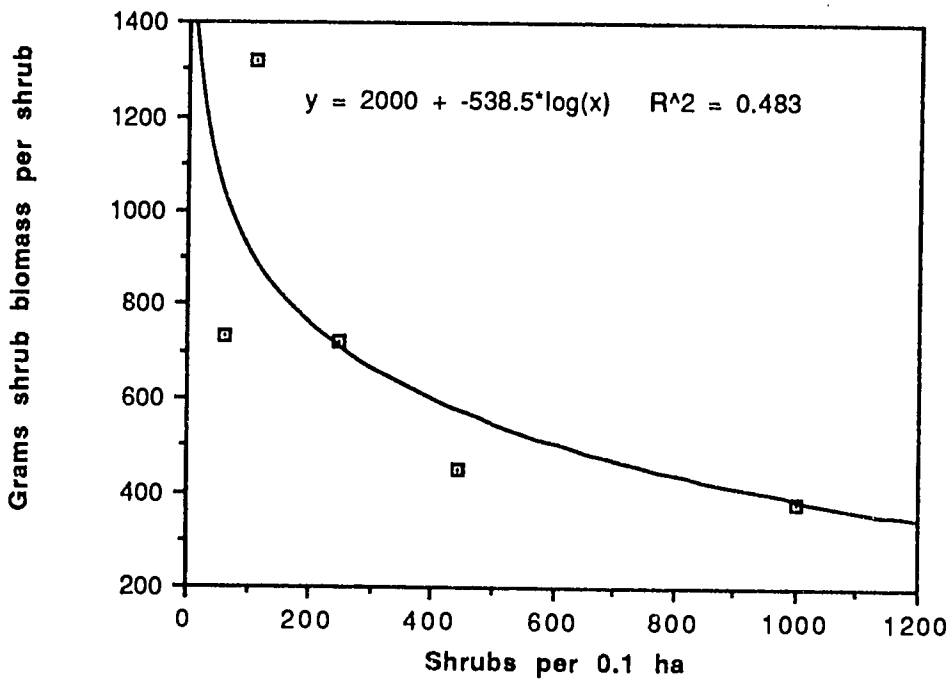


Figure 4

Regrowth (DM) per Shrub of Atriplex nummularia After Grazing at November 1988 and Before Grazing at October 1989 at Different planting Densities



Shrub Regrowth (DM) per 0.1 ha of Atriplex nummularia After Grazing at November 1988 and Before grazing at October 1989 at Different Planting Densities

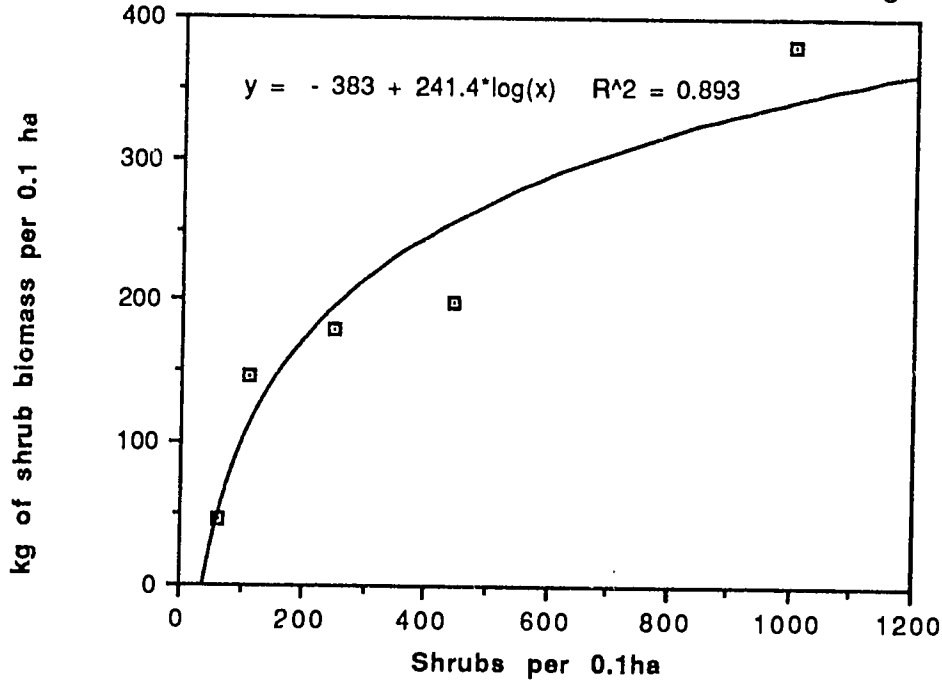
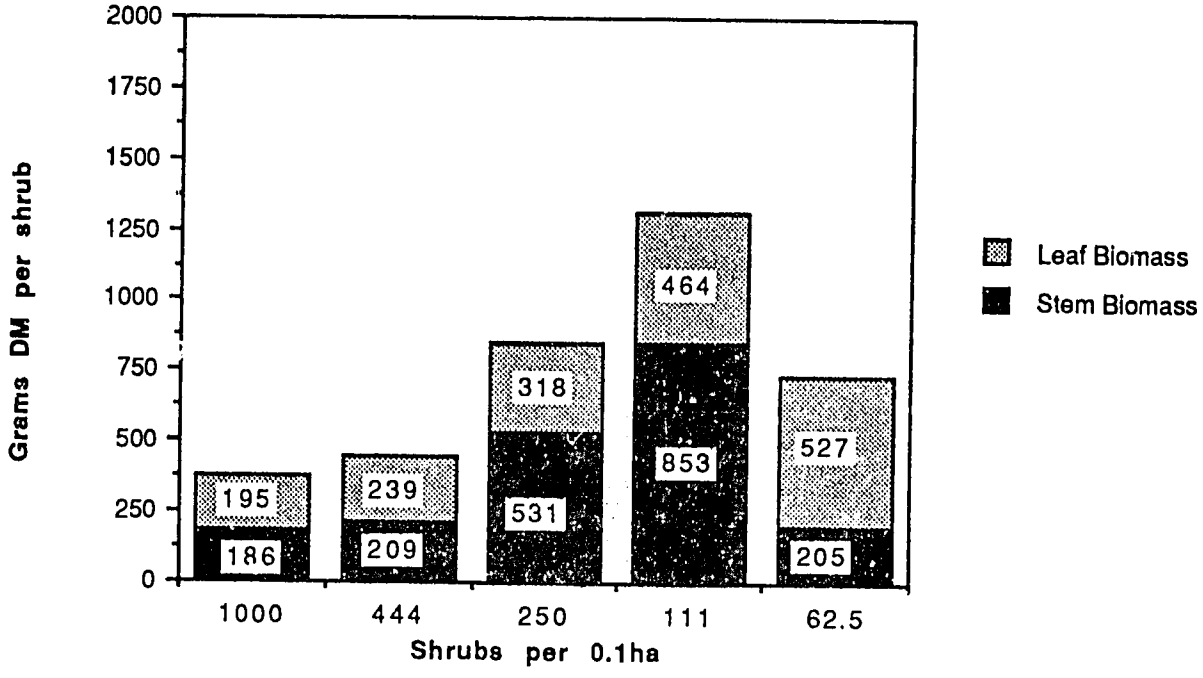


Figure 5

Composition of Atriplex nummularia Regrowth per Shrub After Grazing at November 1988 and Before Grazing at October 1989 at different Planting Densities



Composition of Atriplex nummularia Regrowth per 0.1 ha After Grazing at November 1988 and Before Grazing at October 1989 at Different Planting Densities

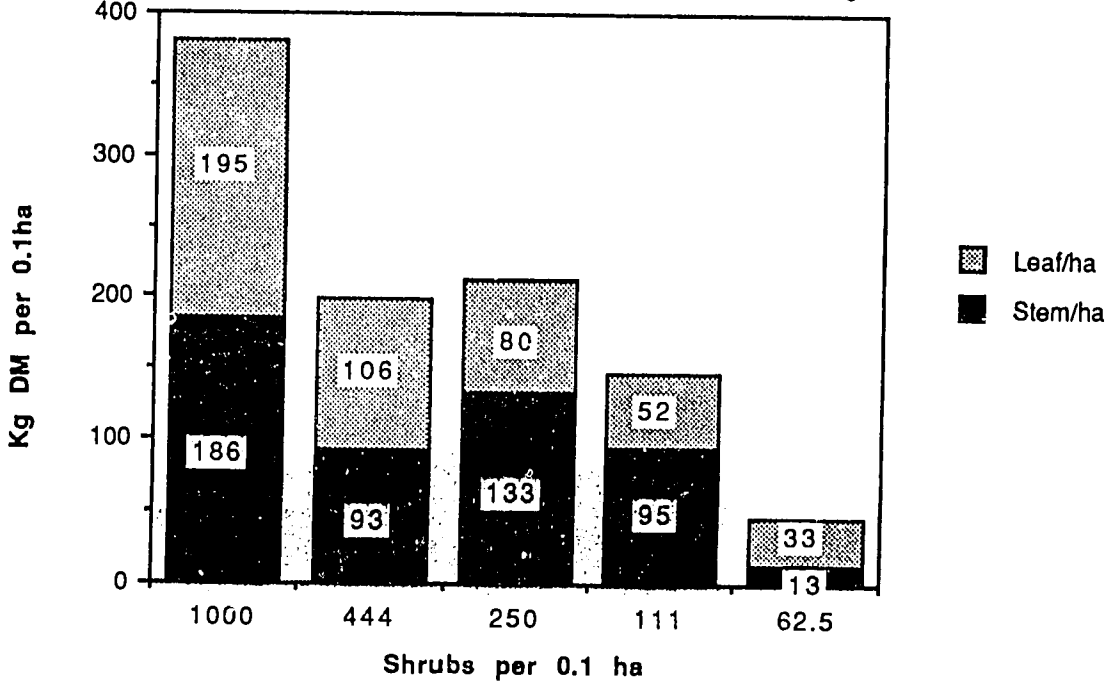
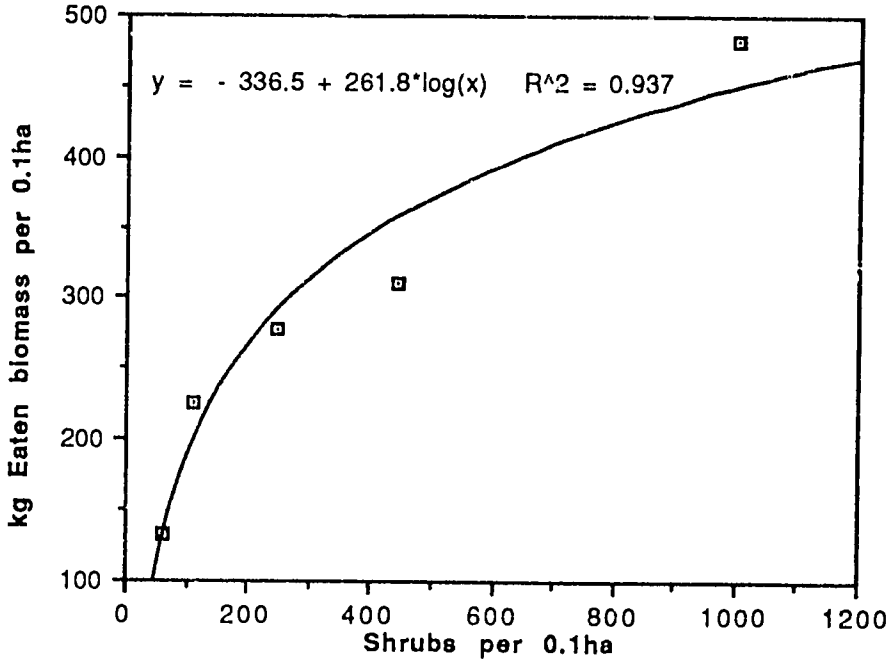


Figure 6

Eaten Biomass per 0.1ha at November 1989 From Atriplex nummularia at Different Planting Densities



Composition of Eaten Biomass per 0.1 ha at November 1989 From Atriplex nummularia

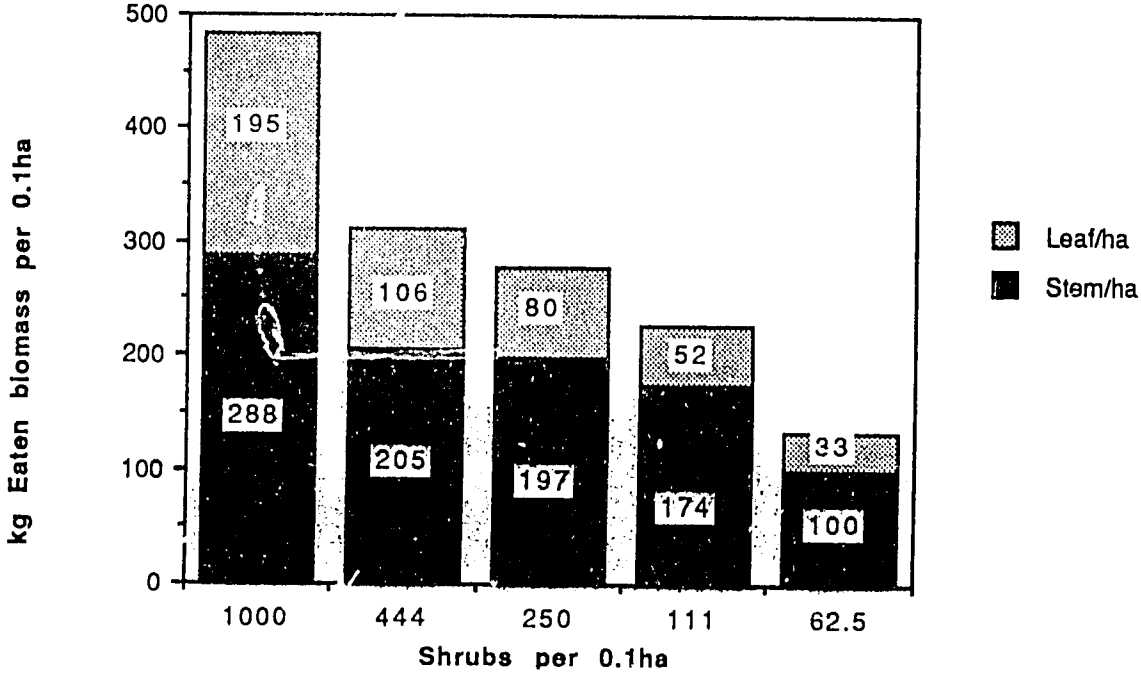
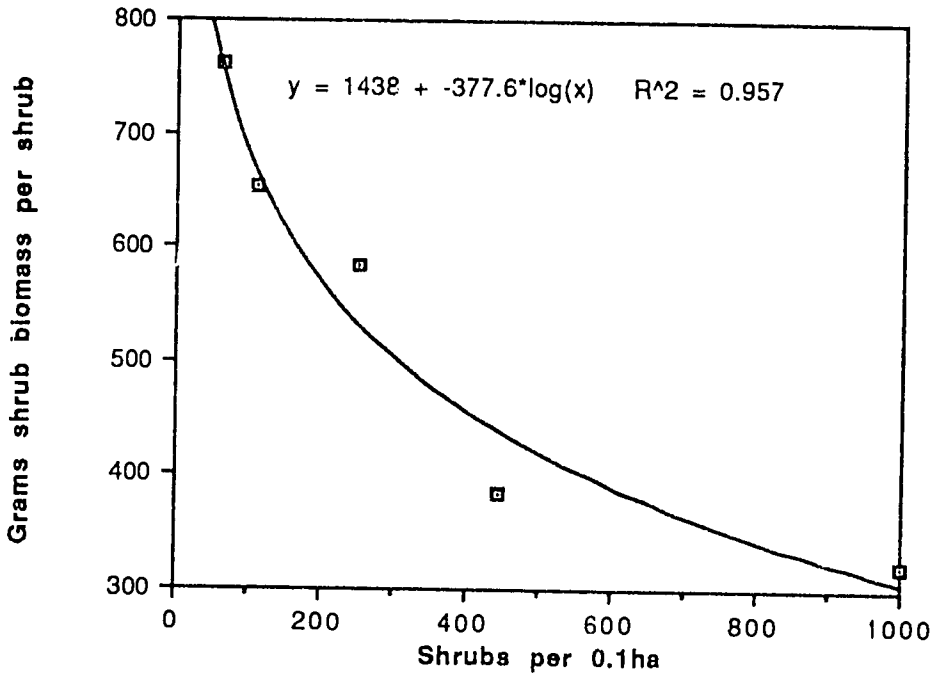


Figure 7

Biomass per Shrub of Atriplex canescens at October 1989, 11 Months After Complete Defoliation



Biomass per 0.1ha of Atriplex canescens at October 1989, 11 Months After Complete Defoliation

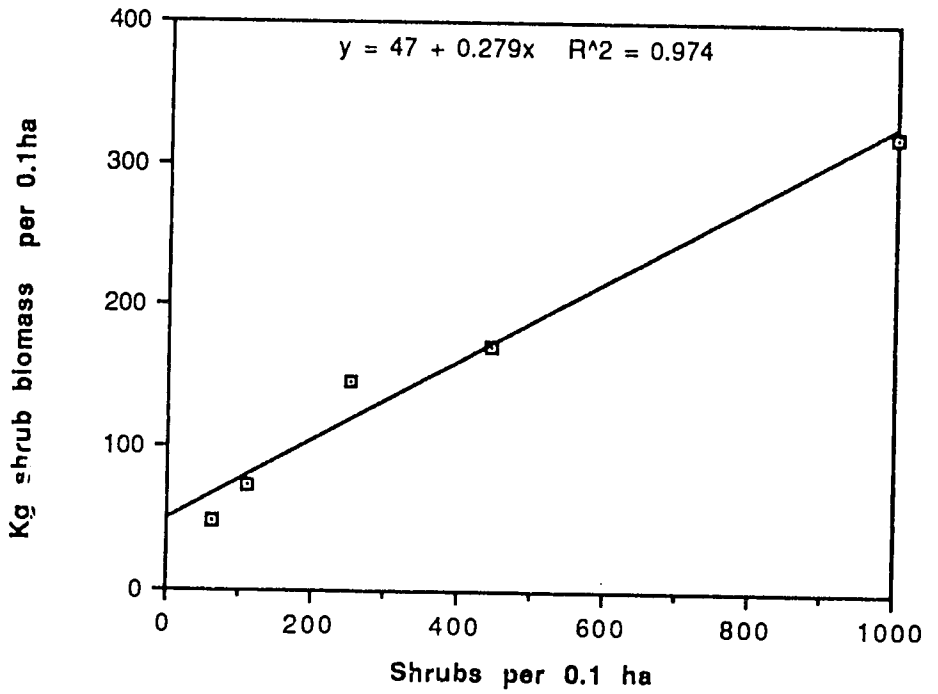
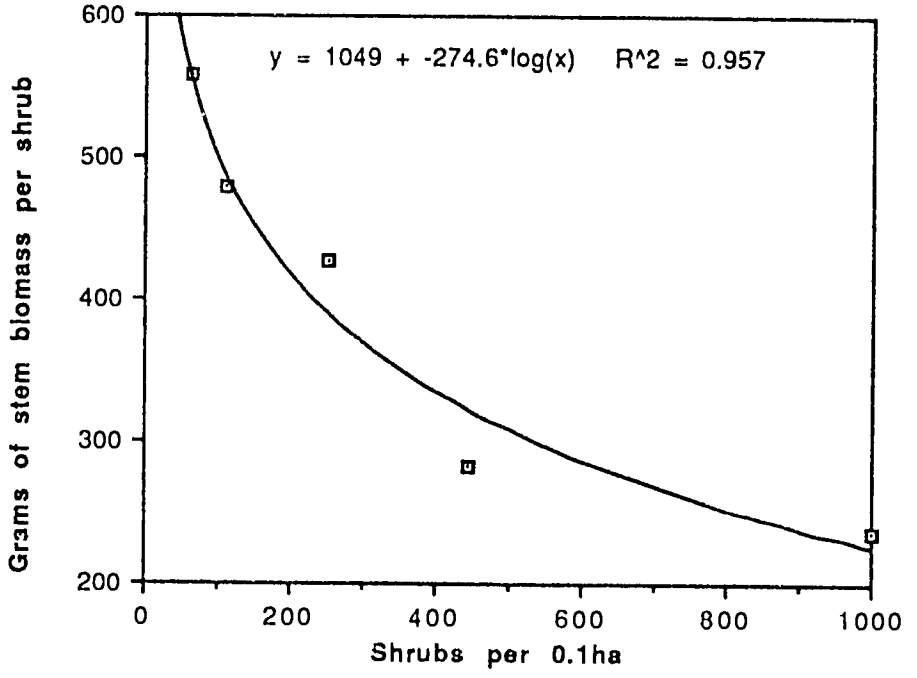


Figure 8

Stem Biomass of Atriplex canescens per Shrub at October 1989, 11 Months After Complete Defoliation



Stem Biomass per 0.1 ha of Atriplex canescens at October 1989, 11 Months After Complete Defoliation

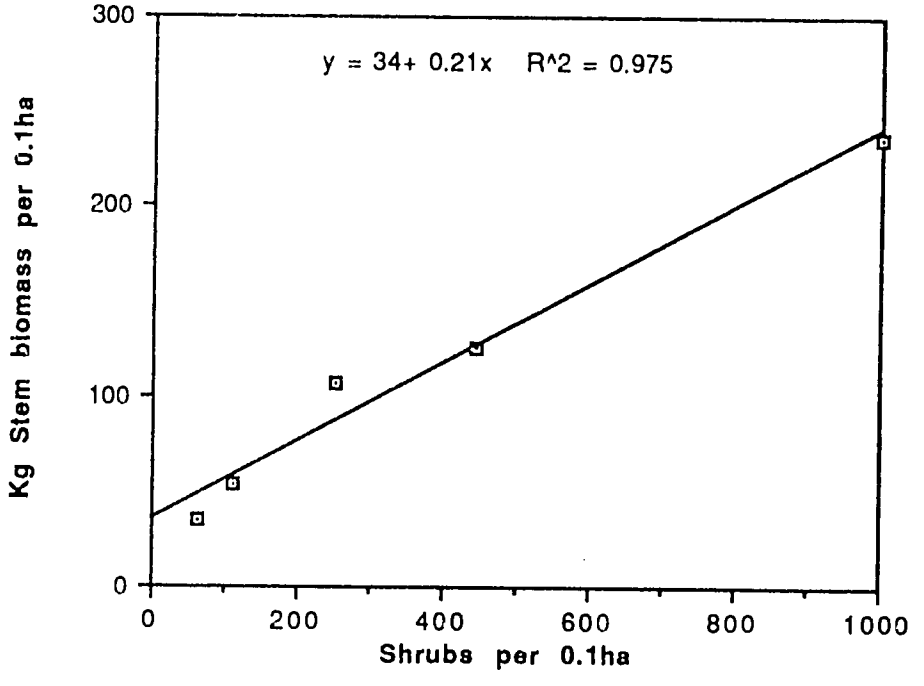
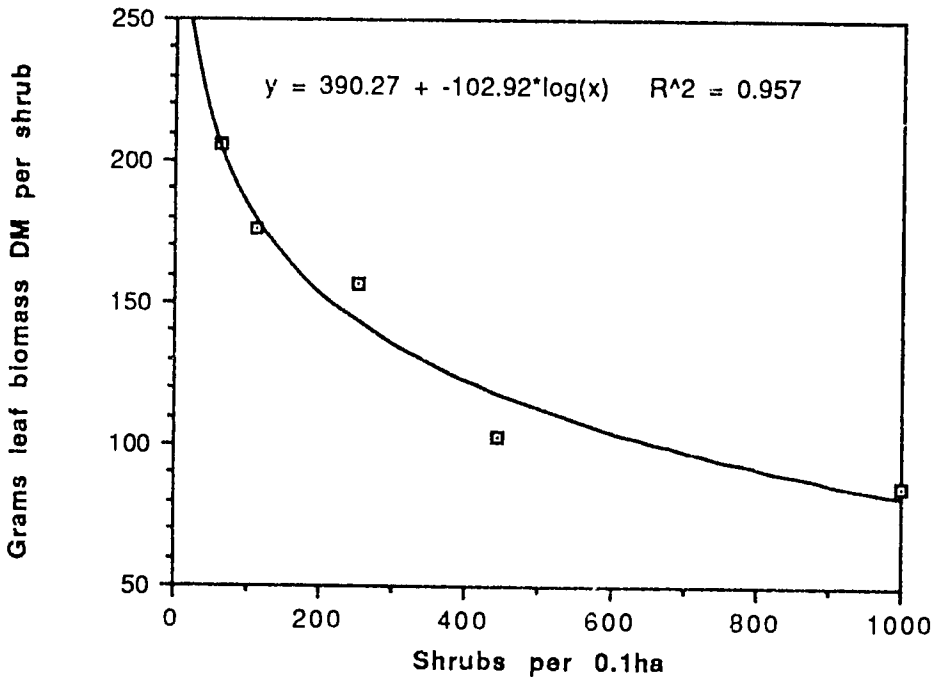


Figure 9

Leaf Biomass per Shrub of Atriplex canescens at October 1989, 11 Months After Complete Defoliation



Leaf Biomass per 0.1ha of Atriplex canescens at October 1989, 11 Months After Complete Defoliation

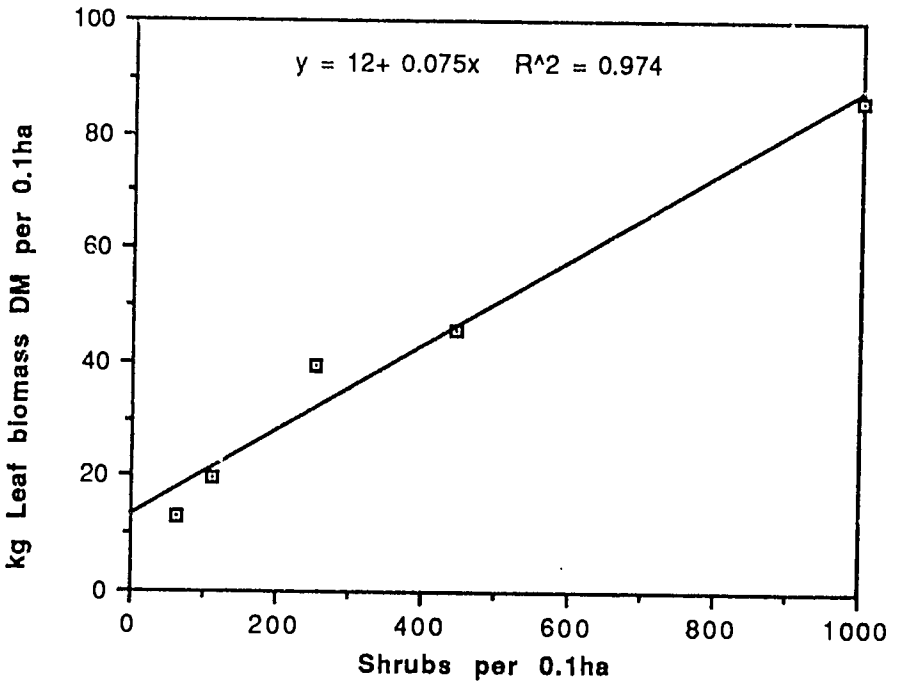
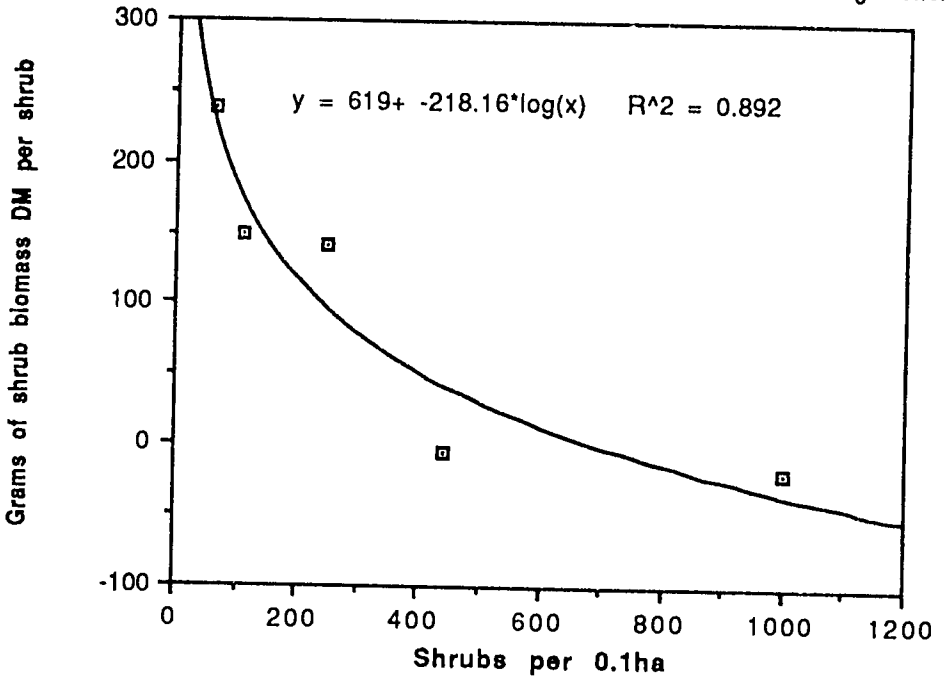


Figure 10

Regrowth per shrub of Atriplex canescens After Grazing at November 1988 and Before Grazing at October 1989 at Different Planting Densities



Shrub Regrowth per 0.1ha of Atriplex canescens After Grazing at November 1988 and Before Grazing at October 1989 at Different Planting Densities

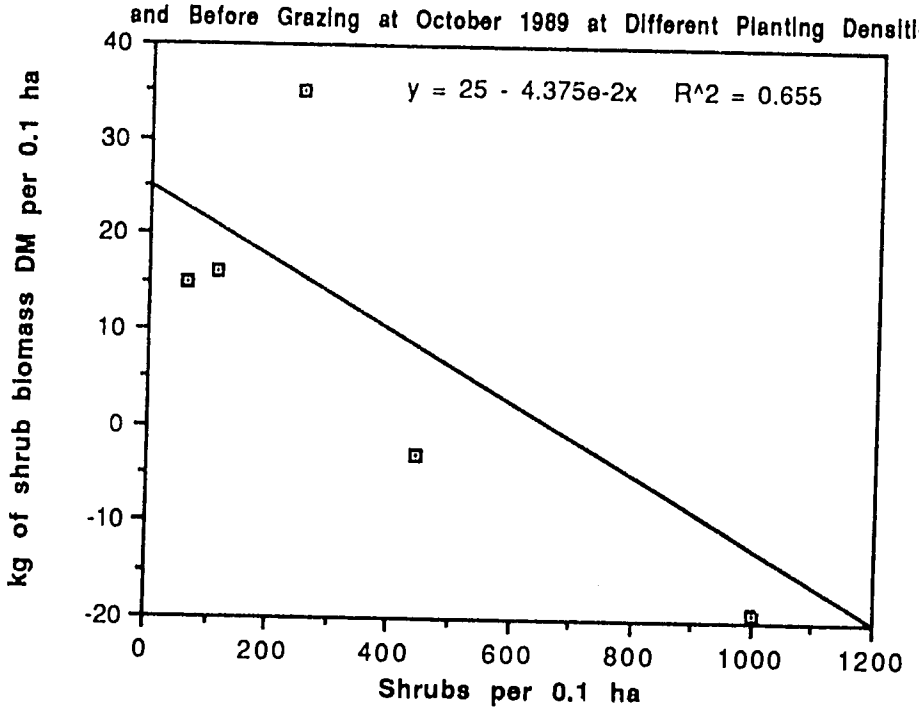
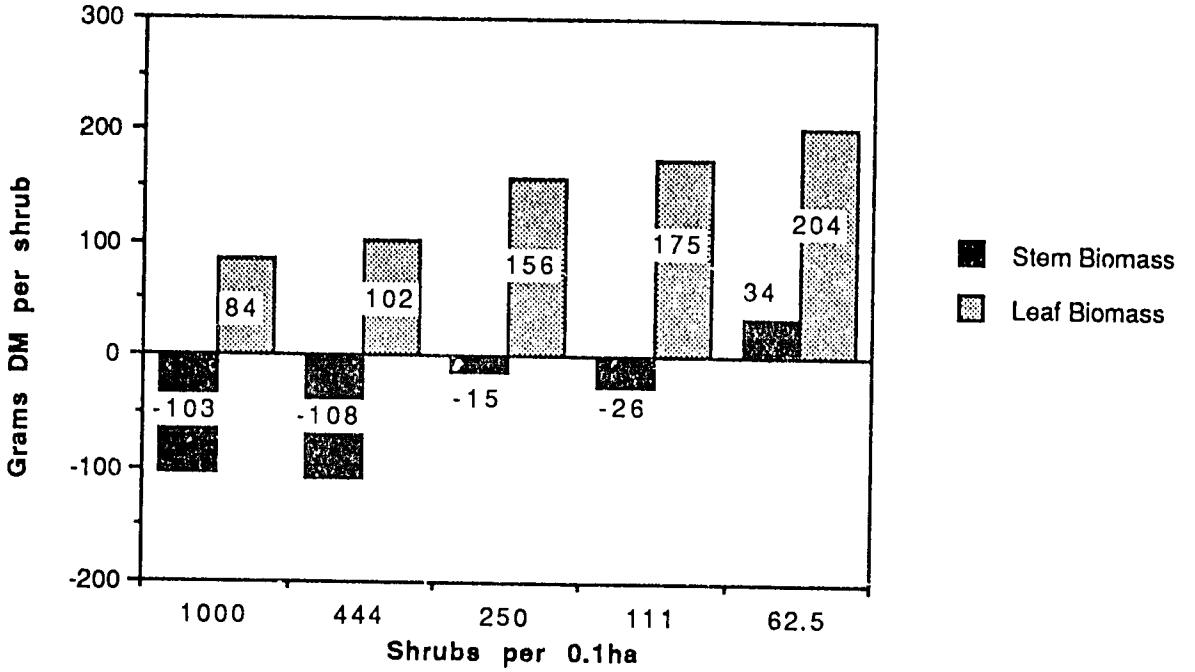


Figure 11

Composition of the Regrowth per Shrub of Atriplex canescens After Grazing at November 1988 and Before Grazing at October 1989 at Different Planting Densities



Composition of Atriplex canescens Regrowth per 0.1 ha After grazing at November 1988 and Before Grazing at October 1989 at Different Planting Densities

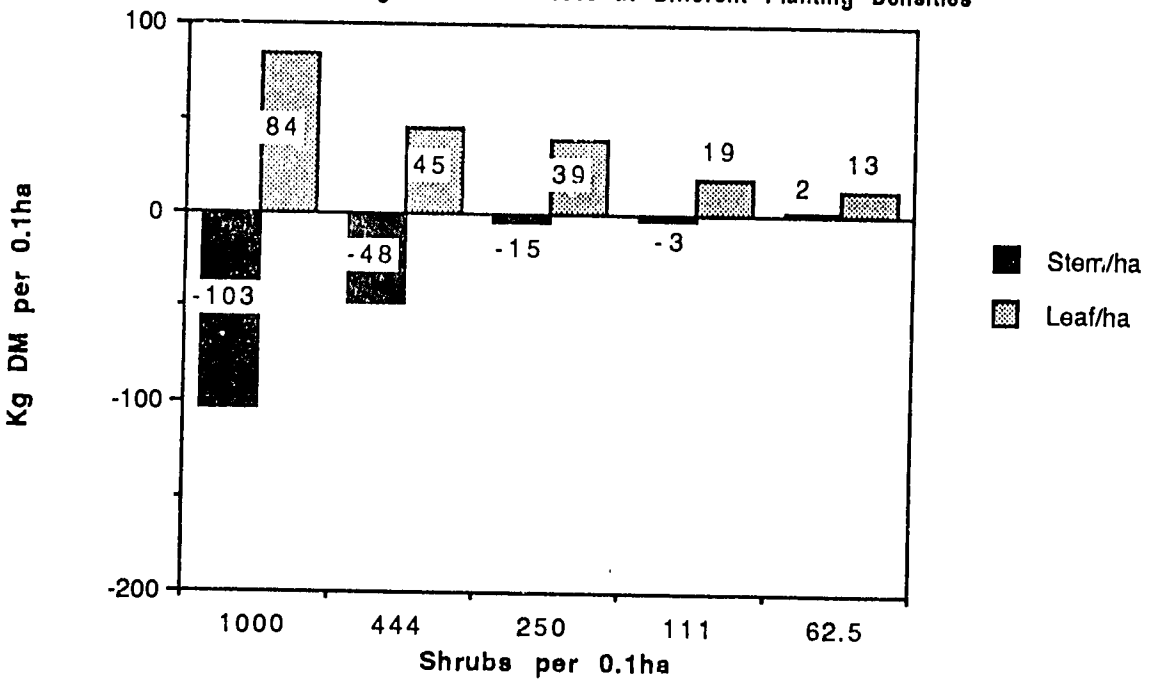
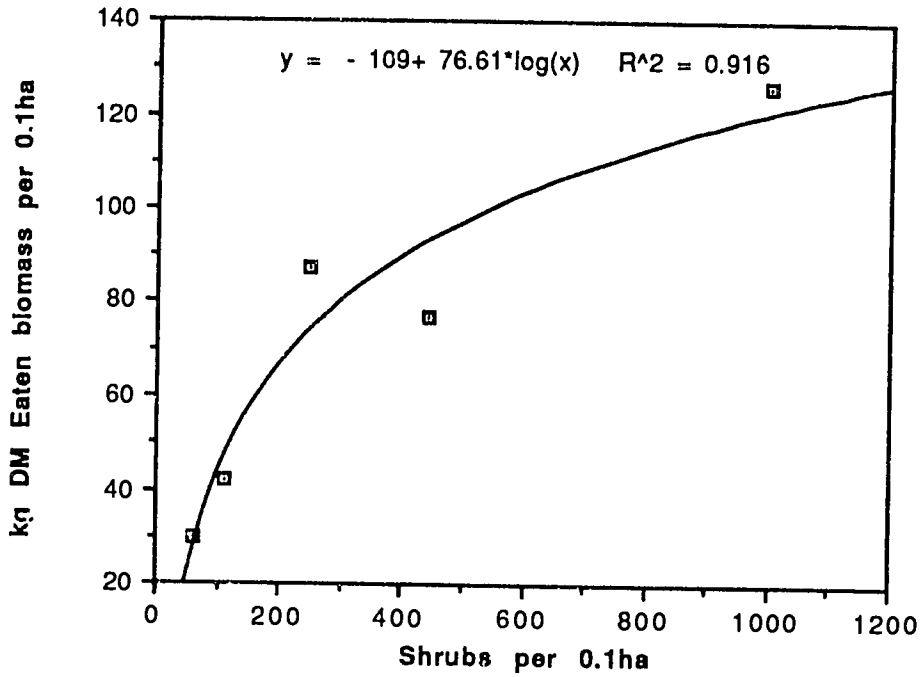


Figure 12

Eaten Biomass per 0.1 ha at November 1989 From Atriplex canescens Planted at Different Densities



Composition of Eaten Biomass per 0.1 ha at November 1989

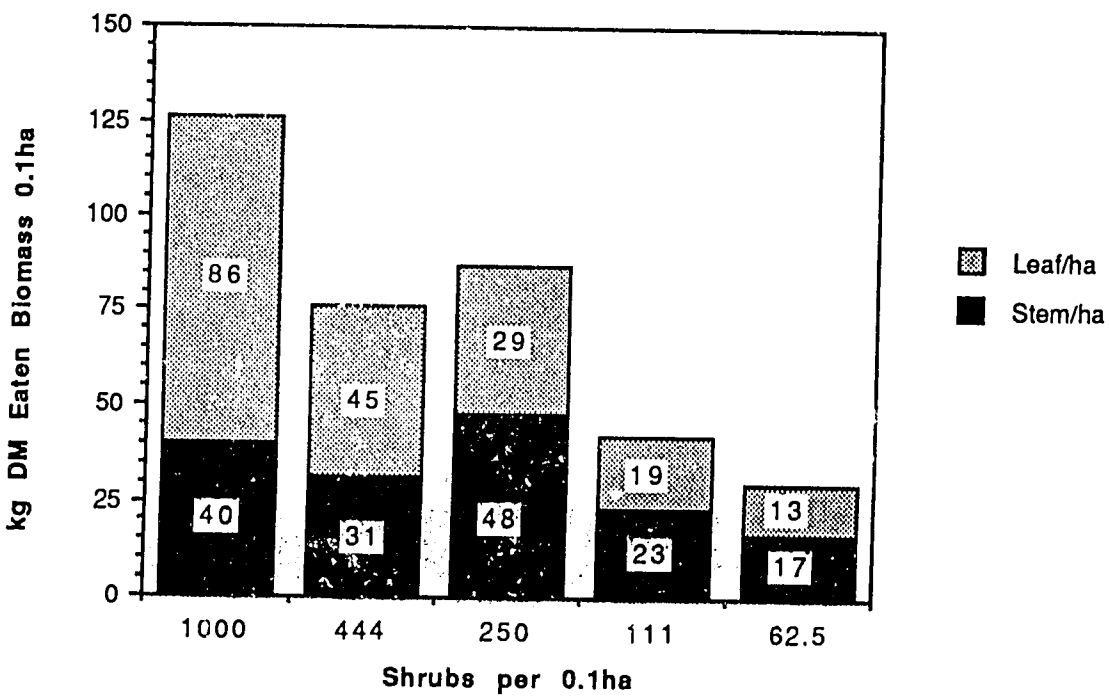
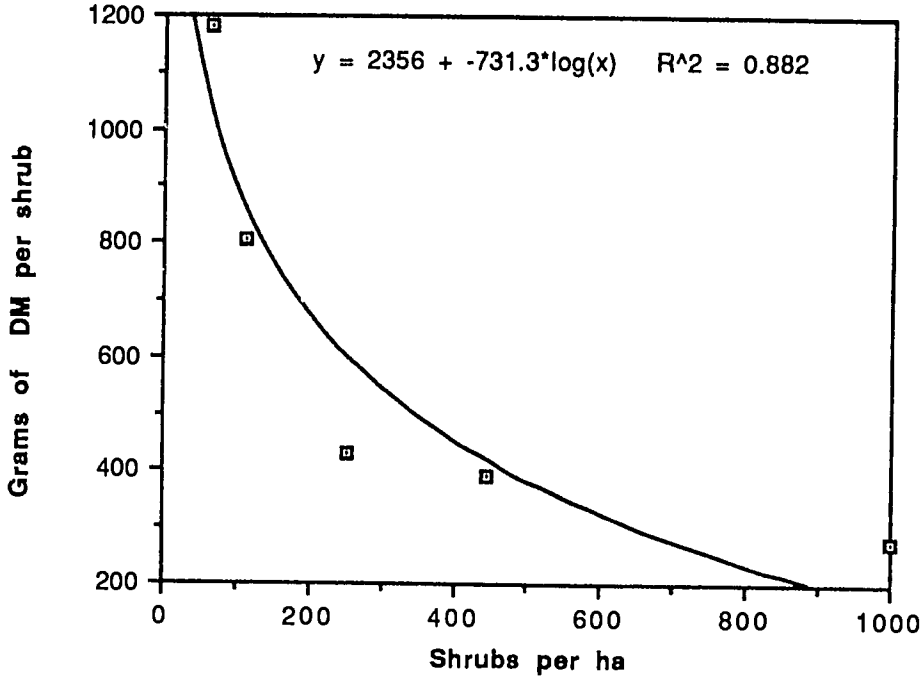


Figure 13

Biomass per Shrub of Cassia sturtii at October 1989, 11 Months After Complete Defoliation



Biomass per 0.1ha of Cassia sturtii at October 1989, 11 Months afer Complete Defoliation

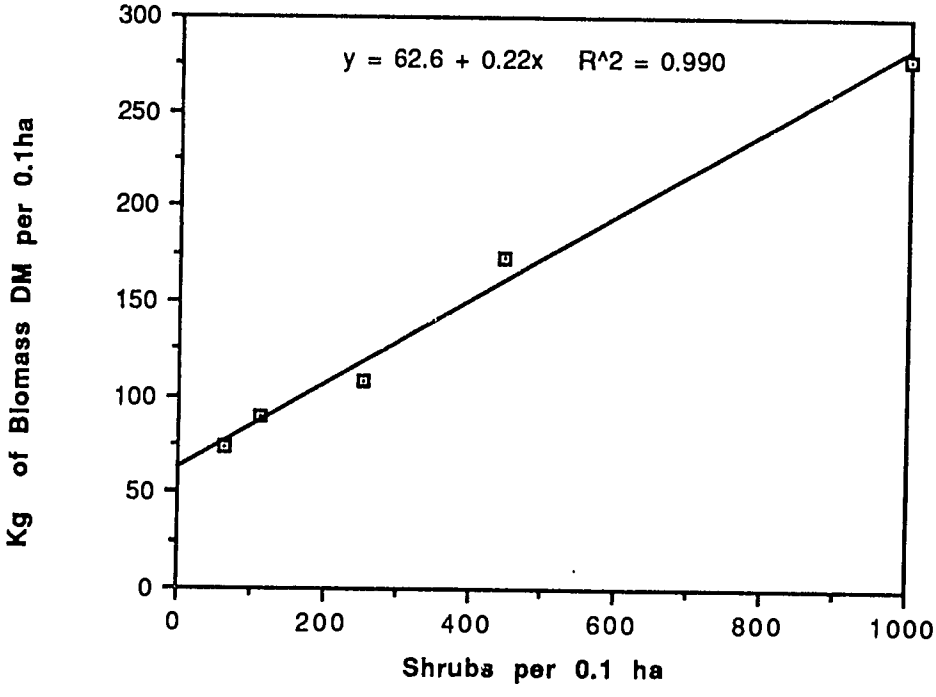
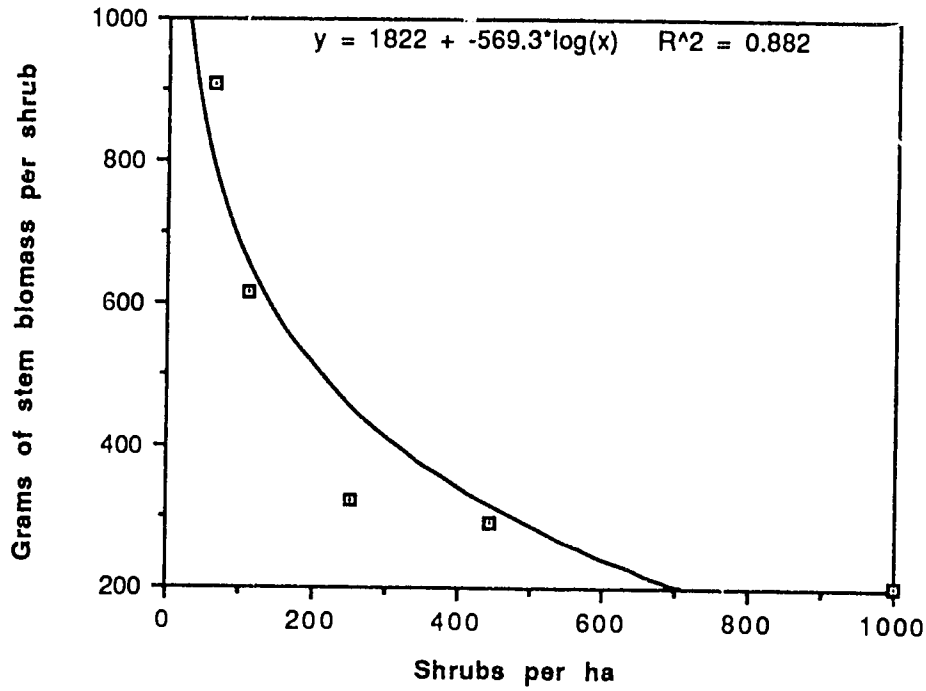


Figure 14

Stem Biomass per Shrub of Cassia sturtii at October 1989, 11 months After Complete Defoliation



Stem Biomass per 0.1ha of Cassia sturtii at October 1989, 11 Months After Complete Defoliation

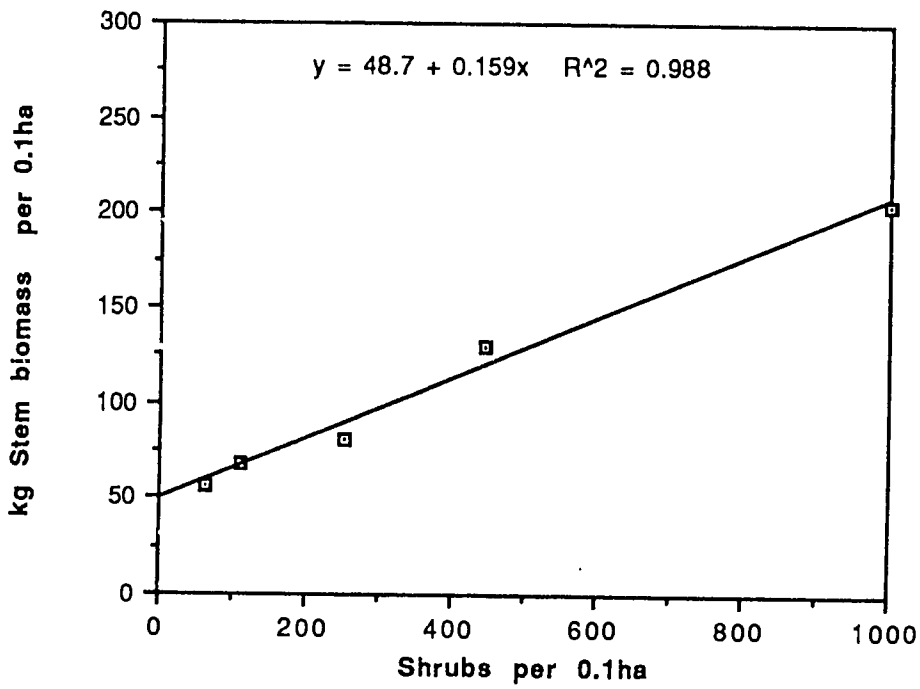
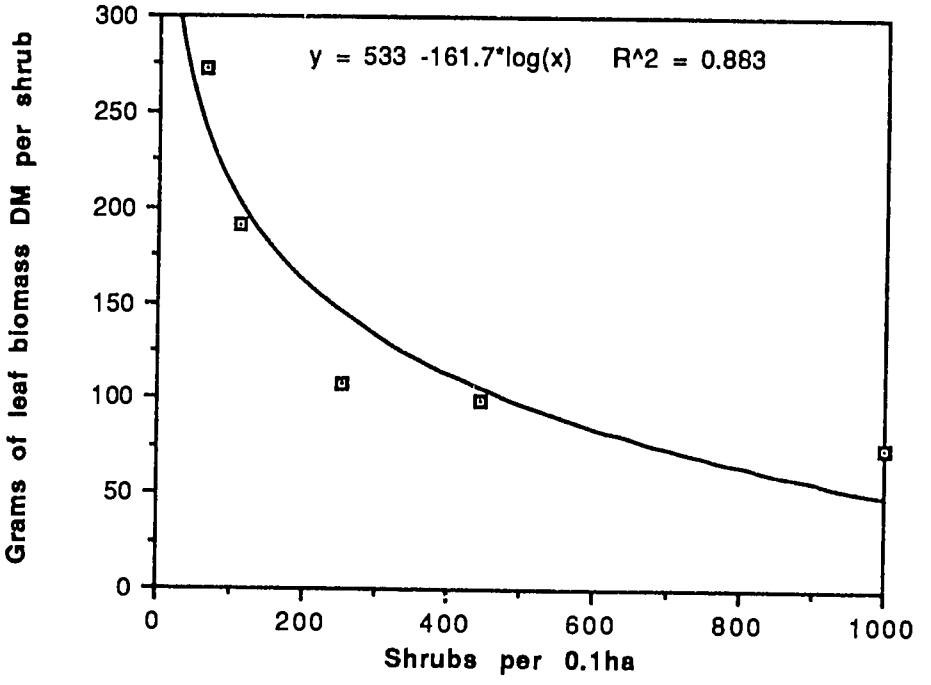


Figure 15

Leaf Biomass per Shrub of Cassia sturtii at October 1989, 11 Months After Complete Defoliation



Leaf Biomass per 0.1ha of Cassia sturtii at October 1989, 11 Months After Complete Defoliation

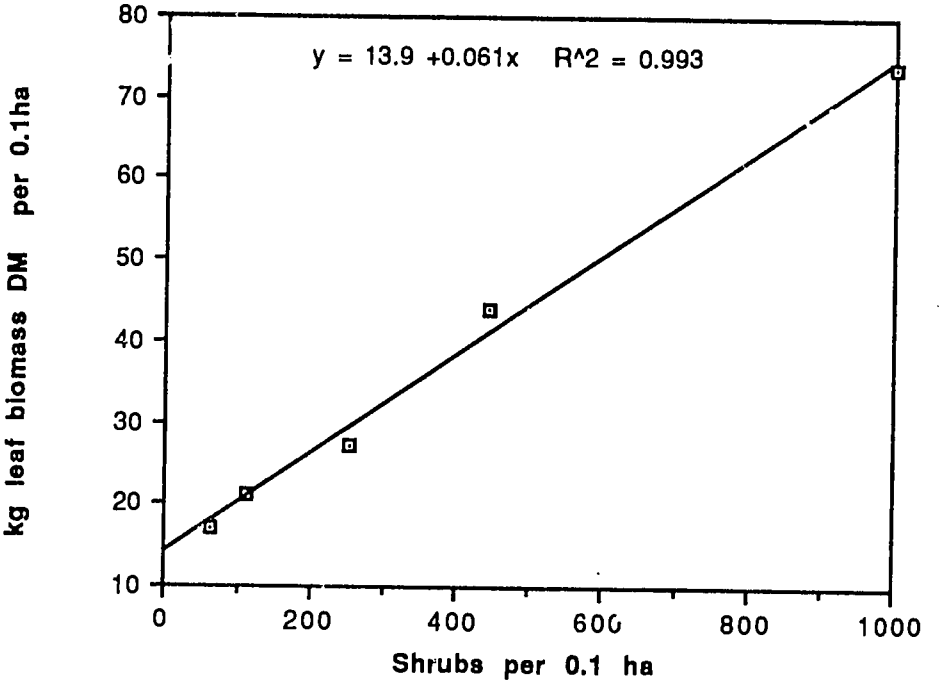
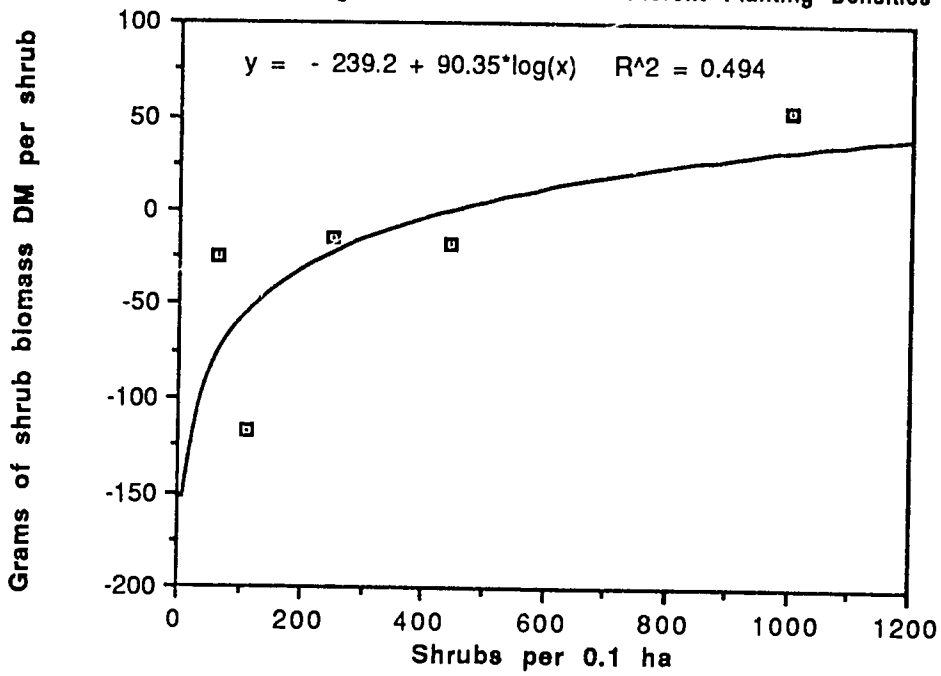


Figure 16

Regrowth per Shrub of Cassia sturtii After Grazing at November 1988 and Before Grazing at October 1989 at Different Planting Densities



Regrowth per 0.1 ha of Cassia sturtii After Grazing at November 1988 and After Grazing at October 1989 at Different Planting Densities

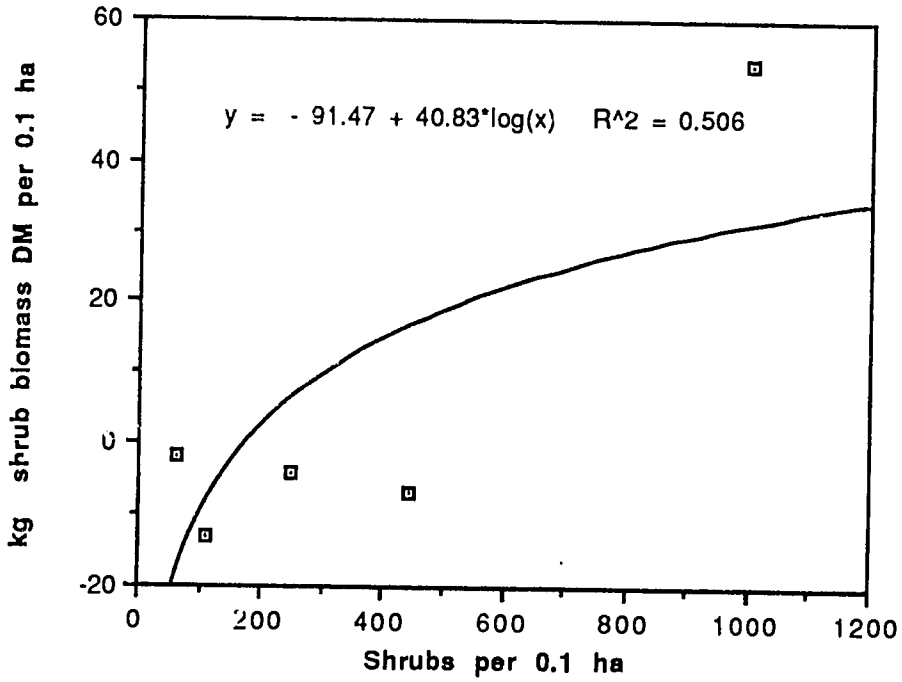
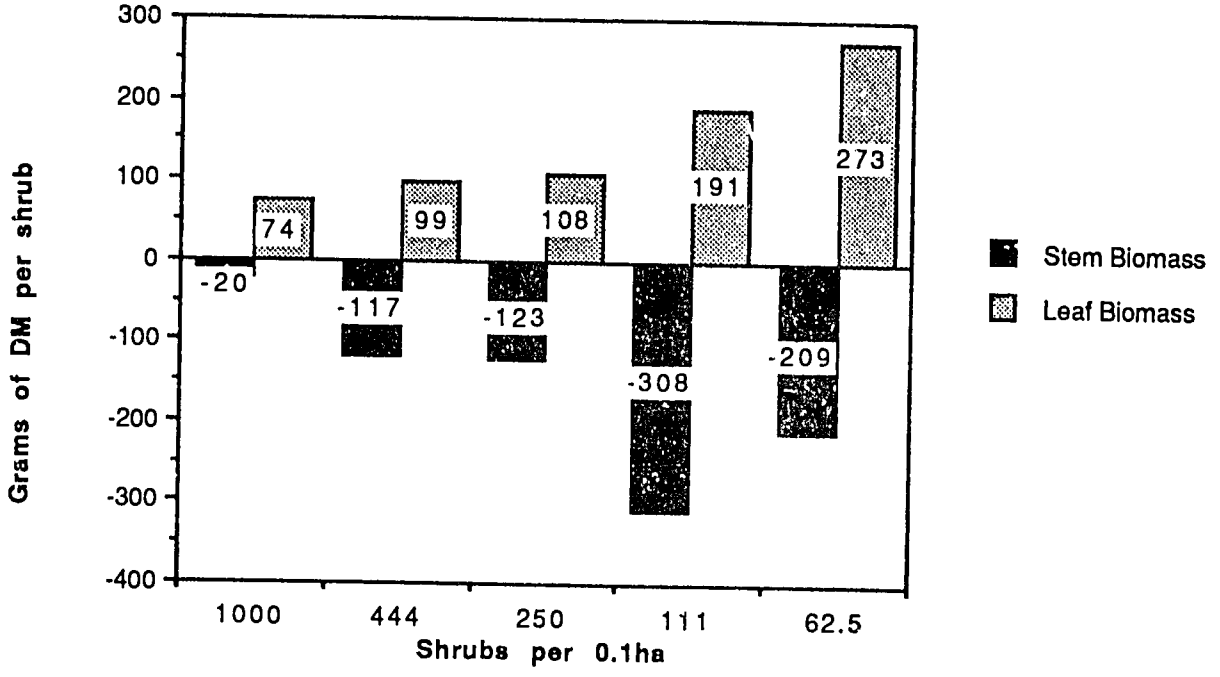


Figure 17

Composition of Cassia sturtii per Shrub Regrowth after Grazing at November 1988 and Before Grazing at October 1989 at Different Grazing Densities



Composition of the Regrowth per 0.1 ha of Cassia sturtii After Grazing at November 1988 and Before Grazing at October 1989 at Different Planting Densities

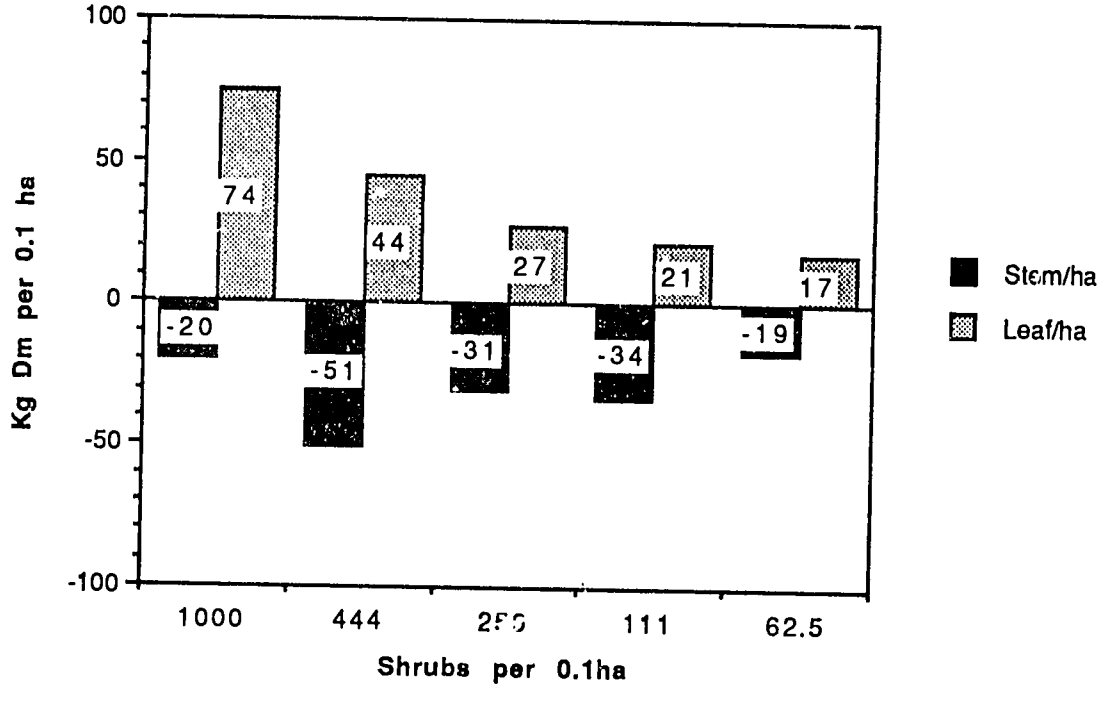
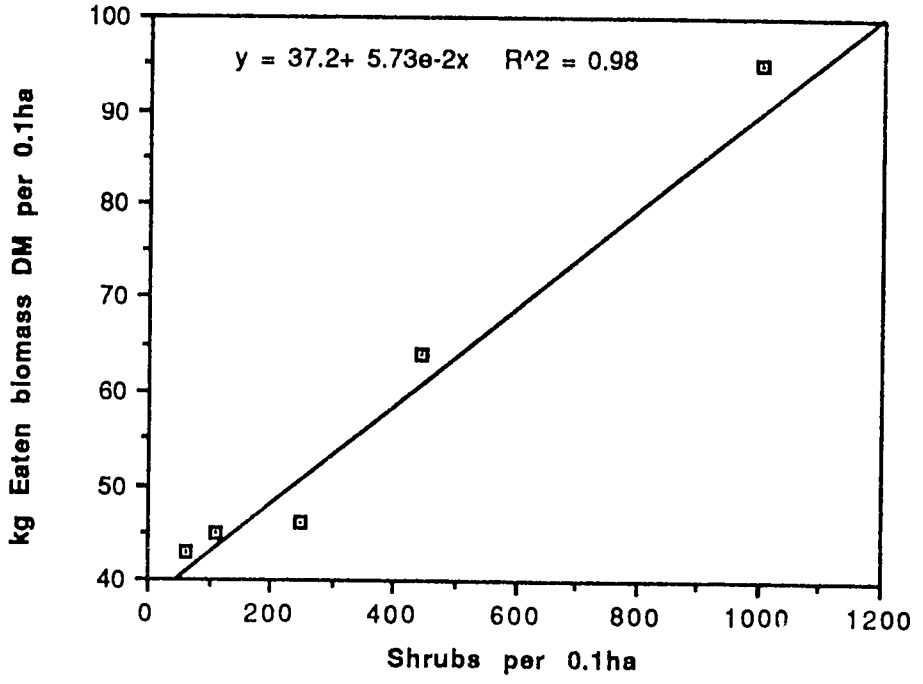


Figure 18

Eaten Biomass per 0.1 ha at November 1989 From Cassia sturtii Planted at different Planting Densities



Composition of Eaten Biomass From Cassia sturtii at November 1989 Planted at Different Planting Densities

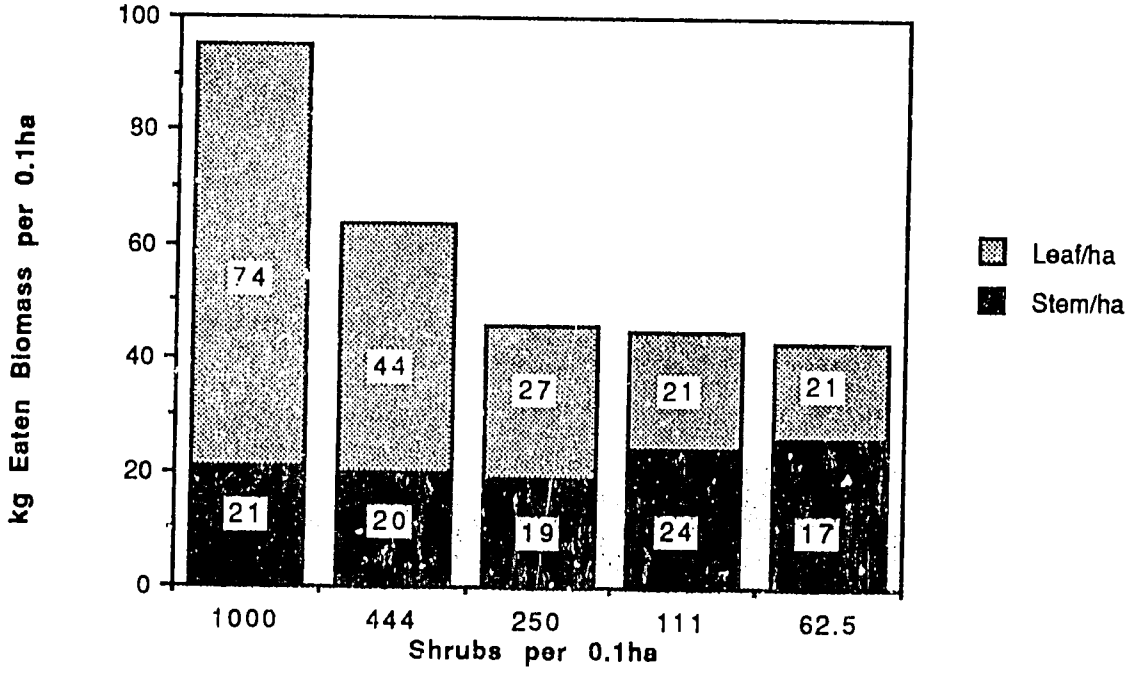


Figure 19

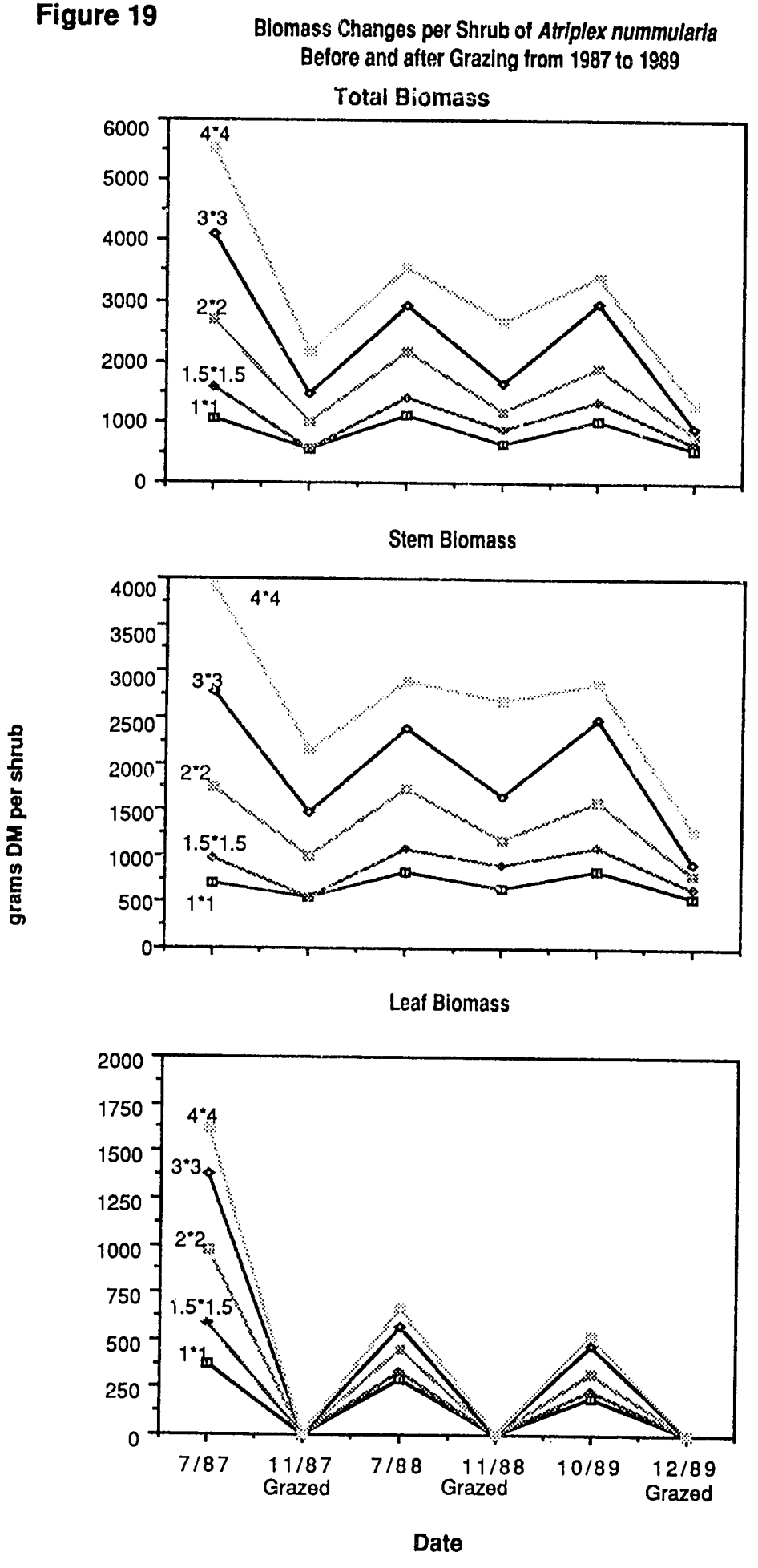

Biomass Changes per Shrub of *Atriplex nummularia*
Before and after Grazing from 1987 to 1989
Total Biomass
6000
5000
4000
3000
2000
1000
0
4*4
3*3
2*2
1.5*1.5
1*1
Stem Biomass
4000
3500
3000
2500
2000
1500
1000
500
0
4*4
3*3
2*2
1.5*1.5
1*1
grams DM per shrub
Leaf Biomass
2000
1750
1500
1250
1000
750
500
250
0
4*4
3*3
2*2
1.5*1.5
1*1
7/87
11/87
Grazed
7/88
11/88
Grazed
10/89
12/89
Grazed
Date

Figure 20

Changes in the Dimensions of *Atriplex nummularia* Shrubs Before and After Grazing 1987 to 1989

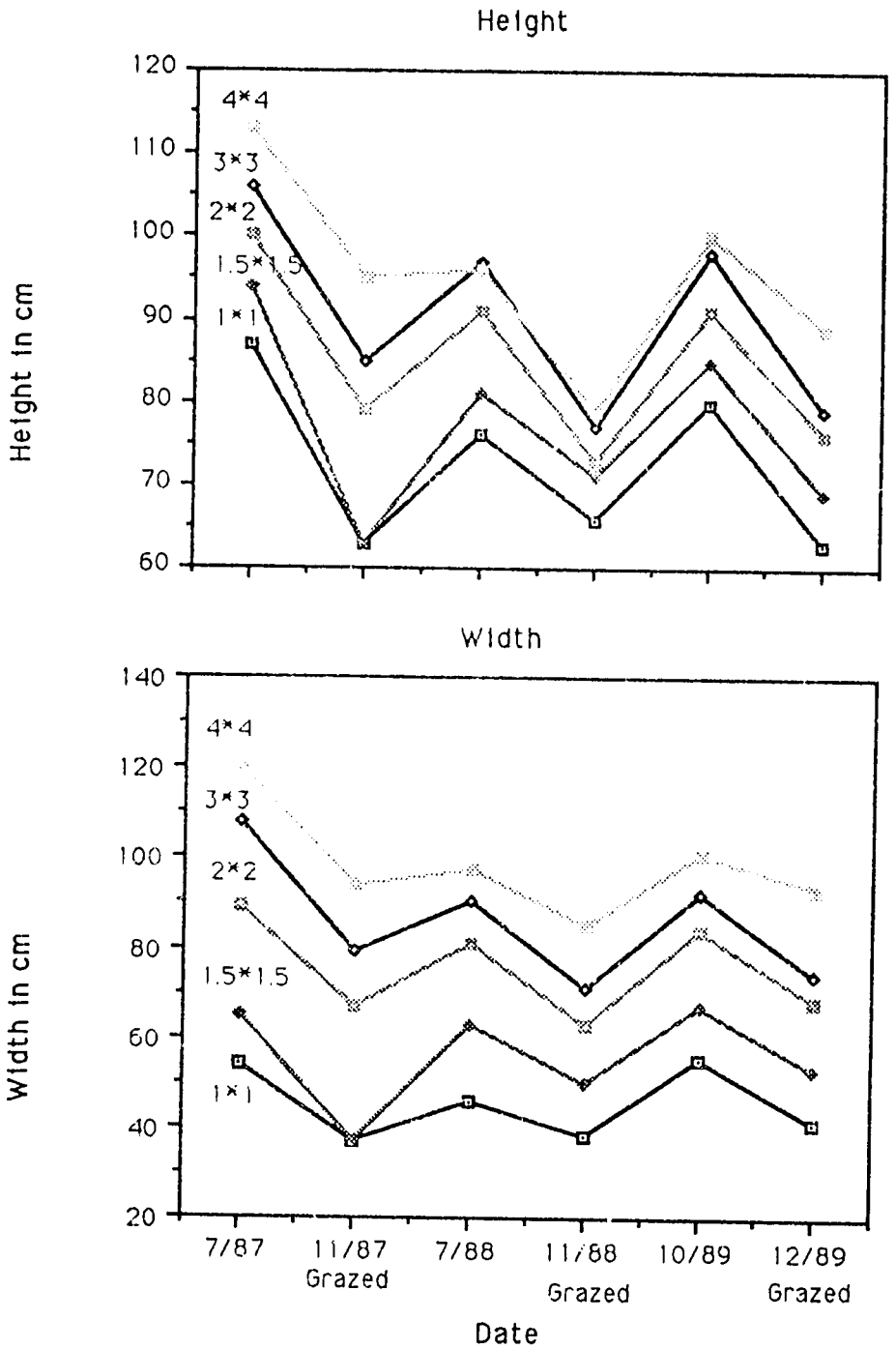


Figure 21

Changes In Shrub Biomass of *Atriplex canescens* Before and After Grazing 1987 to 1989

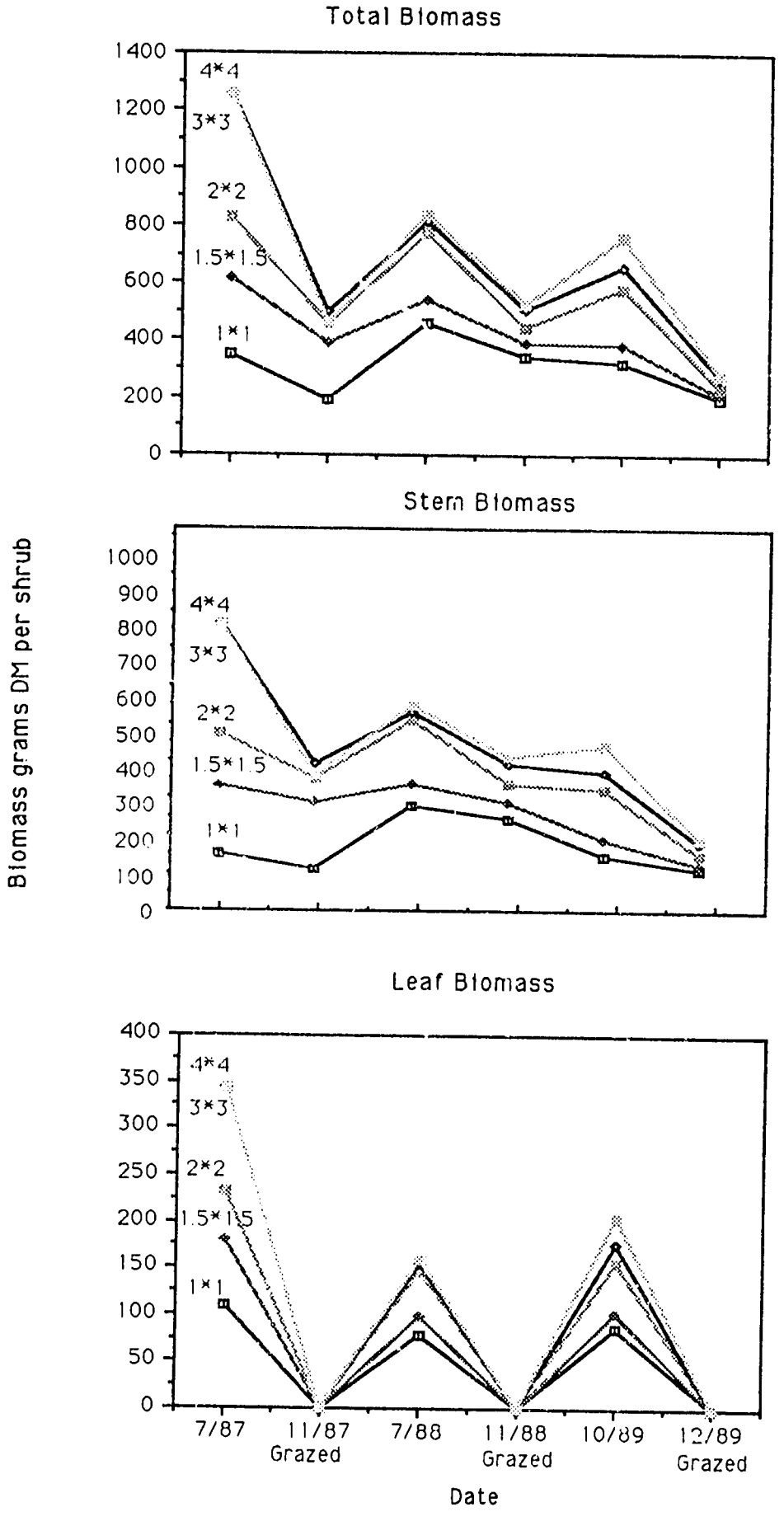


Figure 22

Changes In the Dimensions of *Atriplex canescens* Before and After Grazing 1987 to 1989

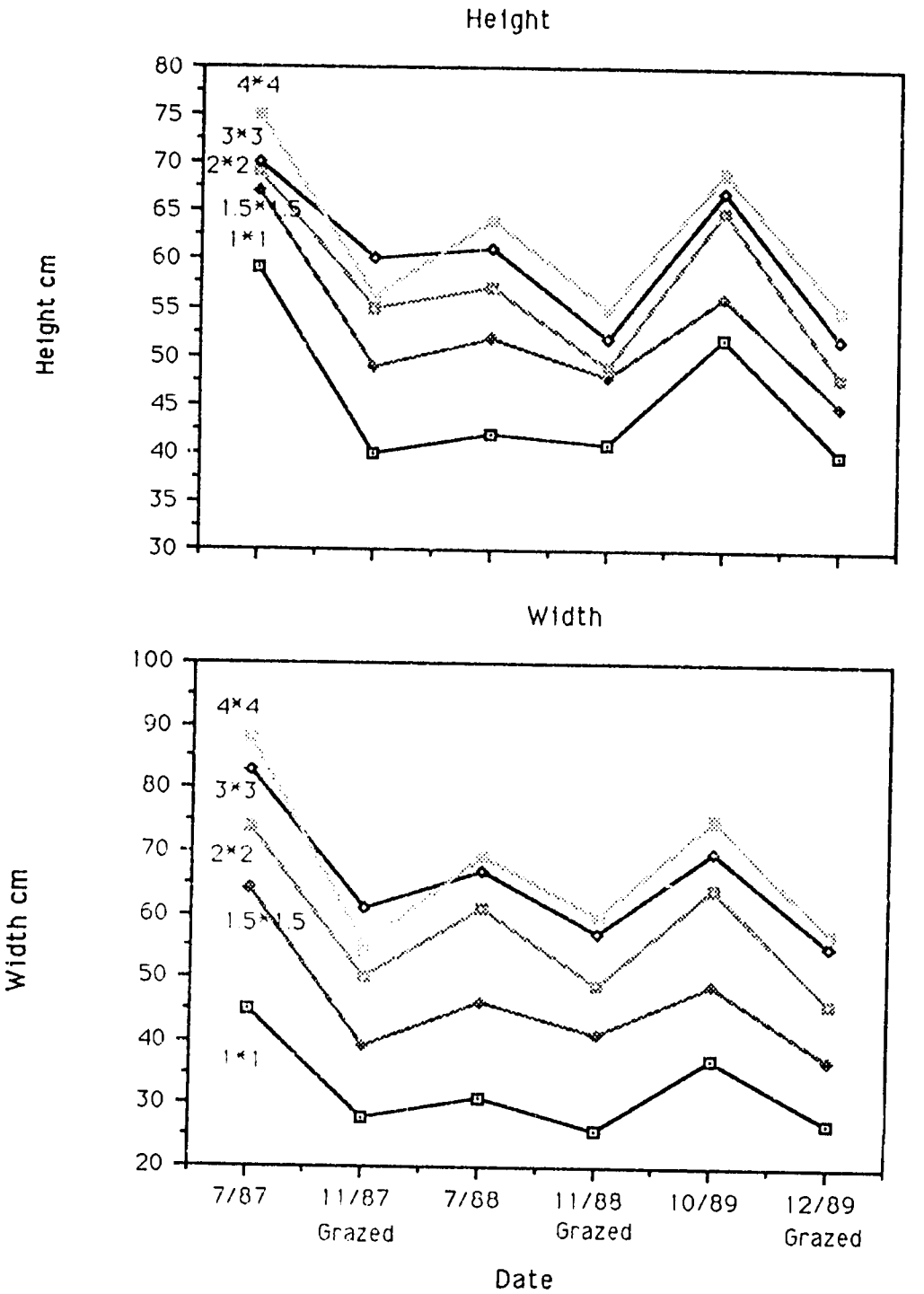


Figure 23

Changes In Shrub Biomass of *Cassia sturtii* Before and After Grazing 1987 to 1989

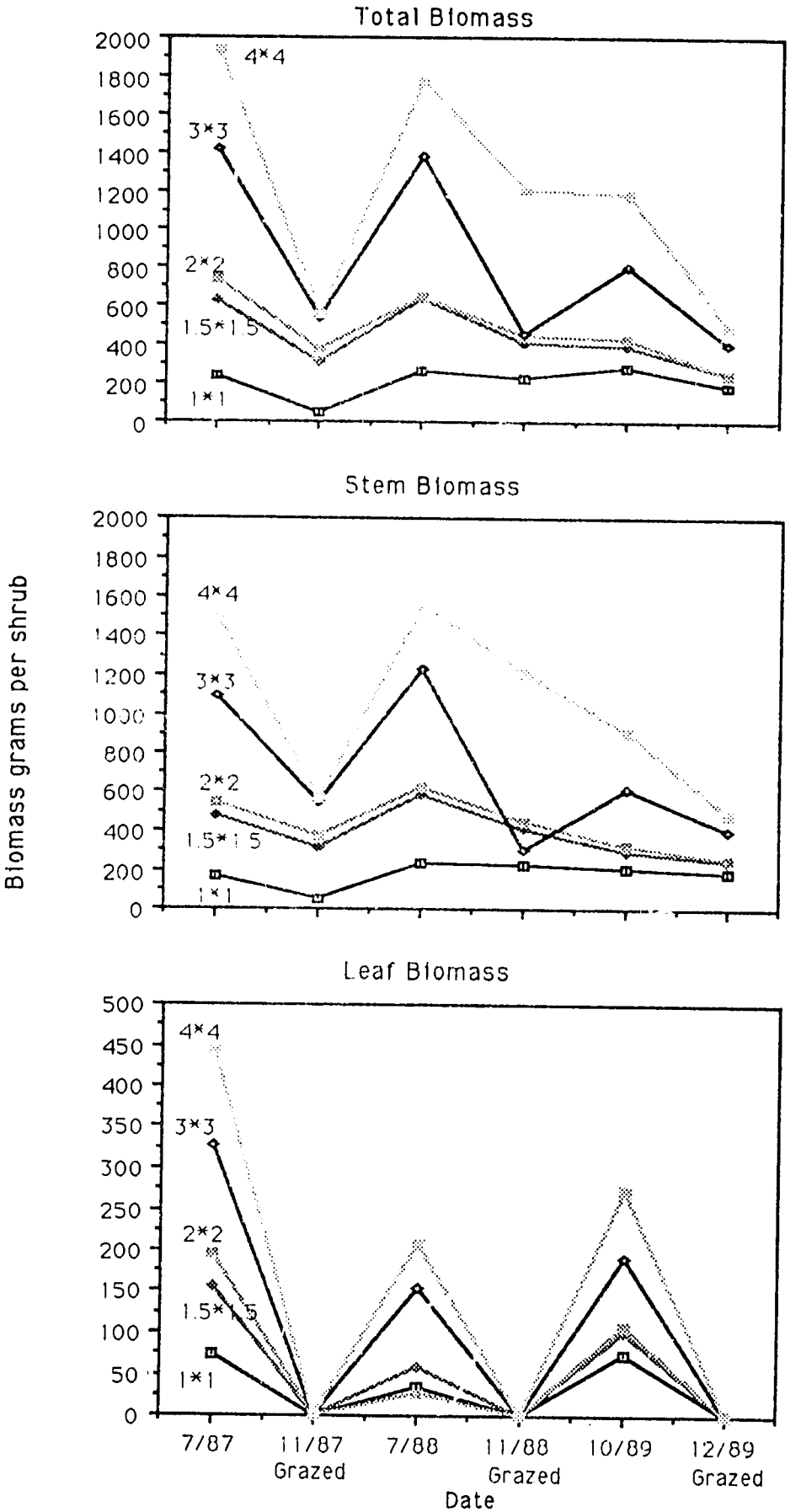
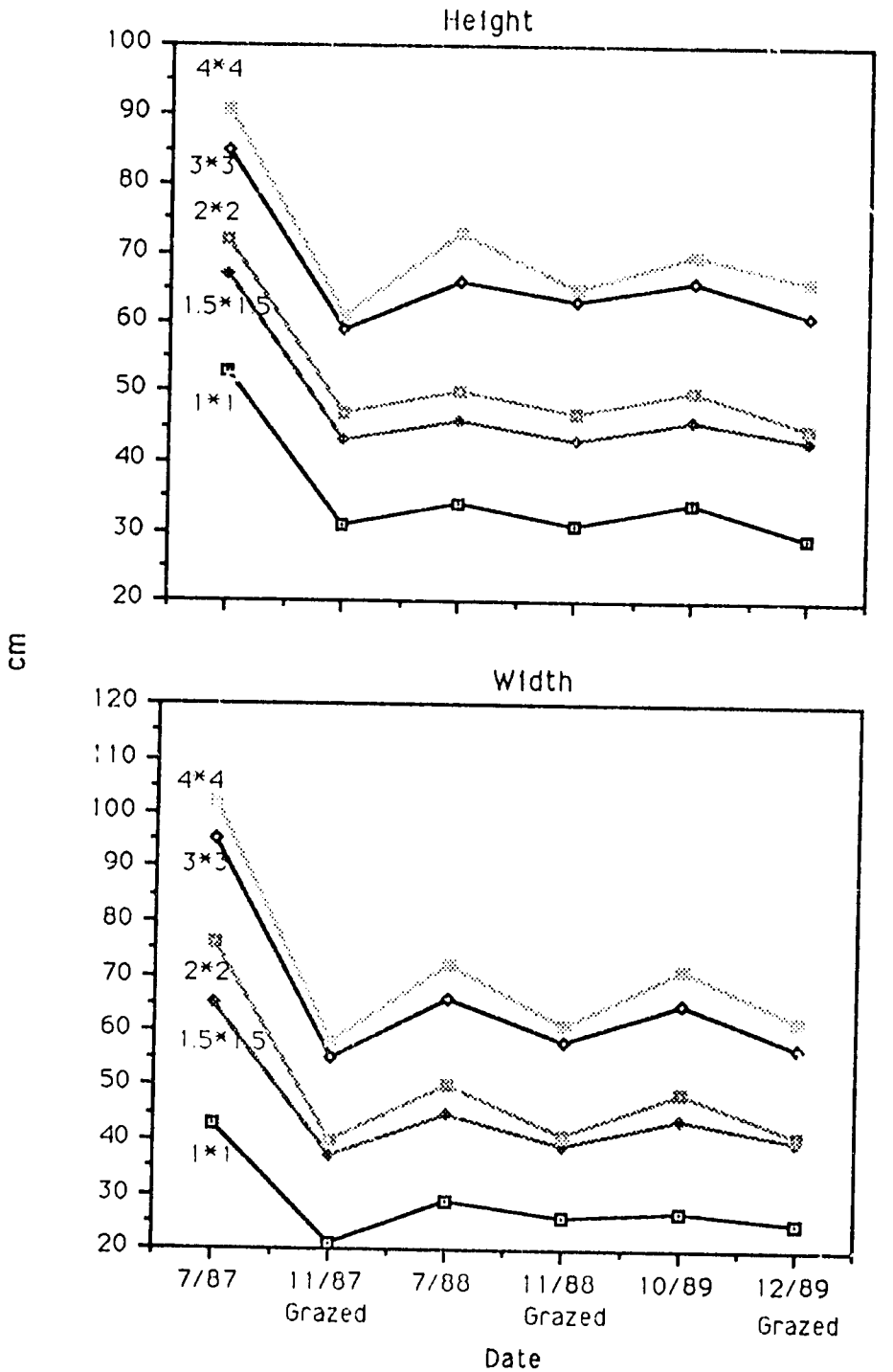


Figure 24

Changes in the Dimensions of *Cassia sturtii* Before and After Grazing 1987 to 1989



EFFECT OF INOCULATION WITH *AZOSPIRILLUM BRASILENSE* STRAIN CD AND *RHIZOBIUM* ON THE ROOT MORPHOLOGY OF BURR MEDIC (*MEDICAGO POLYMORPHA* L.)

Eli Yahalom, Amos Dovrat, Yaacov Okon and Hanoch Czosnek

ABSTRACT

The inoculation of burr medic seedlings grown in pouches with *Azospirillum brasilense* Cd at a concentration of 10^6 cfu/mL significantly increased the number of lateral roots and main root diameter but not the length of roots in the presence or absence of *Rhizobium*. At *Azospirillum* concentrations exceeding 10^8 cfu/mL root elongation was inhibited, although less when combined with *Rhizobium*. Exogenous indole acetic acid (IAA) or benzyl adenine (BA) concentrations of 10^{-8} M stimulated root elongation. Concentrations exceeding 10^{-6}M inhibited root elongation and shortened the length of the root elongation zone. Root hair density was not affected following inoculation with *Azospirillum*, although the number of root hairs per unit of root length increased as a result of the increase in the root circumference. In cross sections taken near the root tip shortly after inoculation with *Azospirillum* (10^9 cfu/ml), larger cortical cells were observed; however their number (in cross section) did not increase compared with the controls. The DNA concentration of root segments formed 24 h after inoculation with 10^9 cfu/mL *Azospirillum* was significantly lower than in roots inoculated with a concentration of 10^8 cfu/mL or the controls. From this finding it was concluded that the reduction in root growth may have resulted from decreased cell division in the apical meristem of the root. The purpose of this research was to study the morphology and anatomy of medic roots following inoculation with *Azospirillum* in the presence or absence of *Rhizobium* and the possibility of mimicking some of these effects by applying auxin and cytokinin.

Keywords: *Azospirillum, Rhizobium, Medicago polymorpha,* root morphology, inoculation

MATERIALS AND METHOD

Organisms and growth conditions - *Rhizobium meliloti* RT-1 was obtained from the South Africa Rhizobium Collection, Rietondale Research Farm, Pretoria. Cultures used for inoculation were grown during 3 days in yeast mannitol broth (YMB). (Vincent 19780) at 30°C in 250 mL Erlenmeyer flasks on a rotary shaker (110 spm). The cells were harvested by centrifugation at 12,000 g and washed twice with sterile distilled

water. *Azospirillum brasilense* Cd (ATCC-29729) was grown on malate liquid medium (Okon et al. 1977) supplemented with 0.25% NH4Cl and 0.01% yeast extract for 20 h at 30°C. Cells were harvested at the late logarithmic growth phase by centrifugation (1,000g) and were washed twice in 30 mM of potassium phosphate-buffered saline (pH 7). The bacterial suspensions that were used for inoculation were diluted to the appropriate number of cells/mL.

Growth of seedlings - Seeds of burr medic (*Medicago polymorpha*) were obtained from the Dept. of Field Crops, Volcani Center, Bet Dagan. The seeds were disinfected as described in Yahalom *et al.* (1987). Swollen seeds were germinated on yeast extract mannitol agar in petri dishes in upside down position. Seedlings free from microbial contamination (bacterial colonies) were transferred to:

1. Paper-towel lined plastic growth pouches, 16x17 cm (diSPo Seed-Pack, Scientific Products, Evanston, IL.) which previously were moistened with 9 mL of sterilized half-strength free Jensen's medium (Vincent 1970). Tiny holes were made in the top of the paper towel prior to inserting the roots of the germinating seeds. Four seedlings were placed in each pouch. All of the above operations were carried out under aseptic conditions. The growth pouches with seedlings were placed in boxes in an upright position, covered with aluminium foil until inoculation to prevent drying and to maintain aseptic conditions. The boxes with seedlings were placed in a growth chamber with 50-70% RH, 25°C light and 25°C dark, 13 h photoperiod with a light intensity at bench level of 150 mE m^{-2} sec^{-1}. The paper towels were kept moist with sterile water throughout the experiment.
2. Petri dishes (9.0 cm diam. X 1.3 cm deep) lined with filter paper moistened with 2 mL of sterile tap water. The tip of each root (RT) was marked by a small piece of paper and the inoculation was carried out as described above. This technique was used in experiments where DNA determinations were made.

Marking and inoculation - The position of the RT was marked at the time of inoculation with a waterproof marking pen by viewing through a stereo microscope. In most experiments *Azospirillum* was applied either alone or together with *Rhizobium*.

Seedlings were inoculated by applying 0.5 mL bacterial suspension of *Rhizobium* or *Azospirillum* to the roots of the seedlings in each pouch. The controls received the identical amount of culture medium.

Indolacetic acid (IAA) and the cytokinin benzyl adenine (BA) (Sigma) were dissolved in 1 M KOH and were diluted to the appropriate concentrations with 30 mM of potassium phosphate buffer pH 7.

Scanning Electron Microscopy (SEM) - Root segments were fixed for 5 h in 3% glutaraldehyde solution and dehydrated by sequential transfer of samples into 30, 50, 70, 90, and 100% acetone diluted with 0.025 M phosphate buffer for 20 min and then to 100% acetone for 1 h. Root segments were dried in a CO_2 atmosphere in a Polaron Critical Point Dryer and coated with gold by a diode sputtering system (type E-5000). The root segments were examined using a Jeol JSM 35 SEM.

Light microscopy - The root segments were fixed as described above and then after one wash with phosphate buffer were postfixed with 1% cold OsO4 (Sigma) in phosphate buffer for 1 h and washed overnight. After dehydration with ethanol, root segments were embedded in an Epon 812 medim and polymerized 3 days at 60°C. Thin section (5μm) were then stained with methylene blue and examined with the "Galai CIS-2 microscope image analyzer" computerized inspection system (Galai Ltd., Israel).

DNA estimation - Equal numbers of roots frozen in liquid nitrogen were ground to powder. DNA was extracted according to the CTAB detergent method after Taylor and Powell (1982). It was electrophorized on a 1% agarose gel, blotted and hybridized (Southern 1975). The probe was purified genome DNA from burr medic, radiolabelled with α^{32}p dCTP (Amersham) by nick-translation (Rigby *et al.* 1977). The DNA amounts were estimated according to Czosnek et al. (1988), after scanning autoradiograms with a Bio-Rad densitometer, model VD 620.

RESULTS

Inoculation with *Azospirillum* of up to 10^7 cfu/mL to pouch-grown seedlings, in the absence or presence of *Rhizobium*, did not affect root length (Fig. 1A, B). However at higher concentrations of *Azospirillum* root elongation was inhibited, though less in the presence of *Rhizobium*. The influence of *Azospirillum* on the length of the root elongation zone was comparable to its effect on root length, although the length of the root elongation zone slightly increased in seedlings inoculated with *Azospirillum* 10^5-10^6 cfu/mL, in the absence or presence of *Rhizobium*. Concentrations greater than 10^7 cfu/mL significantly reduced the length of the root elongation zone (Fig. 1 C, D).

Azospirillum inoculum concentration of 10^{6-7} cfu/mL increased the number of lateral roots almost 3 fold compared with the control (Fig 2A). *Rhizobium* at 105 cfu/ml alone increased the number of laterals (Fig. 2B). The number of laterals formed in the presence of both *Rhizobium* and *Azospirillum* was higher than with *Azospirillum* alone (Fig. 2A, B). Inoculation with *Azospirillum* at concentrations of 10^{-6} and 10^{9} cfu/mL significantly increased the root diameter and the length of the root hairs, compared with the uninoculated controls (Table 1). Root hair density was not affected by inoculation with *Azospirillum*, but the number of root hairs per unit of root length increased as a result of the increase in root diameter (Table 1).

Application of IAA (auxin) or BA (cytokinin) at concentration of 10^{-8}M stimulated root elongation, but concentrations exceeding 10^{-6}M inhibited root elongation and shortened the root elongation zone (Fig. 3). IAA at concentration of 10^{-5}M increased the diameter of the roots and the length of the root hairs, compared with the uninoculated controls, similar to inoculation with *Azospirillum* 10^{9} cfu/mL. BA in concentrations of 10^{-5}M did not alter the diameter of the roots but reduced the number of root hairs per unit of root surface (Table 1).

Cross sections prepared from sites close to the root tip at the time of inoculation with 10^{9} cfu/mL *Azospirillum* showed larger cortical cells than in controls;, however their number across the root was unaffected (Table 2).

We assumed that DNA level in each cell of the plant is identical. Accordingly the level of DNA in root segments formed 24 h after inoculation should be associated with the number of new root cells that formed during this period of time. Inoculation with *Azospirillum* at a concentration of 10^{9} cfu/mL significantly decreased the DNA level whereas inoculation with 10^{7} cfu/mL did not affect the DNA level compared with the control (Table 3).

DISCUSSION

Inoculation of wheat with *Azospirillum* (10^{6} cfu/mL) has been reported to increase root length compared with controls (Kapulnik *et al.* 1985). In medic no increase in root elongation was found. However, inoculum concentrations above 10^{8} cfu/mL *Azospirillum* inhibited root elongation and shortened the length of the root elongation zone. Inhibition of growth associated with high *Azospirillum* inoculum concentration (10^{9} cfu/mL) has also been reported for wheat, sorghum and tomato (Kapulnik *et al.* 1985; Morgenstern and Okon 1987).

The inhibitory effect of high inoculum concentration of *Azospirillum* in medic lessened in the presence of *Rhizobium*. This may be due to competition for adsorption and/or colonization sites on the root surface between *Azospirillum* and *Rhizobium* cells. Mineral and water absorption occurs mainly in the young regions of the root (a few centimeters behind the root tip). Therefore the number of root tips and root hairs in each root is of importance in plant growth (Kramer, 1983). Inoculation of medic with *Azospirillum* (10^6-10^7 cfu./mL) significantly increased the number of lateral roots - by 50% and 100% in the presence or absence of *Rhizobium*, respectively. In addition, *Azospirillum* significantly enhanced the diameter of the main root and the average length of root hairs (Table 1).

The elongation of sub-apical root segments of *Lens culinaris* was slightly promoted in response to exogenous IAA 10^{-8}M. However, concentrations of IAA greater than 10^{-7}M inhibited growth compared with the control (Wareing and Phillips, 1986). External IAA and BA significantly stimulated root elongation in pouch-grown medic at a concentration of 10^{-8}M and significantly decreased root elongation at concentrations above 10^{-7}M compared with the control. The effect of IAA 10^{-5} on root morphology was similar to inoculation by *Azospirillum* 10^9 cfu/mL (Table 3). The concentration of IAA in 3 days old culture of *Azospirillum* in the absence of tryptophan was 5×10^{-7}M (Yahalom *et al.*, unpublished result). The amount of IAA that is produced by the bacteria *in vivo* on the root is not known. It is possible that IAA together with other plant growth promoting substances produced by *Azospirillum* affect auxin metabolism and growth of roots (Okon and Kapulnik, 1986).

Root hair density in wheat was found to increase following *Azospirillum* inoculation. Longer root hairs were observed 2.5 cm from root tip (RT), although at a distance of 5 cm from RT no difference was found either in root hair length or in density between treated and untreated plants.

From this it was concluded tha the inoculation with *Azospirillum* caused earlier root development only (Okon and Kapulnik, 1986). Plazinski and Rolfe (1985b) noted that inoculation of white clover with a *Rhizobium-Azospirillum* mixture markedly increases the length of root hairs of primary roots. In medic the length of root hairs significantly increased, but density of root hairs was not increased after inoculation with *Azospirillum* (Table 1). On the other hand the number of root hairs per root section increased, apparently due to the increase in root diameter (Table 2).

The number of lateral roots increased in seedlings of peal millet, and seedlings inoculated with *Azospirillum* developed more extensive root systems (Tien *et al.* 1979). This effect of inoculum size on root morphology can be mimicked by applying different concentrations of IAA and/or cytokinin to the root (Patriquin *et al.*,1983).

The significant increase in the number of lateral roots in medic seedlings was associated with an increase in the surface area of the root and in the uptake of minerals and water (Yahalom *et al.* 1988). Similar findings were reported for wheat and maize (Kapulnik *et al..*, 1985: Fallik et al., 1988).

Inoculation of wheat and maize with *Azospirillum* caused changes in the arrangement of the cortical cells as observed under the light microscope in root sections. It is not clear if these changes occurred prior or during the fixation of root tissue (Kapulnik *et al.*, 1985; Lin *et al.*, 1983).

In this work no changes in the arrangement of cortical cells were observed. However, lateral sections taken from sites adjacent to the root tip at the time of inoculation with *Azospirillum* 10^9 cfu/mL showed larger cortical cells (double in size) with thinner walls as compared with control roots. On the other hand no increase in the number of cells was observed, so the increase in root diameter was apparently caused by the increase in the size of the cortex cells.

The increase in the volume of the root cells may be due to the higher content of free IAA (Fallik *et al.*, 1989), affecting cell-wall plasticity and leading to an increase in cell size (Wareing and Phillips, 1986).

It is not clear if a high concentration of *Azospirillum* inoculum affects root elongation and/or cells division in the root elongation zone. Assuming that the DNA concentration in each plant cell is constant regardless of their size, the lower DNA concentration found in roots inoculated with *Azospirillum* (10^9 cfu/mL) indicated a reduction of cell division leading to shorter roots.

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Table 1. Effect of *Azospirillum* inoculation, application of cytokinin (BA) and auxin (IAA) on medic root diameter, root hair length and density. Roots were examined 24 h after inoculation and measurements made 5 mm below the position of the root tip at the time of inoculation. Means of 6 seedlings. Numbers followed by the same letter in each column do not differ significantly at P=0.05.

Treatment	Concentration	Root Diameter	Root hair Length (mm)	Root hair Density*
Control		0.39 c	0.05 c	31 a
Azospirillum-Cd	10^6 cfu/mL	0.50 b	0.08 b	32 a
Azospirillum-Cd	10^9 cfu/mL	0.69 a	0.12 a	29 a
BA	10^{-5} M	0.40 c	0.04 c	19 b
IAA	10^{-5} M	0.65 a	0.11 a	30 a

*Number of root hairs per 200 μ2 root surface, measured 5 mm below the position of the root tip at the time of inoculation.

Table 2. The effect of inoculation with *Azospirillum* 10^9 cfu/mL on the number of root hair and cortex cells and cross section in medic roots. Measurements were made 5 mm below the position where the root tip was 24 h earlier at the time of inoculation. Means of 6 replicates ±SE.

	No. cells			Cross section
	Epidermal	Root hair	Cortex*	(mm^2)
Control	106±2	26±1.4	31±2	0.25±0.03
Azospirillum	120±3	30±1.6	14±1	0.65±0.06

* Number of cells per 200 μm^2

Table 3. Effect of *Azospirillum* inoculum concentration on the DNA level of the root segment produced after 24 h from inoculation by 2 days-old seedlings of burr medic. Means of 9 replications. Numbers followed by same letter in each column do not differ significantly at P-0.05.

Treatments	Concentration	DNA (OD*mm)	Root length (mm)	Fresh weight (mg/12 roots)
Control		0.44 a	9.73 a	3.24 a
Azospirillum	10^7 cfu/mL	0.42 a	10.04 a	3.27 a
Azospirillum	10^9 cfu/mL	0.33 b	7.98 b	2.90 a

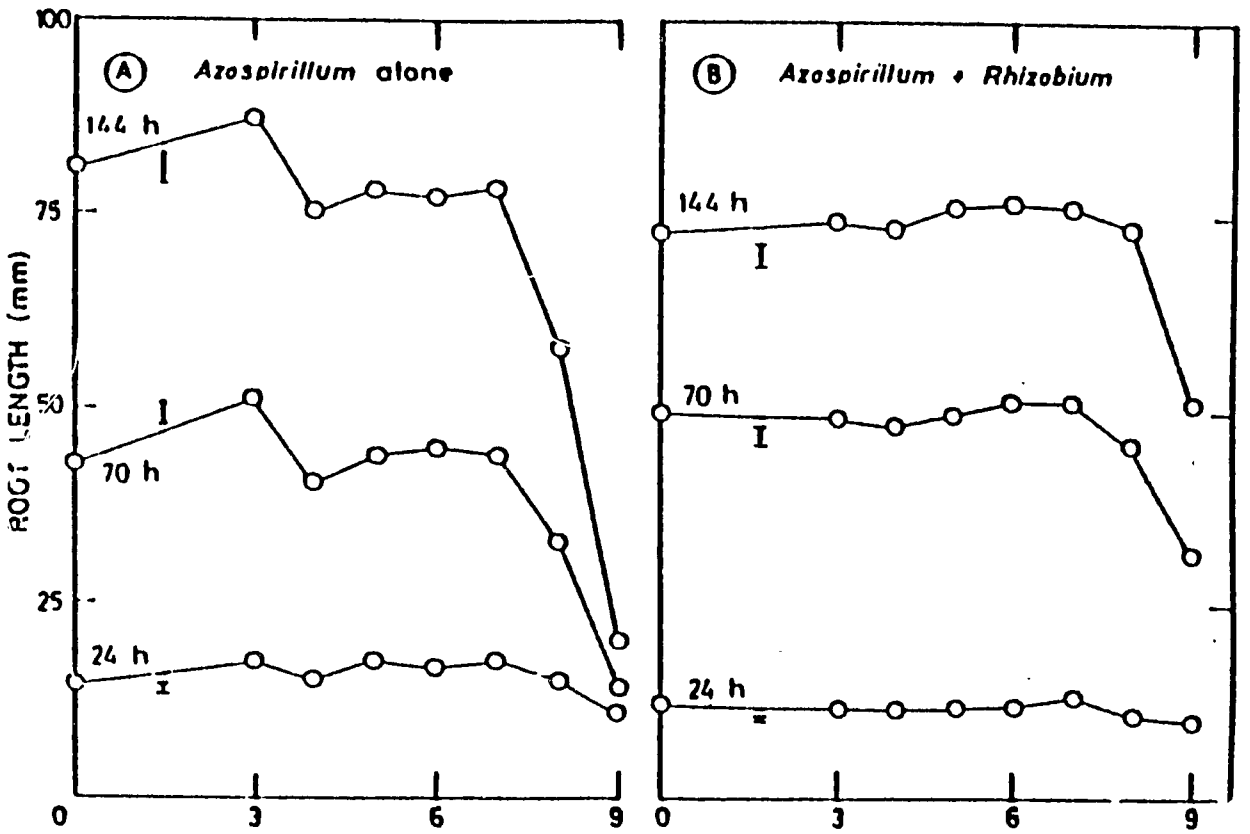

Fig. 1.Effect of *Azospirillum brasilense*-Cd inoculum concentration on root length: (A) in the absence and (B) in the presence of *Rhizobium* (10^6 cfu/mL), in pouch-grown *Medicago polymorpha* seedlings. Measurements were made 24, 70 and 144 h after inoculation. Means of 32 seedlings per treatment. Vertical bars indicate SE.

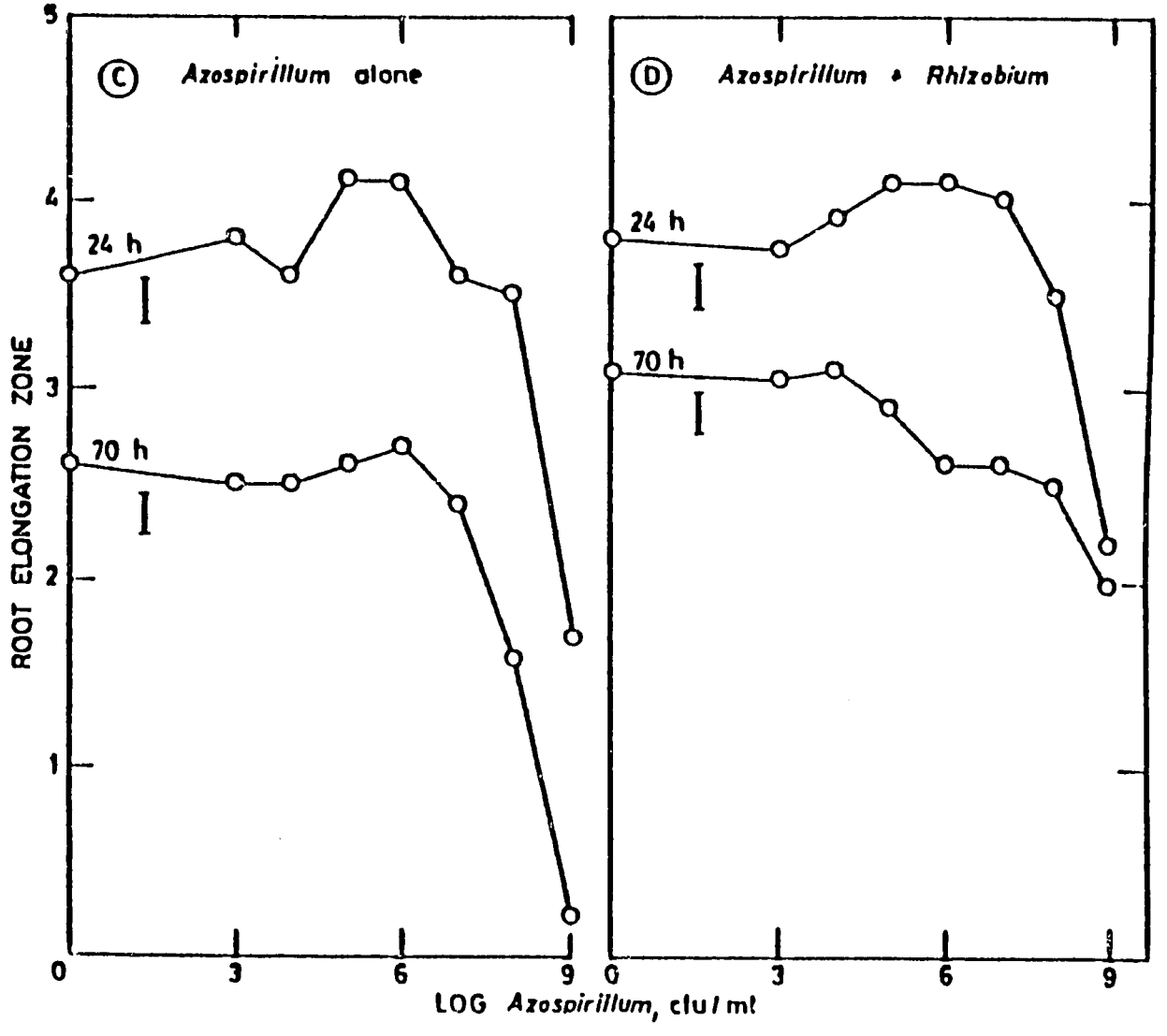

Fig. 1. Effect of *Azospirillum brasilense*-Cd inoculum concentration on the length of the root elongation zone (C) in the absence and (D) in the presence of *Rhizobium* (10^6 cfu/mL) in pouch-grown *Medicago polymorpha* seedlings. Measurements were made 24, 70 and 144 h after inoculation. Means of 32 seedlings per treatment. Vertical bars indicate SE.

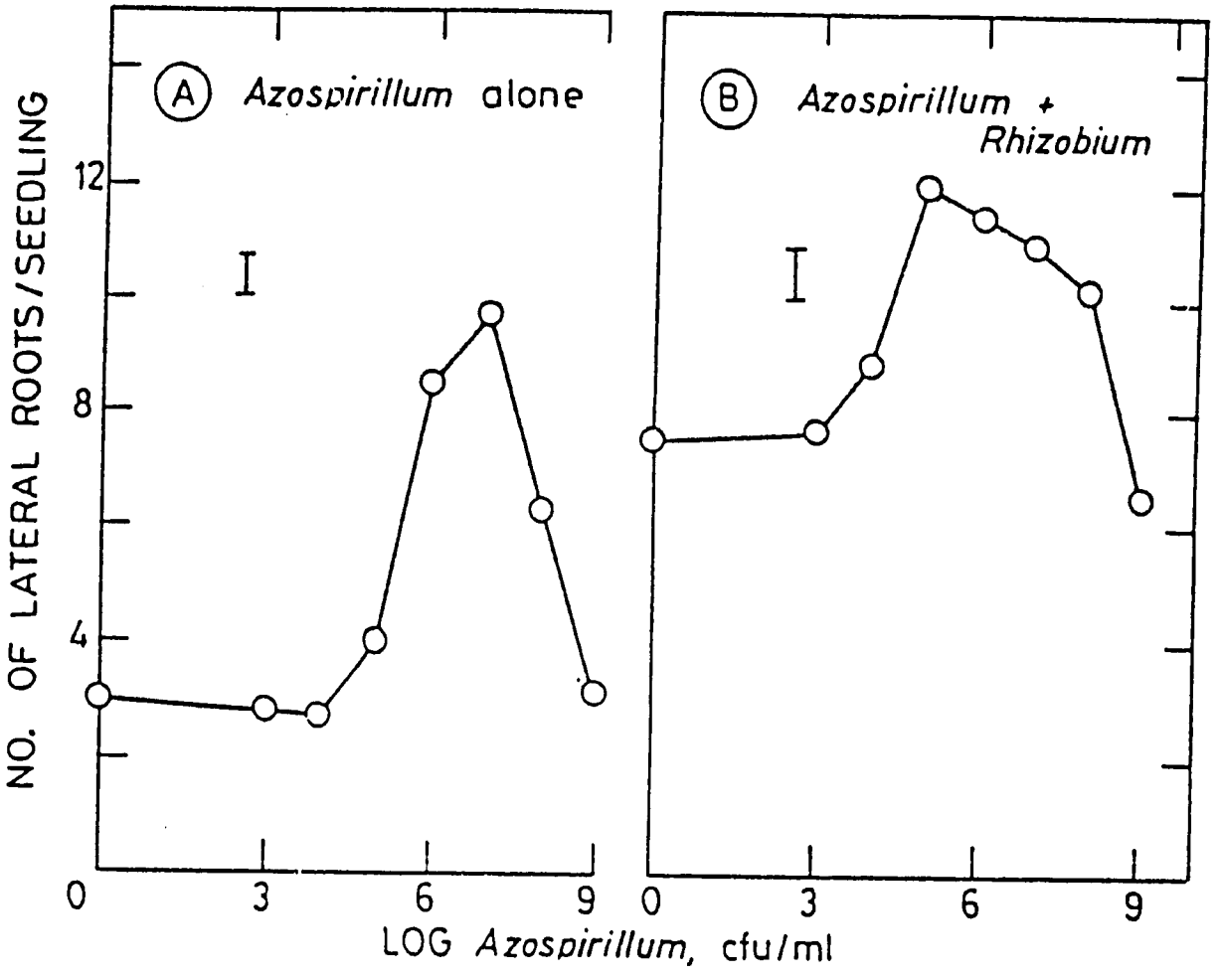

Fig. 2. Effect of *Azospirillum* inoculum concentration on the number of lateral roots in pouch-grown seedlings of burr medic, (A) in the absence and (B) in the presence of *Rhizobium* (10^6 cfu/mL). Measurements were made 12 days after inoculation. Means of 32 seedlings per treatment. Vertical bars indicate SE.

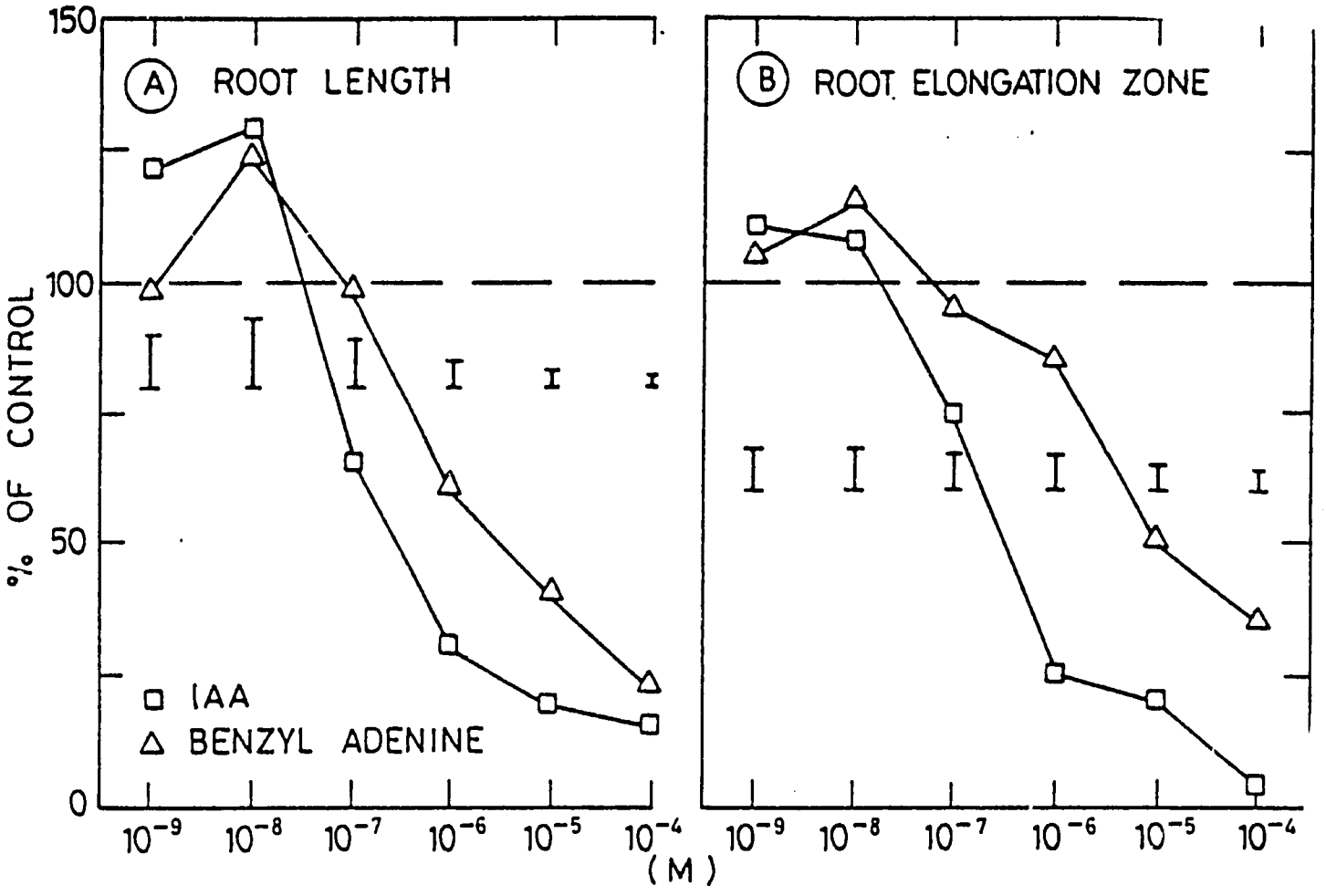

Fig. 3. Effect of auxin (IAA) and cytokinin (BA) concentration on (A) root length and (B) length of the root elongation zone of pouch-grown burr medic seedlings measured 72 h after application. Means of 25 seedlings per treatment. Vertical bars indicate SE.