THE INFLUENCE OF EXOGENOUS AND ENDOGENOUS VITAMINS UPON MUSKRAT FERTILITY AND DISEASE

A Thesis

Presented to

the Faculty of Graduate Studies and Research

University of Manitoba

In Partial Fulfillment

of the Requirements for the degree

Master of Science

by

Alexander Diduch

April 1952

ACKNOWLEDGEMENTS

The author wishes to take this opportunity of expressing his sincere gratitude to Professor R.A.Wardle, Chairman of the Department of Zoology and under whose auspices this work was carried out, for the use of his private laboratory, equipment, kindness and assistance in preparation of this manuscript.

The author is grateful to Dr.J.A.McLeod, Associate Professor, Department of Zoology, for his helpful suggestions, co-operation and interest in carrying out this work.

The author wishes to thank Dr.A.Robinson, Professor, Department of Biochemistry, for making available the facilities of the department and space in which much of the preliminary work was carried out.

To the Department of Mines and Natural Resources, Game and Fisheries Branch, the author is indebted for the availability of the work.

CONTENTS

INTRODUCTION

The fact that many small rodents of the north temperate zone die off in prodigous numbers at regular intervals has been established. No satisfactory explanation, however, has yet been given regarding the cause of these cyclic declines. In the case of muskrats in Manitoba, the cause is definitely a disease traceable to a filterable virus according to an interim report in 1951 to the Manitoba Government by J. A. McLeod and G. F. Bondar. Why the disease should recur cyclically is a matter for speculation. The virulence of a low grade organism may build up by passage through a series of hosts but this should take place sporadically. The resistance of the host to infection varies with its nutritional condition, etc., and dietary deficiency would appear to be the most likely event to recur in a regular fashion.

Starvation in the sense of a shortage of energy yielding material is seldom an accompaniment of an epizootic in muskrats and there is little evidence that the host's resistance to infection is seriously reduced from this cause except in pronounced cases. However, little or nothing is known about the vitamin intake or the vitamin level of the muskrat body from season to season or year to year. It is well known that the taking in of vitamins is usually incidental to the consumption of energy-yielding materials and that avitaminosis frequently predisposes to infection.

The following pages represent the results of an effort to determine the vitamin content of the principal food of muskrats in Manitoba from different places and at different times and to correlate the value of vitamins with the increase and decrease of the muskrat population. The completion of the work is necessary over a period of five to six years in order to establish the relationship, if any, between the concentration

of the vitamins in plants, muskrat fertility and lowered resistance in the animals.

Some experimental work has been done concerning muskrats and their food preferences, but to the author's knowledge there appears to be no available literature on vitamin analyses of marsh plants. The cattail - Typha latifolia - is one of the most desirable muskrat foods. Others are: the river bulrush - Scirpus fluviatilus, soft stem bulrush - Scirpus validus, bur-reed - Sparganium, and the flag-reed - Phragmites maximus. The object of the work was to concentrate on preferred foods and to select only a few of the vitamins which would be of significant value regarding fertility and disease. The vitamins chosen were: Pro-vitamin A or carotene, vitamin B_2 or riboflavin and vitamin C or ascorbic acid. There are other vitamins which would be of equal importance, but since time, equipment and transportation were limiting factors in this work, it was a problem to consider other important vitamins and other selected food of these animals.

METHOD

DETERMINATION OF PRO-VITAMIN A (CAROTENE) IN PLANT TISSUE

The method of analysis used was based upon those suggested by Moore and Ely (1941) and by Wall and Kelly (1943).

A 10 gm. sample of minced plant material is extracted with 150 ml. of 95% ethanol plus 75 ml. of a petroleum ether fraction " Skellysolve B " in a Waring Blendor for about five minutes. Extract and residue are then transferred to a fritted-glass filter and the filter attached to a suction flask. 100 ml. of water containing about 5 gms. of sodium sulfate (anhydrous) are added to the alcohol-skellysolve mixture and the lower aqueous alcohol solution is drawn off. The sodium sulfate checks any tendency towards emulsification. 50 ml. of water are poured into the separatory flasks, then the water is drawn off and the petroleum ether is concentrated to approximately 25 ml. A few grams of anhydrous sodium sulfate (nitrogen free) are added to the skellysolve to remove any water. The adsorbent is composed of three parts of diatomaceous silica (Celite No.503) and one part of magnesium oxide (Micron Brand). The adsorption tube is connected to a suction flask and a plug of cotton placed at the bottom of the tube. The suction source is from a water jacket and with the tap open, adsorbent is added to a height of about two-thirds of the adsorption tube. The column is washed repeatedly with a mixture of 3 to 5% acetone in skellysolve until the solvent comes through colorless. The chlorophyll (green layer) and the xanthophyll (yellow layer) are held firmly at the top of the column as are most of the other non-carotene pigments. The carotene (orange layer) is held about half way down. This is washed with an acetone-skellysolve mixture. The acetone is necessary

because pure skellysolve elutes the carotene from the column too slowly. The whole procedure of adsorption and elution takes about five minutes. The estimation of the carotene is determined by making up the carotene solution to volumes of 100, 200 or 250 cc. The wavelength used is that of 440 millimicrons and the transmission is compared with that of 90% beta and 10% alpha carotene. It is estimated as beta carotene.

Standard Curve for Carotene using the Genco Photelometer

mc. gm/ml.	Yo	Log Yo	Log Yc	antilog.
.1	94.0	1.9731	1.96898	93.1
.2	86.5	1.9370	1.93546	86.2
.3	79.0	1.8976	1.90194	79.8
.4	73.0	1.8633	1.86842	73.9
.5	68.0	1.8325	1.83490	68.3
.6	63.8	1.8048	1.80138	63.3
.7	58.7	1.7686	1.76786	58.6
.8	54.8	1.7388	1.73434	54.2
.9	50.0	1.6990	1.70082	50.2
1.0	46.4	1.6665	1.66730	46.5
		18.1812		

1). $E \log Y = mEx_2 + 10\ b.$ $\quad 18.1812 = m5.5 + 10\ b.$

2). $Ex \log Y = mEx^2 + bEx.$ $\quad 9.7231 = m3.85 + 5.5\ b.$

Solve for m.

$$m = \frac{18.1812 \quad 10}{9.7231 \quad 5.5}$$

$$\frac{5.5 \quad 10}{3.85 \quad 5.5}$$

$$m = -.3352$$

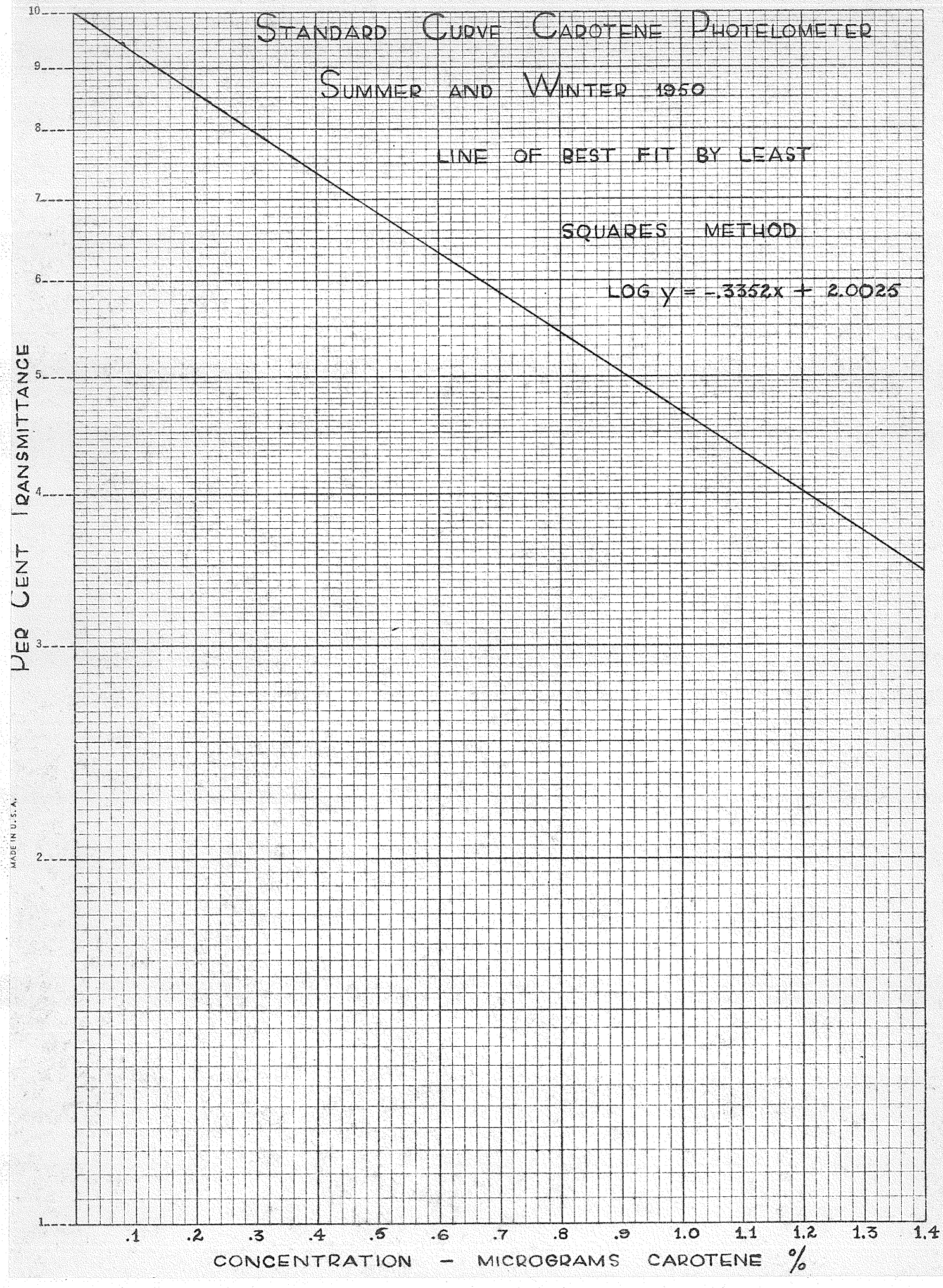
Solve for b.

$$b = \frac{5.5 \quad 18.1812}{3.85 \quad 9.7231}$$

$$-8.25$$

$$b = \frac{-16.5205}{-8.25}$$

$$\text{Log } Y = mx - b = .3352x - 2.0025$$


STANDARD CURVE CAROTENE PHOTELOMETER
SUMMER AND WINTER 1950
LINE OF BEST FIT BY LEAST
SQUARES METHOD
LOG y = -.3352x + 2.0025
PER CENT TRANSMITTANCE
10
9
8
7
6
5
4
3
2
1
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
1.1
1.2
1.3
1.4
CONCENTRATION — MICROGRAMS CAROTENE %
MADE IN U.S.A.

Photograph No.1. - The Extraction Stage

The plant material is weighed out and placed in a Waring Blender jar. Alcohol and skellysolve B are two solvents used to extract the plant pigments. Carotene (Pro-vitamin A) is one of the plant pigments.

Photograph No. 2 - The Filtration Stage

The plant fibers remain in the glass filter funnel and the extract is collected under vacuum in conical flasks.

Photograph No. 3 - The Separation Stage

The top portion of the separatory flask contains the ether and plant pigments. The bottom portion of the flask contains the alcohol-water and other impurities.

Photograph No. 5 - The Reading Stage

A - Coleman Junior Spectrophotometer Model 6A.

B - Photelometer.

C - Coleman Spectrophotometer Model 11.

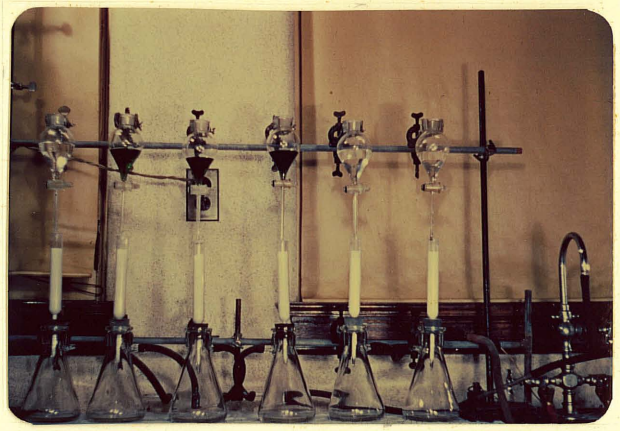


Table I

Pro-vitamin A (Carotene) Summer 1950 Cenco Photelometer

Plant	Wt. of plant sample	% Moisture	Average Photo-Reading	Volume	Carotene ug/gm.	Dry Basis
Typha latifolia						
Whole Plant:						
1.	10 gms.	45.5	82.5	200 ml.	5.1	9.18
2.	10 gms.	48.2	88.6	200 ml.	3.4	6.46
3.	10 gms.	45.8	88.5	200 ml.	5.1	9.18
4.	10 gms.	47.9	87.4	200 ml.	3.5	6.65
5.	10 gms.	47.9	88.6	200 ml.	3.4	6.46
				(average)		7.58
Scirpus validus						
Whole Plant:						
1.	10 gms.	38.5	34.3	200 ml.	27.86	44.57
2.	10 gms.	38.2	36.2	200 ml.	27.20	43.52
3.	10 gms.	40.2	68.0	200 ml.	10.08	16.12
4.	10 gms.	40.2	67.5	200 ml.	10.30	16.12
				(average)		30.08
Phragmites maximus						
Whole Plant:						
1.	10 gms.	35.5	69.5	200 ml.	9.50	14.25
2.	10 gms.	35.8	68.5	200 ml.	9.90	14.85
3.	10 gms.	31.3	59.0	200 ml.	13.80	19.32
4.	10 gms.	31.6	60.0	200 ml.	13.14	18.39
5.	10 gms.	31.8	59.5	200 ml.	13.70	19.18
				(average)		17.19
Scirpus fluviatilus						
Whole Plant:						
1.	10 gms.	36.2	46.5	200 ml.	20.90	31.35
2.	10 gms.	36.5	47.5	200 ml.	20.30	30.45
3.	10 gms.	35.8	41.5	200 ml.	23.70	35.55
				(average)		32.64
Sparganium						
Whole Plant:						
1.	10 gms.	38.5	60.5	200 ml.	13.10	20.96
2.	10 gms.	37.3	52.5	200 ml.	17.00	27.20
3.	10 gms.	37.1	52.5	200 ml.	17.00	27.20
				(average)		25.12

Table II

Pro-vitamin A (Carotene) Winter 1950 Cenco Photelometer

Plant	Wt. of Plant sample	% Moisture	Average Photo-Reading	Volume	Carotene ug/gm.	Dry Basis
Typha latifolia						
Leaf:						
1.	5 gms.	0.78	98	200 ml.	1.2	1.2
2.	5 gms.	0.62	98	200 ml.	1.2	1.2
3.	5 gms.	1.00	96.5	200 ml.	2.0	2.20
4.	5 gms.	0.68	97	200 ml.	1.6	1.6
5.	5 gms.	0.90	96.5	200 ml.	2.0	2.20
				(average)		1.60
Stem:						
1.	5 gms.	0.13	98	200 ml.	1.2	1.2
2.	5 gms.	0.13	99	200 ml.	0.60	0.60
3.	5 gms.	3.42	98	200 ml.	1.2	1.2
4.	5 gms.	0.76	99	200 ml.	0.60	0.60
5.	5 gms.	1.83	100	200 ml.	0.00	0.00
				(average)		0.90
Root:						
1.	10 gms.	1.16	97	100 ml.	0.40	0.40
2.	10 gms.	1.07	98	100 ml.	0.30	0.30
3.	10 gms.	2.25	97	100 ml.	0.40	0.40
4.	10 gms.	2.03	98	100 ml.	0.30	0.30
5.	10 gms.	1.34	96	100 ml.	0.55	0.55
				(average)		0.39
Scirpus validus						
Stem:						
1.	10 gms.	0.35	69.5	200 ml.	9.5	9.5
2.	10 gms.	0.38	59.5	200 ml.	13.7	13.7
3.	10 gms.	0.34	66.5	200 ml.	10.7	10.7
				(average)		11.3
Root:						
1.	10 gms.	0.80	95.5	100 ml.	0.65	0.65
2.	10 gms.	0.80	97.0	100 ml.	0.40	0.65
3.	10 gms.	0.80	97.5	100 ml.	0.35	0.35
				(average)		0.46

Table II (cont'd)

Pro-vitamin A (Carotene) Winter 1950 Cenco Photelometer

Plant	Wt. of plant sample	% Moisture	Average Photo-Reading	Volume	Carotene ug/gm.	Dry Basis
Phragmites maximus						
Stem:						
1.	20 gms.	0.21	76.5	100 ml.	1.82	1.82
2.	20 gms.	0.24	85.5	100 ml.	1.25	1.25
3.	20 gms.	0.17	81.5	100 ml.	0.67	0.67
				(average)		1.24
Root:						
1.	20 gms.	0.42	98	100 ml.	0.15	0.15
2.	20 gms.	0.46	97.5	100 ml.	0.17	0.17
3.	20 gms.	0.24	98.5	100 ml.	0.10	0.10
				(average)		0.14

Table III

Pro-vitamin A (Carotene) Spring 1951 Cenco Photelometer

Plant	Wt. of plant sample	% Moisture	Average Photo-Reading	Volume	Carotene ug/gm.	Dry Basis
Typha latifolia						
Leaf:						
1.	5 gms.	76.84	96	200 ml.	28	6.48
2.	5 gms.	76.77	97	200 ml.	20	4.64
				(average)		5.56
Stem:						
1.	20 gms.	92.32	93	200 ml.	1.00	0.076
2.	20 gms.	92.07	94	200 ml.	0.90	0.071
				(average)		0.074
Root:						
1.	25 gms.	89.36	97	200 ml.	0.40	0.04
2.	25 gms.	89.28	97	200 ml.	0.40	0.04
				(average)		0.04
Phragmites maximus						
Leaf:						
1.	5 gms.	45.75	80	200 ml.	24.0	13.02
2.	5 gms.	45.85	83	200 ml.	20.8	11.26
				(average)		12.14
Stem:						
1.	20 gms.	68.20	91	200 ml.	6.5	2.07
2.	20 gms.	67.99	90	200 ml.	7.5	2.40
				(average)		2.24
Root:						
1.	25 gms.	83.05	96	200 ml.	0.56	0.095
2.	25 gms.	83.43	97	200 ml.	0.40	0.066
				(average)		0.081
Scirpus validus						
Leaf:	Not available					
Stem:						
1.	5 gms.	82.06	92	200 ml.	48.00	8.77
2.	5 gms.	81.83	94	200 ml.	36.00	6.54
				(average)		7.66
Root:						
1.	25 gms.	87.19	92	100 ml.	0.48	0.0615
2.	25 gms.	87.14	93	100 ml.	0.40	0.0510
				(average)		0.0563

Table IV

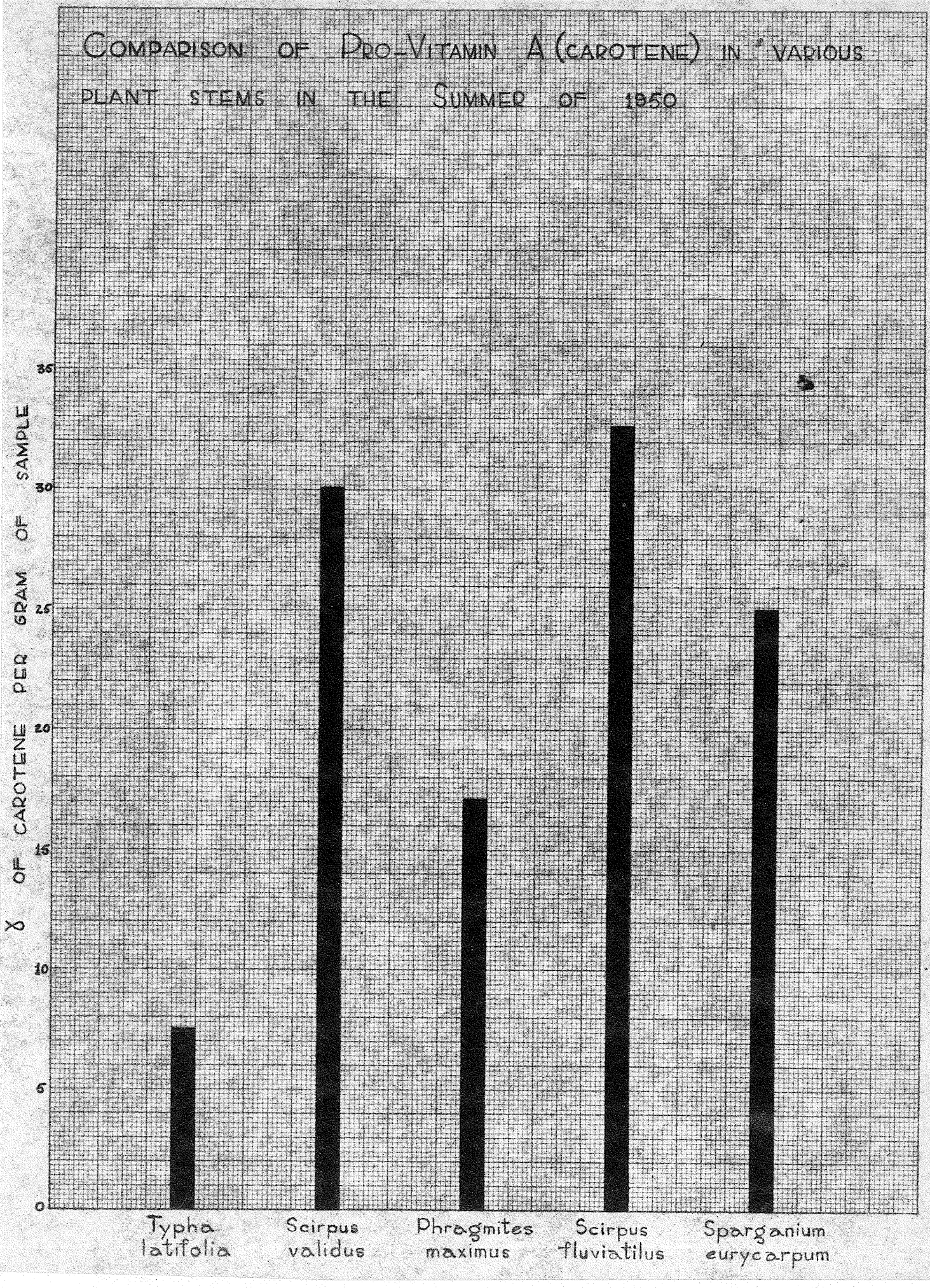
Pro-vitamin A (Carotene) Summer 1951 Genco Photelometer

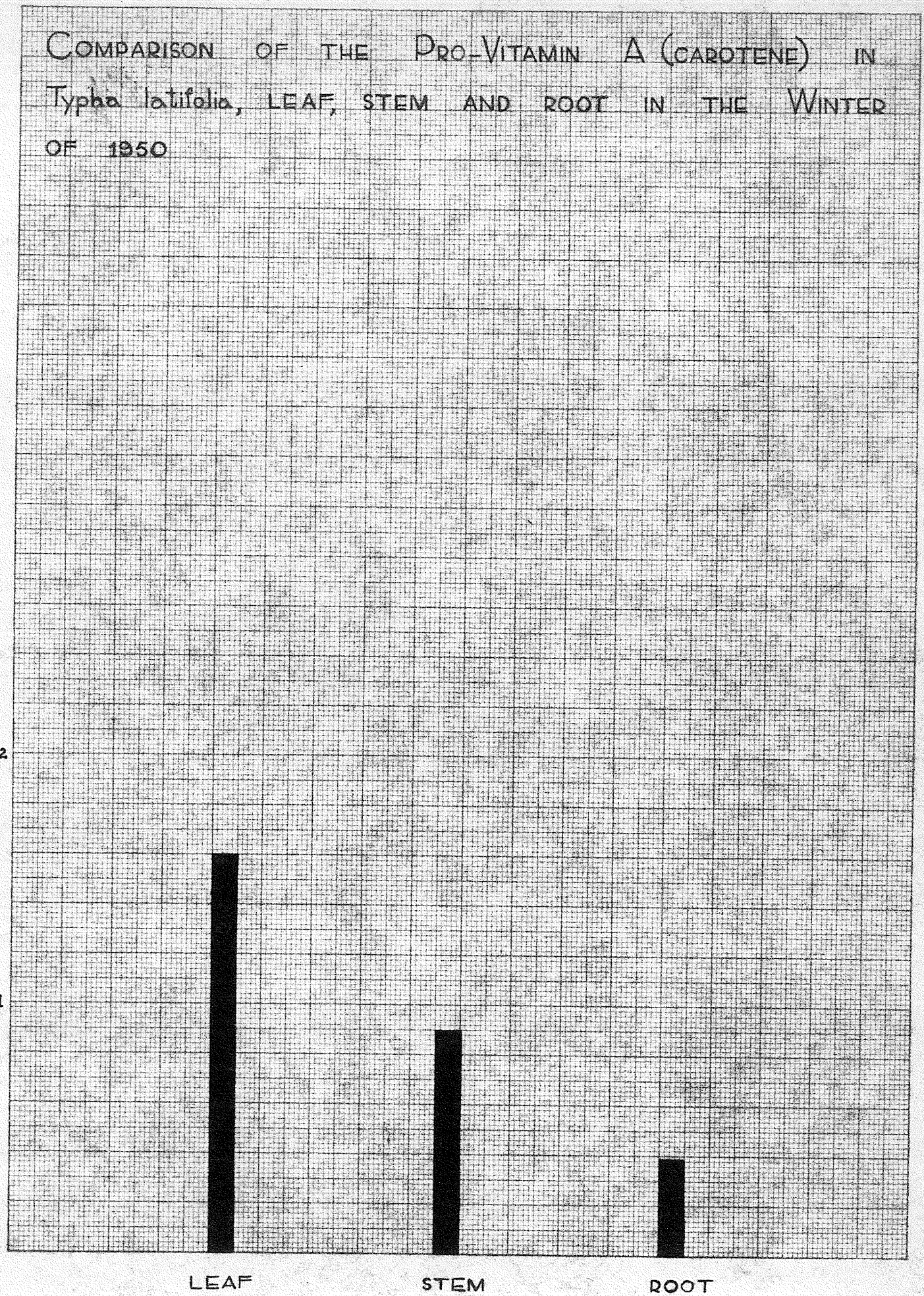
Plant	Wt. of plant sample	% Moisture	Average Photo-Reading	Volume	Carotene ug/gm.	Dry Basis
Typha latifolia						
Leaf:						
1.	5 gms.	67.03	88	200 ml.	7.20	2.37
2.	5 gms.	65.15	89	200 ml.	6.40	2.23
				(average)		2.30
Stem:						
1.	20 gms.	90.49	92	200 ml.	0.60	0.057
2.	20 gms.	90.69	92	200 ml.	0.60	0.058
				(average)		0.0575
Root:						
1.	25 gms.	86.53	92	100 ml.	0.48	0.056
2.	25 gms.	85.82	93	100 ml.	0.40	0.064
				(average)		0.060
Phragmites maximus						
Leaf:	Not available					
Stem:						
1.	20 gms.	68.22	96	200 ml.	7.00	2.224
2.	20 gms.	67.98	97	200 ml.	5.00	1.601
				(average)		1.913
Root:						
1.	20 gms.	69.65	92	100 ml.	0.60	0.182
2.	20 gms.	70.36	93	100 ml.	0.50	0.148
				(average)		0.165
Scirpus validus						
Leaf:	Not available					
Stem:						
1.	15 gms.	71.49	90	200 ml.	20.0	5.70
2.	15 gms.	71.94	93	200 ml.	13.3	3.73
				(average)		4.72
Root:						
1.	25 gms.	76.08	94	200 ml.	0.72	0.172
2.	25 gms.	75.62	96	200 ml.	0.56	0.137
				(average)		0.155

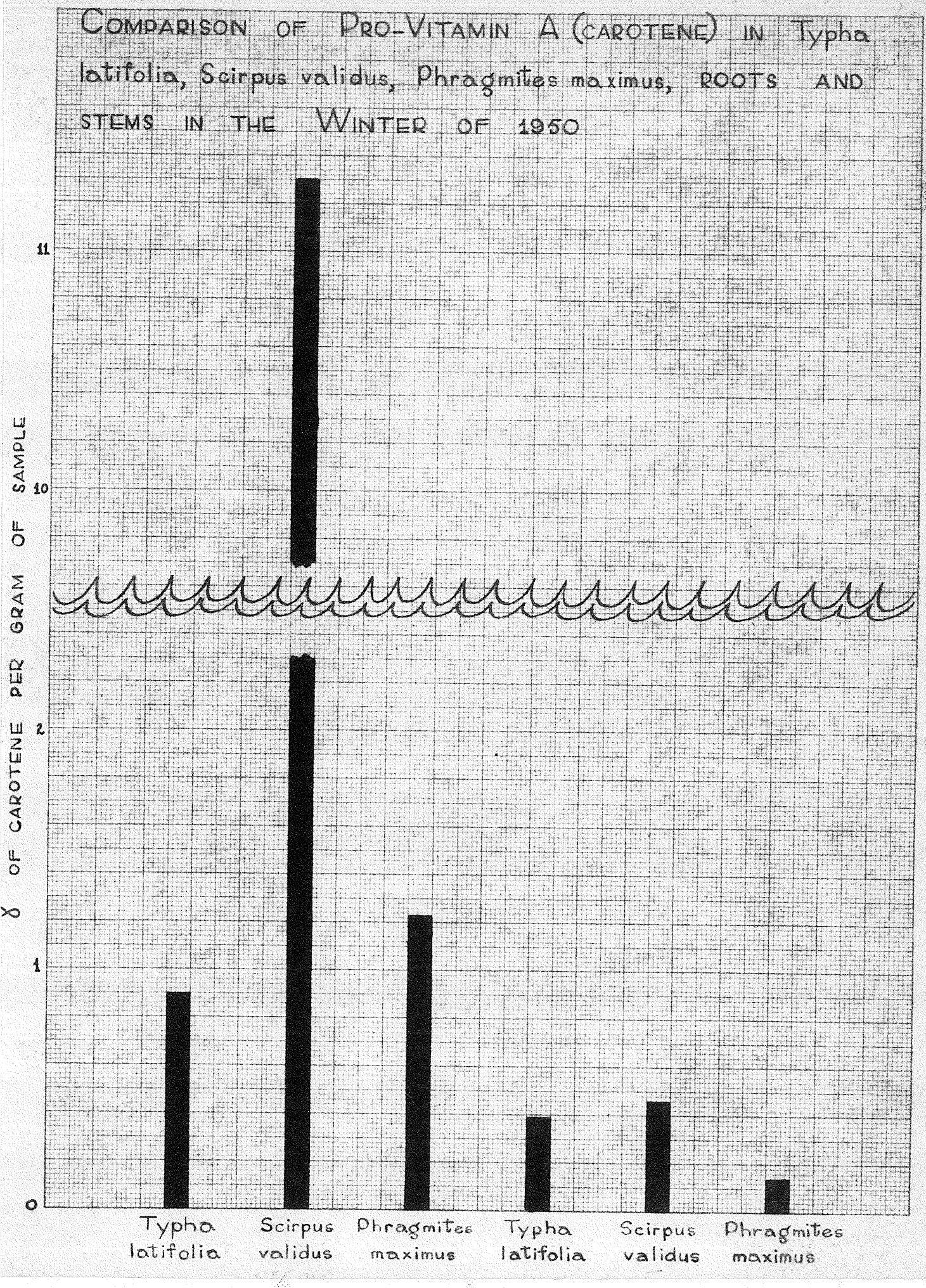
Table V

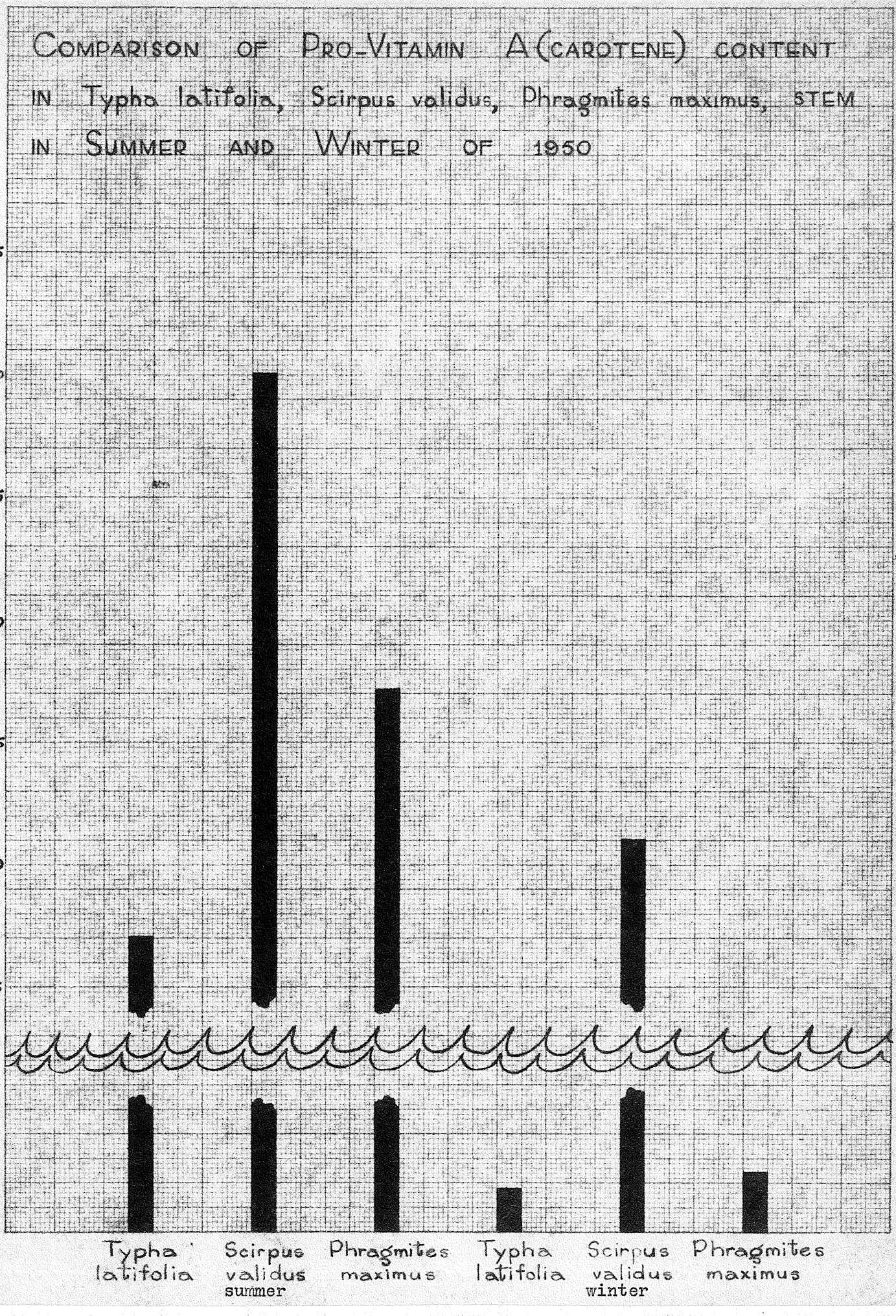
Pro-vitamin A (Carotene) Fall 1951 Cenco Photelometer

Plant	Wt. of plant sample	% Moisture	Average Photo-Reading	Volume	Carotene ug/gm.	Dry Basis
Typha latifolia						
Leaf:						
1.	3 gms.	60.71	96	200 ml.	47.0	18.46
2.	3 gms.	61.18	95	200 ml.	53.0	20.57
				(average)		19.52
Stem:						
1.	20 gms.	84.88	96	200 ml.	0.70	0.105
2.	20 gms.	85.21	97	200 ml.	0.50	0.073
				(average)		0.089
Root:						
1.	10 gms.	79.49	98	100 ml.	0.40	0.082
2.	10 gms.	80.04	97	100 ml.	0.50	0.099
				(average)		0.091
Phragmites maximus						
Leaf:						
1.	10 gms.	17.33	84	500 ml.	120.0	99.204
2.	10 gms.	17.01	85	500 ml.	110.0	91.289
				(average)		95.247
Stem:						
1.	16 gms.	58.26	82	200 ml.	5.4	2.253
2.	10 gms.	58.21	80	200 ml.	6.0	2.507
				(average)		2.380
Root:						
1.	10 gms.	67.99	98	100 ml.	0.4	0.128
2.	10 gms.	68.11	97	100 ml.	0.5	0.159
				(average)		0.144
Scirpus validus						
Leaf:	Not available					
Stem:						
1.	20 gms.	68.61	63	200 ml.	61.0	19.15
2.	20 gms.	67.35	65	200 ml.	57.0	18.61
				(average)		18.88
Root:						
1.	25 gms.	72.39	94	200 ml.	0.72	0.199
2.	25 gms.	72.45	95	200 ml.	0.64	0.180
				(average)		0.190

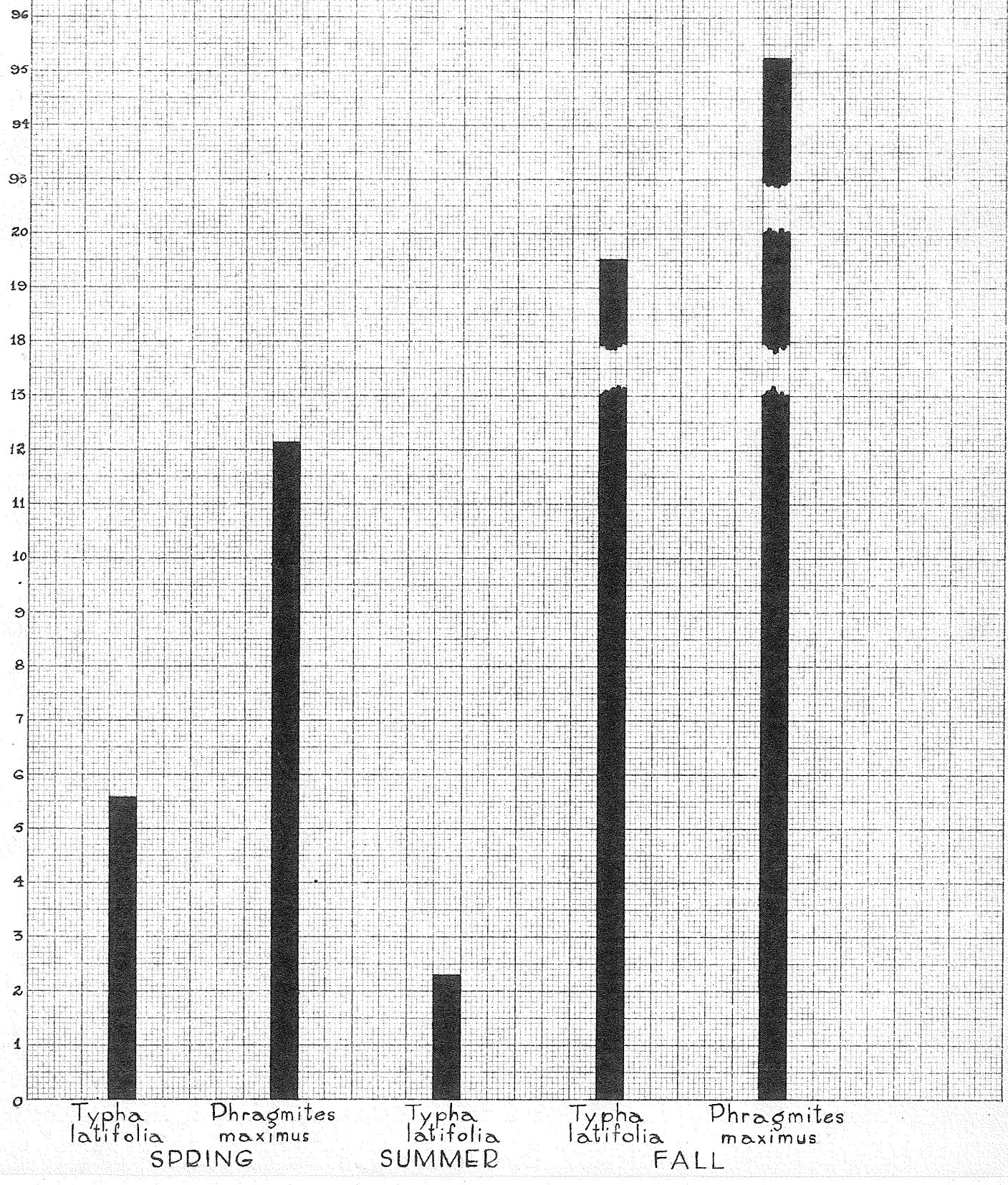

COMPARISON OF PRO-VITAMIN A (CAROTENE) IN VARIOUS PLANT STEMS IN THE SUMMER OF 1950
γ OF CAROTENE PER GRAM OF SAMPLE
0
5
10
15
20
25
30
35
Typha latifolia
Scirpus validus
Phragmites maximus
Scirpus fluviatilus
Sparganium eurycarpum

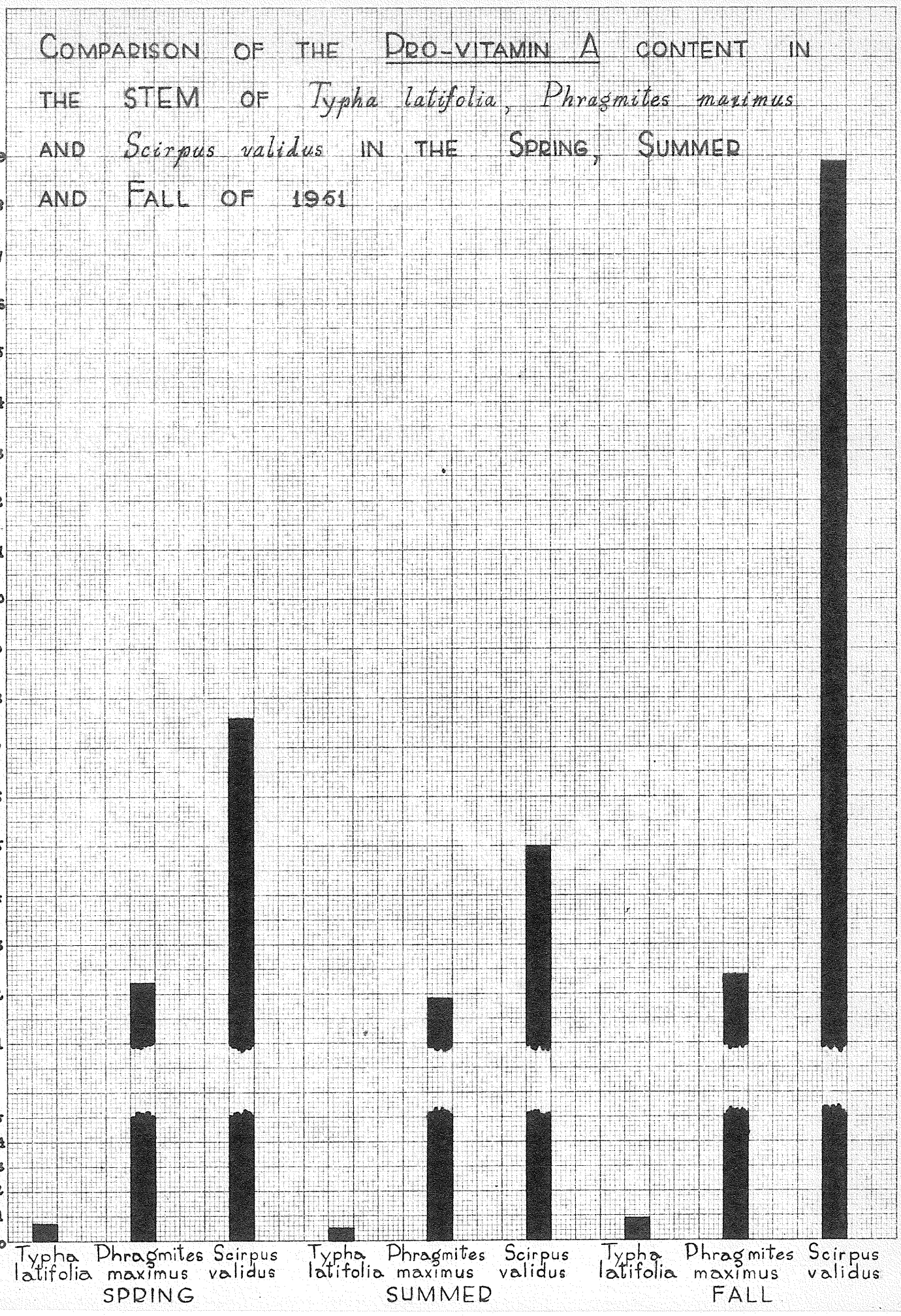

COMPARISON OF THE PRO-VITAMIN A (CAROTENE) IN Typha latifolia, LEAF, STEM AND ROOT IN THE WINTER OF 1950
γ OF CAROTENE PER GRAM OF SAMPLE
0
1
2
LEAF
STEM
ROOT


COMPARISON OF PRO-VITAMIN A (CAROTENE) IN Typha latifolia, Scirpus validus, Phragmites maximus, ROOTS AND STEMS IN THE WINTER OF 1950
γ OF CAROTENE PER GRAM OF SAMPLE
11
10
2
1
0
Typha latifolia
Scirpus validus
Phragmites maximus
Typha latifolia
Scirpus validus
Phragmites maximus

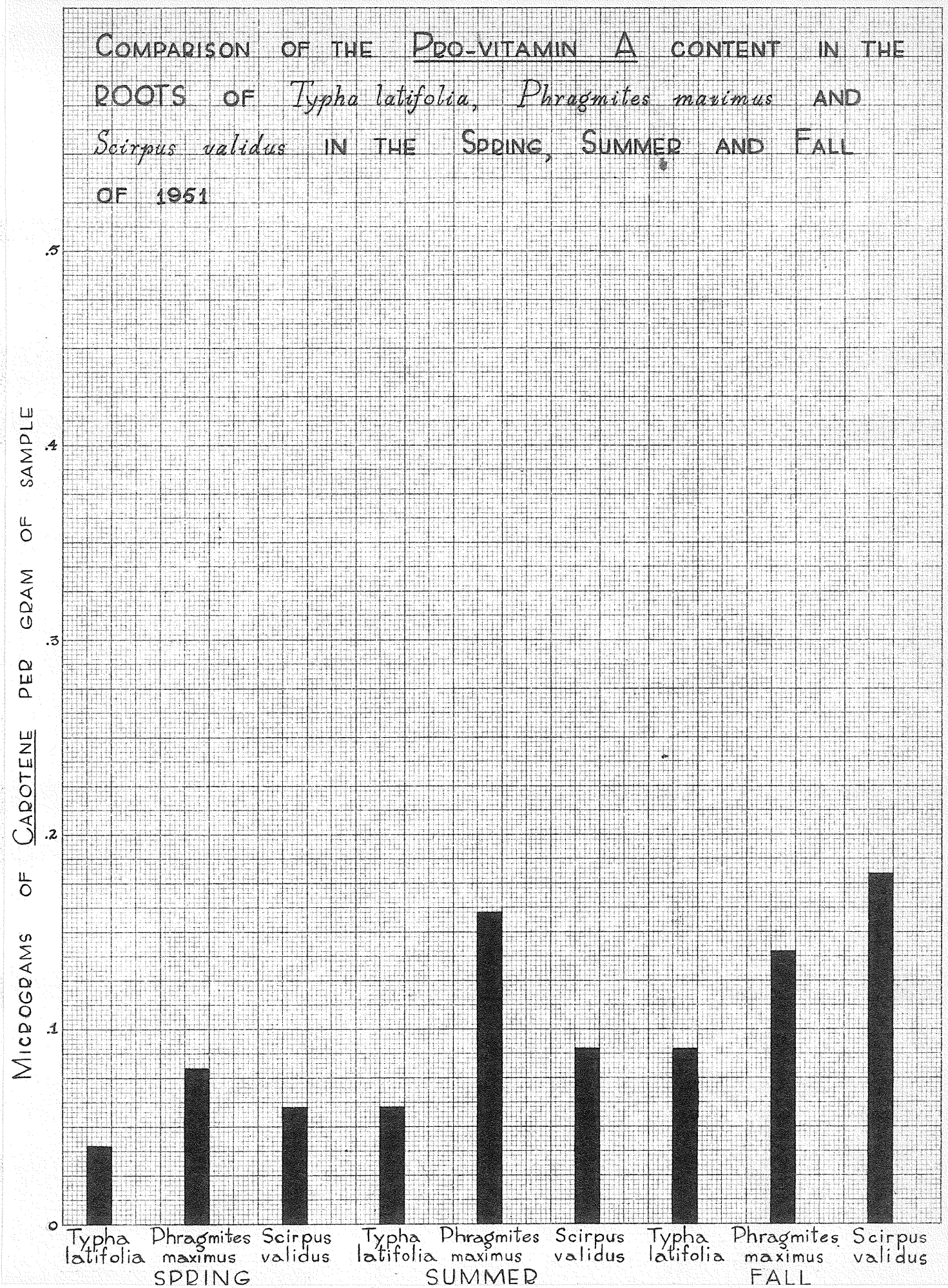

COMPARISON OF PRO-VITAMIN A (CAROTENE) CONTENT
IN Typha latifolia, Scirpus validus, Phragmites maximus, STEM
IN SUMMER AND WINTER OF 1950
γ OF CAROTENE PER GRAM OF SAMPLE
35
30
25
20
15
10
5
2
1
0
Typha latifolia
Scirpus validus
summer
Phragmites maximus
Typha latifolia
Scirpus validus
winter
Phragmites maximus

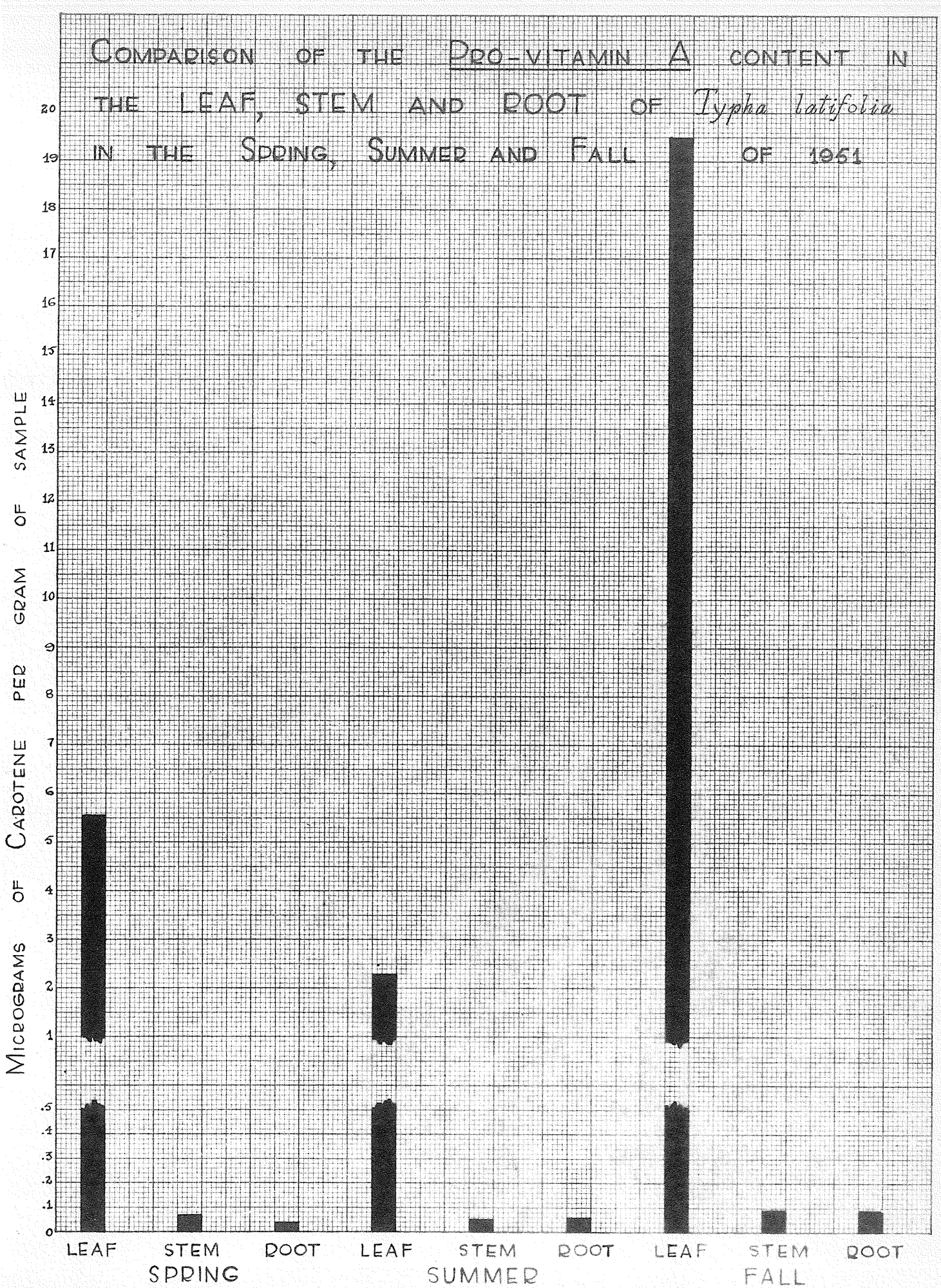
COMPARISON OF THE PRO-VITAMIN A CONTENT IN THE LEAVES OF *Typha latifolia* AND *Phragmites maximus* IN THE SPRING, SUMMER AND FALL OF 1951

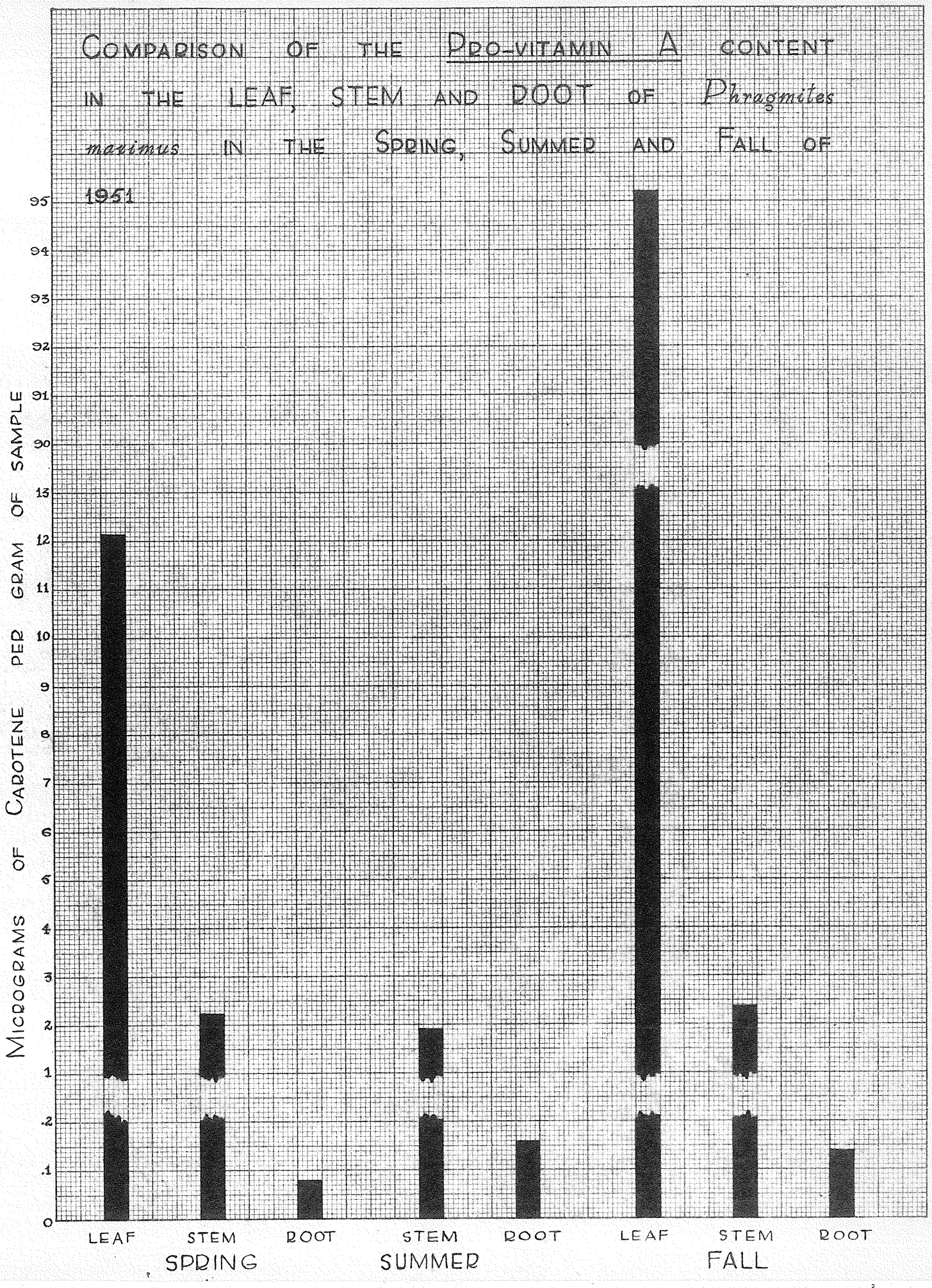


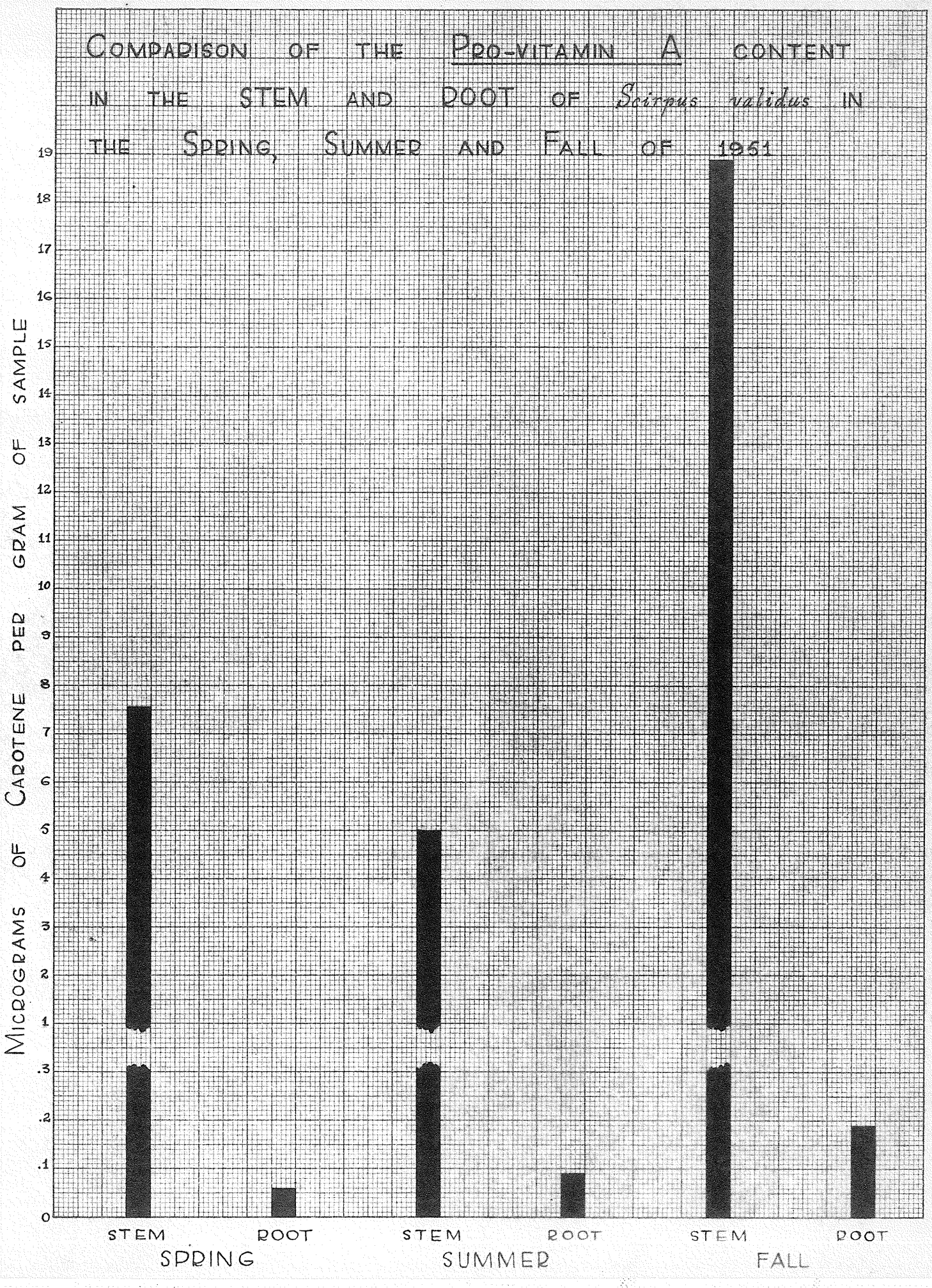

COMPARISON OF THE PRO-VITAMIN A CONTENT IN THE STEM OF Typha latifolia, Phragmites maximus AND Scirpus validus IN THE SPRING, SUMMER AND FALL OF 1961
MICROGRAMS OF CAROTENE PER GRAM OF SAMPLE
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
.6
.4
.3
.2
.1
0
Typha latifolia
Phragmites maximus
Scirpus validus
SPRING
SUMMED
FALL

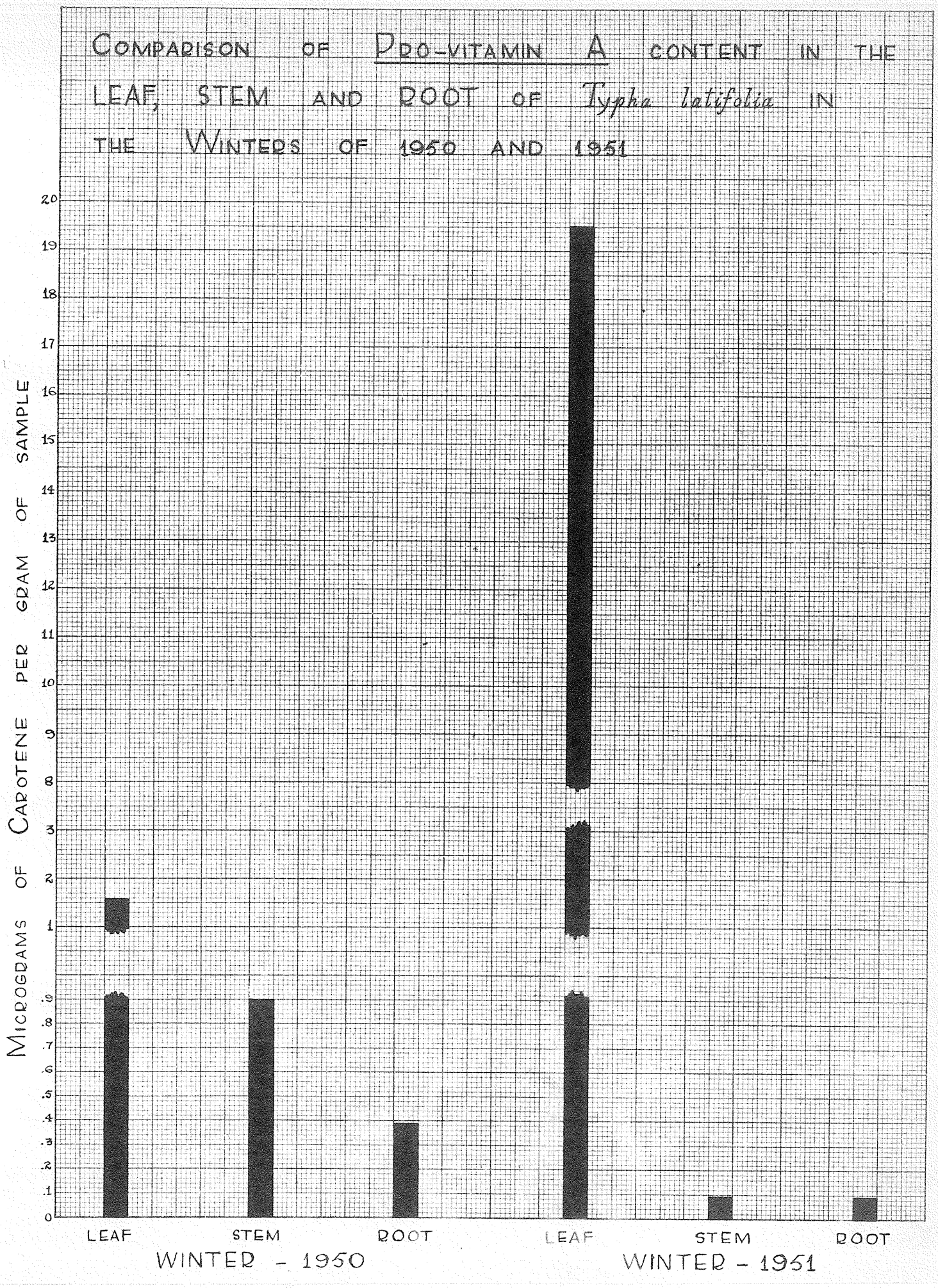
COMPARISON OF THE PRO-VITAMIN A CONTENT IN THE ROOTS OF *Typha latifolia*, *Phragmites maximus* AND *Scirpus validus* IN THE SPRING, SUMMER AND FALL OF 1951



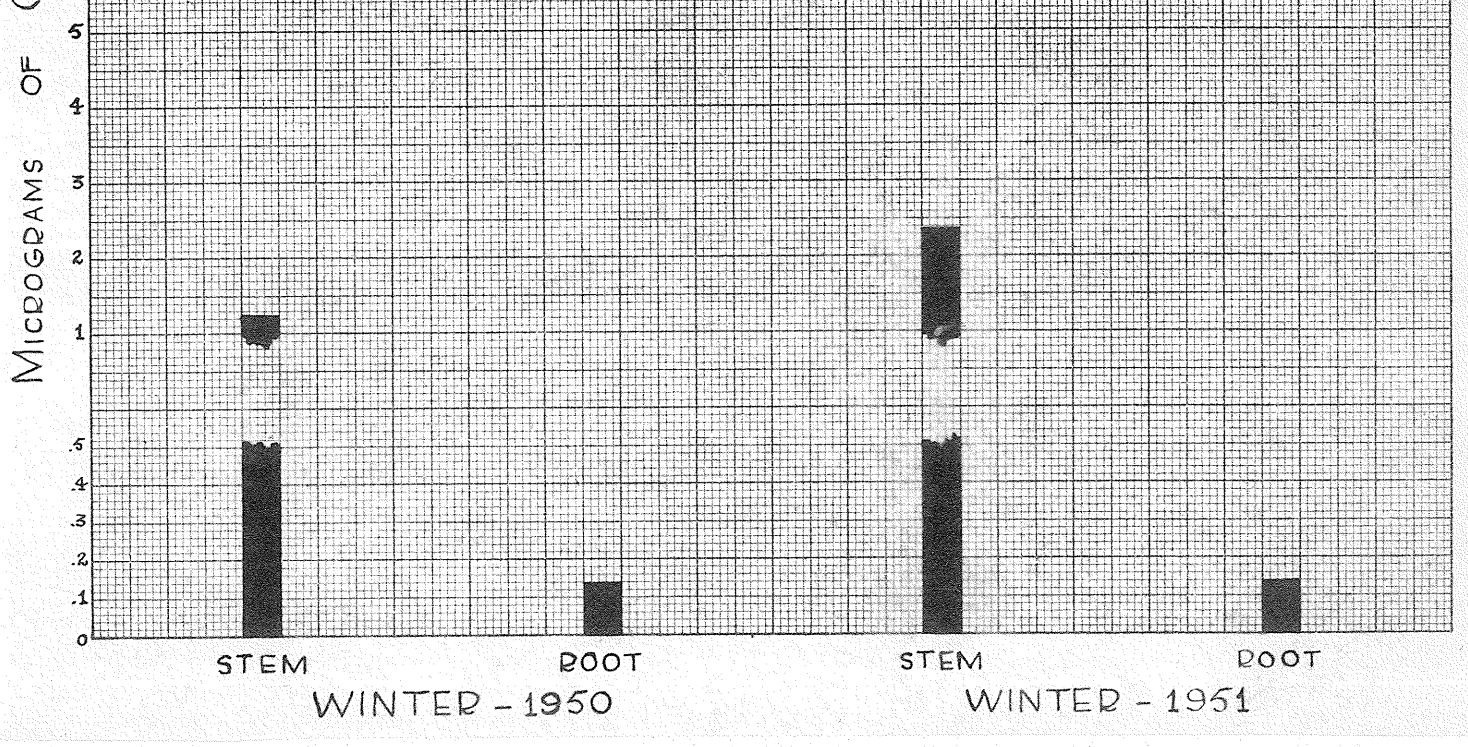

COMPARISON OF THE PRO-VITAMIN A CONTENT IN THE LEAF, STEM AND ROOT OF Typha latifolia IN THE SPRING, SUMMER AND FALL OF 1951
MICROGRAMS OF CAROTENE PER GRAM OF SAMPLE
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
.5
.4
.3
.2
.1
0
LEAF
STEM
ROOT
LEAF
STEM
ROOT
LEAF
STEM
ROOT
SPRING
SUMMER
FALL

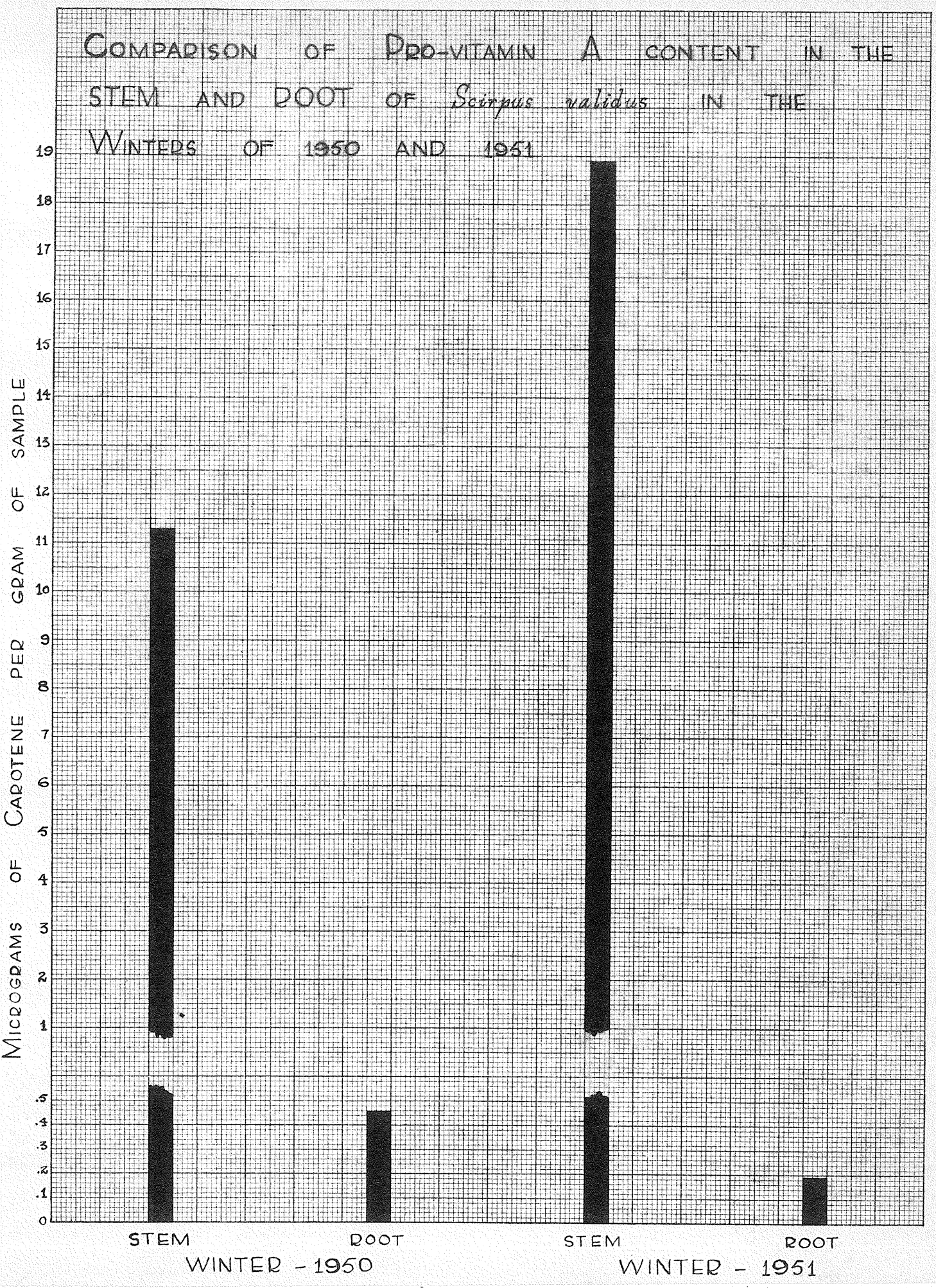



COMPARISON OF THE PRO-VITAMIN A CONTENT IN THE STEM AND ROOT OF Scirpus validus IN THE SPRING, SUMMER AND FALL OF 1951
MICROGRAMS OF CAROTENE PER GRAM OF SAMPLE
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
.3
.2
.1
0
STEM
ROOT
STEM
ROOT
STEM
ROOT
SPRING
SUMMER
FALL


COMPARISON OF PRO-VITAMIN A CONTENT IN THE LEAF, STEM AND ROOT OF Typha latifolia IN THE WINTERS OF 1950 AND 1951
MICROGRAMS OF CAROTENE PER GRAM OF SAMPLE
20
19
18
17
16
15
14
13
12
11
10
9
8
3
2
1
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
LEAF
STEM
ROOT
LEAF
STEM
ROOT
WINTER - 1950
WINTER - 1951

COMPARISON OF THE PRO-VITAMIN A CONTENT IN THE STEM AND ROOT OF *Phragmites maximus* IN THE WINTERS OF 1950 AND 1951




COMPARISON OF PRO-VITAMIN A CONTENT IN THE STEM AND ROOT OF Scirpus validus IN THE WINTERS OF 1950 AND 1951
MICROGRAMS OF CAROTENE PER GRAM OF SAMPLE
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
.5
.4
.3
.2
.1
0
STEM
ROOT
STEM
ROOT
WINTER - 1950
WINTER - 1951

DISCUSSION

RESULTS OF PRO-VITAMIN A (CAROTENE) ANALYSES OF SUMMER AND WINTER PLANTS OF 1950.

1. Comparing the five different types of plants studied, Scirpus fluviatilus contained 32.64 micrograms of carotene per gram of dry plant material, Scirpus validus came second with 30.08 grams, Phragmites maximus 17.19 grams and Typha latifolia 7.58 grams. However the analyses were restricted to three main plants: Typha latifolia, Scirpus validus and Phragmites maximus. It appears that the pro-vitamin A contents of these plants were very high in the summer as compared with the vitamin contents during the winter.
2. Scirpus validus tends to have the highest concentration of this vitamin both during the summer and winter.
3. The concentration of the vitamin in the stem of Scirpus validus during the summer is approximately $2\frac{1}{2}$ times as much as that in the winter.
4. The stem during the winter contains approximately $2\frac{1}{2}$ times as much as the root.
5. Due to the difficulty of obtaining root samples from these plants during the summer, vitamin assays were restricted to leaves and stems. However, roots are eaten very little in summer but comprise much of the diet in winter.

RESULTS OF PRO-VITAMIN A (CAROTENE) ANALYSES OF SUMMER AND WINTER PLANTS OF 1951

1. Typha latifolia

(i) Leaf - The concentration of carotene was highest in the fall; four times more than in the spring and nine times more than in the summer.

(ii) Stem - The concentration of carotene was more or less constant in the spring, summer and fall.

(iii) Root - A gradual increase from spring to fall was evident, with a concentration of the latter being almost two and a half times as much as that of the spring.

2. Phragmites maximus

(i) Leaf - Almost eight times as much carotene was obtained in the fall leaf as compared to that found in the spring leaf. No results were available for the summer.

(ii) Stem - The results were more or less constant throughout, with the fall being slightly higher.

(iii) Root - Very little difference in concentration of carotene was observed in summer and fall. Summer appeared to be slightly higher, whereas spring showed only one half as much as the others.

3. Scirpus validus

(i) Stem - The concentration appeared highest in the fall, two and a half times more than that in the spring.

(ii) Root - The concentration was about three times greater in the fall than that of the spring. There was a gradual increase from spring to summer to fall.

COMPARISON

CAROTENE CONCENTRATIONS - 1950 and 1951

Comparisons are based on the highest concentrations obtained from the plants in 1950 and 1951.

(i) Typha latifolia

1. Summer - The concentration of carotene showed a considerable decrease in 1951; almost one-quarter that of 1950.
2. Winter - The concentration of carotene showed a remarkable increase in 1951; twelve times that of 1950. This increase may indicate a very high resistance set up in the animal with perhaps the mortality rate being almost negligible.

(ii) Phragmites maximus

1. Summer - The concentration of carotene showed a decrease in 1951; being slightly over two-thirds that of 1950.
2. Winter - The concentration showed a most remarkable increase in 1951; seventy-six times that of 1950. Is there a relationship between the vitamin concentration and disease?

(iii) Scirpus validus

1. Summer - The concentration of carotene showed a decrease in 1951; about one-sixth that of 1950.
2. Winter - The concentration of carotene showed an increase in 1951; about one and one-half times that of 1950.

METHOD

DETERMINATION OF VITAMIN B_2 (RIBOFLAVIN) IN PLANT TISSUE

The method of analysis used was that adopted by the sub-committee on Vitamin Assays of the Associate Committee on Grain Research.

A sample of 1-4 gms. of plant material is added to 50 ml. of 0.1N. sulfuric acid in a 100 ml. volumetric flask and boiled on a water bath for one hour. Then it is cooled. To 12 ml. of 6.5% sodium phosphate solution and 2 ml. of redistilled glacial acetic acid are then added 0.01 gms. of takadiastase and the mixture allowed to stand for 45 minutes in the dark, in an incubator at 45-50° centigrade. The mixture is then diluted to 100 ml. with distilled water, mixed and filtered through a filter paper (Whatman No.40). To an aliquot (50 ml.) in a small Erlenmeyer flask, 1 ml. of freshly prepared permanganate solution is added. The aliquot is allowed to stand for 1 minute with frequent swirling of the flask. Enough hydrogen peroxide to decolorize the solution is added. The adsorption column is prepared by placing a wisp of cotton in the bottom of the tube and adding sodium fluorescein in hot water until a height of about two inches is obtained. After one washing with distilled water, the decolorized extract is added to the drained column and allowed to flow on its own accord. The sodium fluorescein column is washed twice with 5 ml. portions of distilled water. The adsorbed riboflavin is removed by passing 15 ml. of pyridine acetic acid solution through the column. The solution is collected in a graduated cylinder and made up to a convenient volume. The fluorometer is standardized and the galvanometer needle set at a desired setting by using dilute sodium fluorescein as a standard. In the cuvette, 15 ml. of the solution is placed and any deflection in the needle is corrected by adding 0.25 ml. of ice cold sodium hydrosulfite-sodium bicarbonate

solution. The deflection is noted again (C). This is the blank reading due to the fluoresence of the reagents. At the same time a reading (B) is made of the solution by diluting 1 ml. of riboflavin standard solution with pyridine-acetic acid solution to the same volume as that used of the eluate. This, less reading C is the deflection due to 1.0 micrograms of riboflavin. The riboflavin content of the sample is calculated from the following equation:

$$\text{micrograms } B_2 \text{ /gm} = \frac{A-C}{B-C} \times \frac{100}{\text{Vol. of Aliquot}} \times \frac{\text{Vol. of eluate}}{\text{Vol. of standard}}$$

Table VI

Vitamin B_2 (Riboflavin) Summer 1950 Coleman Photofluorometer

Plant	Sample Weight	Sample Reading	Sample Blank	Standard Reading	B_2 ug/gm.	% Moisture	Dry Basis
Typha maximus							
1.	3 gms.	30 - 11	19	19-10 9	1.40	50.9	2.84
2.	3 gms.	31 - 12	19	19-10 9	1.40	49.0	2.75
3.	3 gms.	34 - 12	19	19-10 9	1.60	49.7	3.04
4.	3 gms.	32- 12	22	19-10 9	1.40	50.2	2.80
5.	3 gms.	20 - 11	9	19-10 9	0.85	54.0	1.87
6.	3 gms.	20 - 11	9	19-10 9	0.85	53.0	1.78
7.	3 gms.	18 - 12	6	19-10 9	0.56	54.5	1.17
8.	3 gms.	19 - 11	8	19-10 9	0.76	53.5	1.59
							2.23
Scirpus validus							
1.	3 gms.	67 - 14	53	17-10 7	5.0	40.5	8.35
2.	3 gms.	65 - 14	51	17-10 7	4.8	40.9	8.16
3.	3 gms.	62 - 12	50	17-10 7	4.7	40.9	7.52
4.	3 gms.	65 - 13	52	17-10 7	4.9	40.6	7.84
5.	3 gms.	60 - 10	50	17-10 7	4.7	40.8	7.52
6.	3 gms.	62 - 13	49	17-10 7	4.6	41.0	7.36
7.	3 gms.	62 - 12	50	17-10 7	4.7	40.8	7.52
8.	3 gms.	65 - 12	53	17-10 7	5.0	40.4	8.35
							7.85
Phragmites maximus							
1.	3 gms.	72 - 12	60	19-10 9	4.4	31.4	6.16
2.	3 gms.	75 - 13	62	19-10 9	4.6	31.5	6.44
3.	3 gms.	75 - 12	63	19-10 9	4.6	31.4	6.44
4.	3 gms.	77 - 13	64	19-10 9	4.7	31.0	6.58
5.	3 gms.	75 - 12	63	19-10 9	4.6	31.4	6.44
6.	3 gms.	76 - 12	64	19-10 9	4.7	31.4	6.59
7.	3 gms.	70 - 14	56	19-10 9	4.1	31.5	5.74
8.	3 gms.	72 - 13	59	19-10 9	4.3	31.5	6.02
							6.30

Table VI (cont'd)

Vitamin B_2 (Riboflavin) Summer 1950 Coleman Photofluorometer

Plant	Sample Weight	Sample Reading - Sample Blank	Standard Reading	B_2 ug/gm.	% Moisture	Dry Basis
Scirpus fluviatilus						
1.	3 gms.	70 - 16 = 54	17-10 7	5.1	44.3	8.67
2.	3 gms.	75 - 15 = 60	17-10 7	5.6	44.0	9.52
3.	3 gms.	62 - 12 = 50	17-10 7	4.7	44.8	8.46
4.	3 gms.	75 - 17 = 58	17-10 7	5.5	44.1	9.35
5.	3 gms.	65 - 12 = 53	17-10 7	5.0	44.3	8.50
6.	3 gms.	67 - 11 = 56	17-10 7	5.3	44.1	9.01
7.	3 gms.	72 - 15 = 57	17-10 7	5.4	44.0	9.18
8.	3 gms.	60 - 12 = 48	17-10 7	4.5	44.8	8.10
						8.84
Sparganium						
1.	3 gms.	36 - 12 = 24	19-10 9	1.7	38.2	2.72
2.	3 gms.	37 - 12 = 25	19-10 9	1.8	38.0	2.88
3.	3 gms.	31 - 11 = 20	19-10 9	1.4	38.0	2.24
4.	3 gms.	31 - 11 = 20	19-10 9	1.4	38.0	2.24
5.	3 gms.	35 - 12 = 23	19-10 9	1.7	38.4	2.72
6.	3 gms.	35 - 11 = 24	19-10 9	1.7	38.2	2.72
7.	3 gms.	36 - 12 = 24	19-10 9	1.7	38.2	2.72
8.	3 gms.	35 - 11 = 24	19-10 9	1.7	38.3	2.72
						2.62

Table VII

Vitamin B_2 (Riboflavin) Winter 1950 Coleman Photofluorometer

Plant	Sample Reading	Sample Reading - Sample Blank	Standard Reading	B_2 ug/gm.	% Moisture	Dry Basis
Typha latifolia						
Leaf:						
1.	4 gms.	53 - 17 36	28-11 17	0.21	7.31	0.21
2.	4 gms.	52 - 16 36	28-11 17	0.21	6.92	0.21
3.	4 gms.	48 - 16 32	28-11 17	0.19	7.89	0.19
						0.20
Stem:						
1.	4 gms.	31 - 17 14	28-11 17	0.41	23.71	0.41
2.	4 gms.	30 - 16 14	28-11 17	0.43	25.46	0.43
3.	4 gms.	31 - 17 14	28-11 17	0.41	7.80	0.41
						0.41
Root:						
1.	4 gms.	29 - 14 15	28-11 17	0.43	14.10	0.43
2.	4 gms.	29 - 14 15	28-11 17	0.43	10.70	0.43
3.	4 gms.	29 - 14 15	28-11 17	0.43	19.87	0.43
						0.43
Scirpus validus						
1.	4 gms.	27 - 17 10	28-11 17	0.29	6.54	0.29
2.	4 gms.	30 - 17 13	28-11 17	0.38	6.62	0.38
3.	4 gms.	32 - 18 14	28-11 17	0.43	6.94	0.43
						0.36
Phragmites maximus						
1.	4 gms.	36 - 15 21	28-11 17	0.62	29.02	0.62
2.	4 gms.	34 - 14 20	28-11 17	0.58	29.02	0.58
3.	4 gms.	38 - 16 22	28-11 17	0.63	29.02	0.63
						0.61

Table VIII

Vitamin B_2 (Riboflavin) Spring 1951 Coleman Photofluorometer

Plant	Sample Reading	Sample Reading - Sample Blank	Standard Reading	B_2 ug/gm.	% Moisture	Dry Basis
Typha latifolia						
Leaf:						
1.	1 gm.	38 - 10.5 = 27.5	15	1.83	76.81	0.44
		40 - 11.0 = 29.0	15	1.93		
		41 - 12.0 = 29.0	15	1.93		
			mean -	1.90		
Stem:						
1.	3 gms.	19 - 11 = 8	15	0.36	92.20	0.28
		18 - 10 = 8	15	0.36		
		19 - 11 = 8	15	0.36		
			mean -	0.36		
Root:						
1.	3 gms.	23 - 13 = 10	15	0.44	89.32	0.05
		22 - 12 = 10	15	0.44		
		23 - 12 = 11	15	0.44		
			mean -	0.44		
Scirpus validus						
Stem:						
1.	3 gms.	57 - 12 = 45	15	2.00	81.95	0.35
		55 - 11 = 44	15	1.95		
		56 - 12 = 44	15	1.95		
			mean -	1.97		
Root:						
1.	3 gms.	25.0 - 12.0 = 13.0	15	0.58	87.17	0.08
		27.0 - 13.0 = 14.0	15	0.62		
		25.5 - 12.0 = 13.5	15	0.59		
			mean -	0.60		

Table VIII (cont'd)

Vitamin B_2 (Riboflavin) Spring 1951 Coleman Photofluorometer

Plant	Sample Reading	Sample Reading - Sample Blank	Standard Reading	B_2 ug/gm.	% Moisture	Dry Basis
Phragmites maximus						
Leaf:						
1.	1 gm.	80 - 13 = 67	15	8.92	45.80	4.79
		81 - 13 = 68	15	9.06		
		76 - 12 = 64	15	8.55		
			mean -	8.84		
Stem:						
1.	3 gms.	28 - 13 = 15	15	0.66	68.10	0.22
		28 - 12 = 16	15	0.71		
		27 - 12 = 15	15	0.66		
			mean -	0.68		
Root:						
1.	3 gms.	29 - 12 = 17	15	0.75	83.24	0.13
		30 - 12.5 = 17.5	15	0.78		
		29 - 12 = 17	15	0.76		
			mean -	0.76		

Table IX

Vitamin B_2 (Riboflavin) Summer 1951 Coleman Photofluorometer

Plant	Sample Reading	Sample Reading - Sample Blank		Standard Reading	B_2 ug/gm.	% Moisture	Dry Basis
Typha latifolia							
Leaf:							
1.	1 gm.	48 - 11	37	16	4.63	66.09	1.60
		50 - 12	38	16	4.75		
		50 - 12	28	16	4.75		
				mean -	4.71		
Stem:							
1.	3 gms.	25 - 11	14	16	0.58	90.59	0.05
		26 - 12	14	16	0.58		
		25 - 11.5	13.5	16	0.56		
				mean -	0.57		
Root:							
1.	3 gms.	24 - 12	12	16	0.50	86.18	0.07
		24 - 12.5	11.5	16	0.48		
		23 - 11	12	16	0.50		
				mean -	0.49		
Scirpus validus							
Stem:							
1.	3 gms.	38 - 16	22	15	0.98	71.72	0.28
		39 - 17	22	15	0.98		
		42 - 19	23	15	1.02		
				mean -	0.99		
Root:							
1.	3 gms.	32 - 12.5	19.5	15	0.87	75.85	0.12
		30 - 12.5	17.5	15	0.78		
		34 - 14.0	20.0	15	0.89		
				mean -	0.85		

Table IX (cont'd)

Vitamin B_2 (Riboflavin) Summer 1951 Coleman Photofluorometer

Plant	Sample Reading	Sample Reading - Sample Blank		Standard Reading	B_2 ug/gm.	% Moisture	Dry Basis
Phragmites maximus							
Stem:							
1.	3 gms.	24 - 14	10	15	0.44	68.10	0.13
		22 - 13	9	15	0.40		
		24 - 14.5	9.5	15	0.42		
				mean -	0.42		
Root:							
1.	3 gms.	24.5 - 13.0	11.5	15	0.51	70.01	0.15
		24.0 - 12.5	11.5	15	0.49		
		24.0 - 12.5	11.5	15	0.51		
				mean -	0.50		

Table X

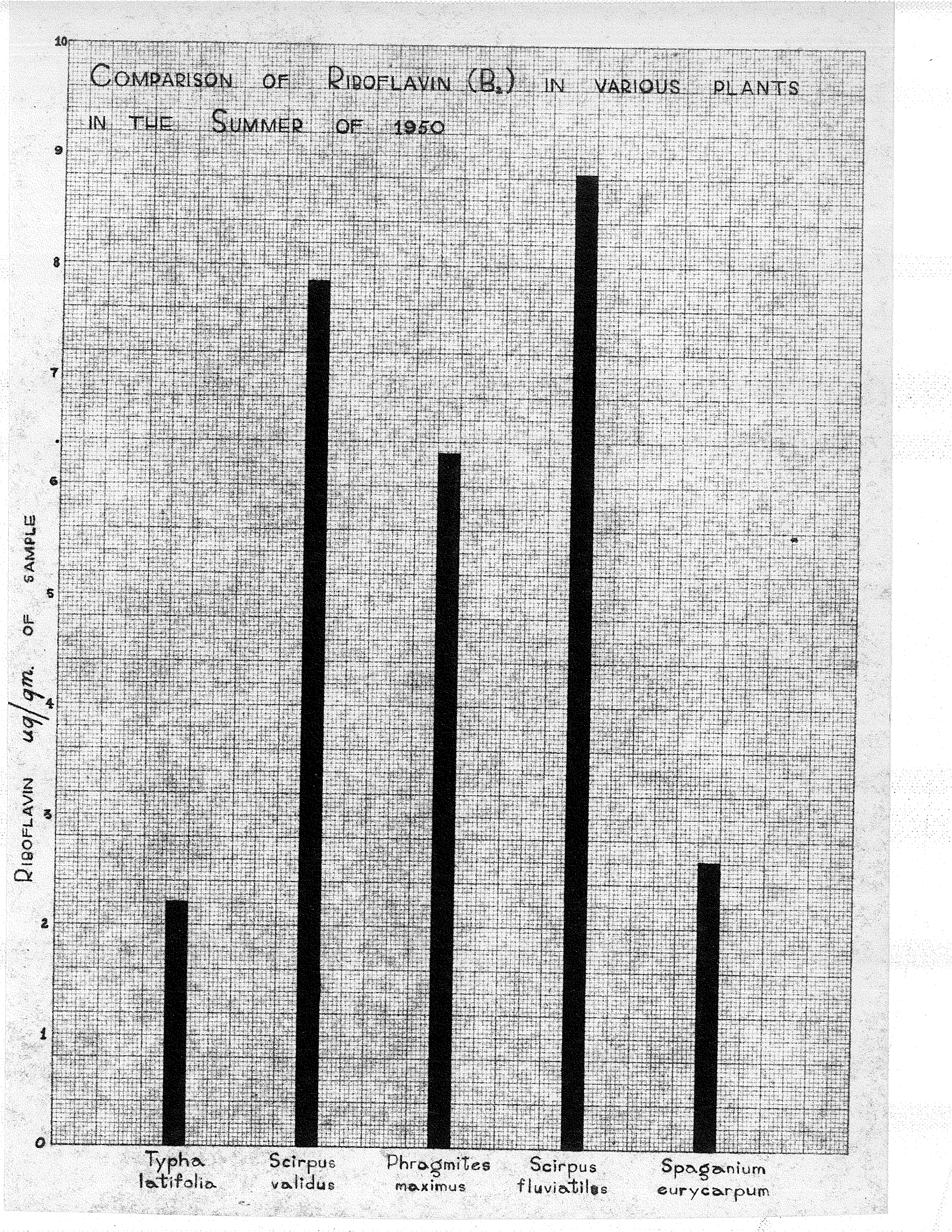
Vitamin B_2 (Riboflavin) Fall 1951 Coleman Photofluorometer

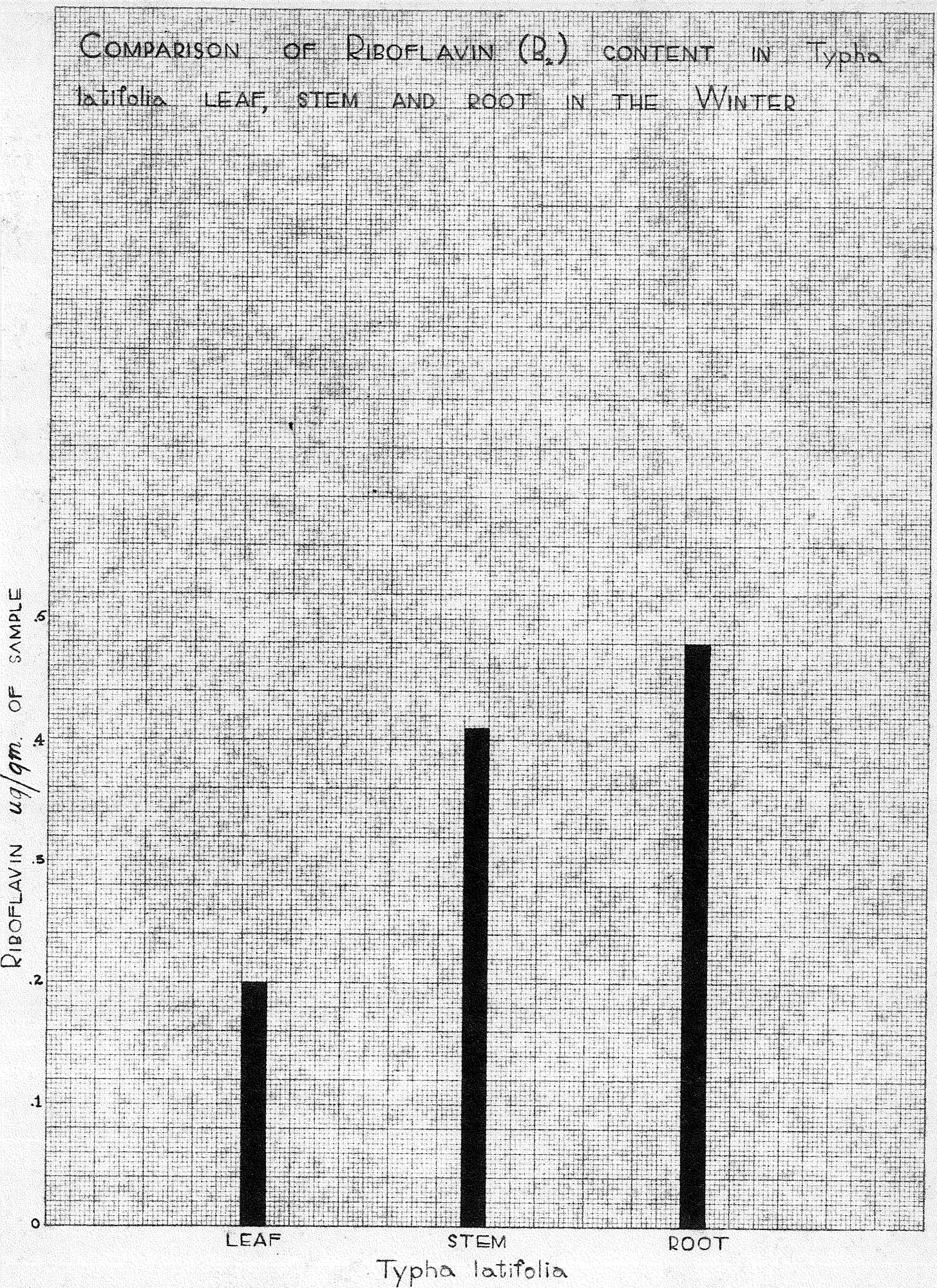
Plant	Sample Reading	Sample Reading - Sample Blank	Standard Reading	B_2 ug/gm.	% Moisture	Dry Basis
Typha latifolia						
Leaf:						
1.	2 gms.	74 - 14 60	17	3.53	60.95	1.38
		75 - 14 61	17	3.59		
		74 - 14 60	17	3.53		
			mean -	3.55		
Stem:						
1.	3 gms.	26 - 13 13	17	0.51	85.05	0.07
		27 - 14.5 12.5	17	0.49		
		25 - 13 12	17	0.47		
			mean -	0.49		
Root:						
1.	3 gms.	27 - 14 13	17	0.51	79.77	0.10
		26 - 13 12	17	0.51		
		25 - 13 12	17	0.47		
			mean -	0.49		
Scirpus validus						
Stem:						
1.	3 gms.	28 - 12 16	16	0.66	67.98	0.22
		30 - 13 17	16	0.71		
		31 - 14 17	16	0.71		
			mean -	0.69		
Root:						
1.	3 gms.	29 - 13 16	16	0.66	72.42	0.49
		28 - 12 16	16	0.66		
		29 - 12 17	16	0.71		
			mean -	0.68		

Table X (cont'd)

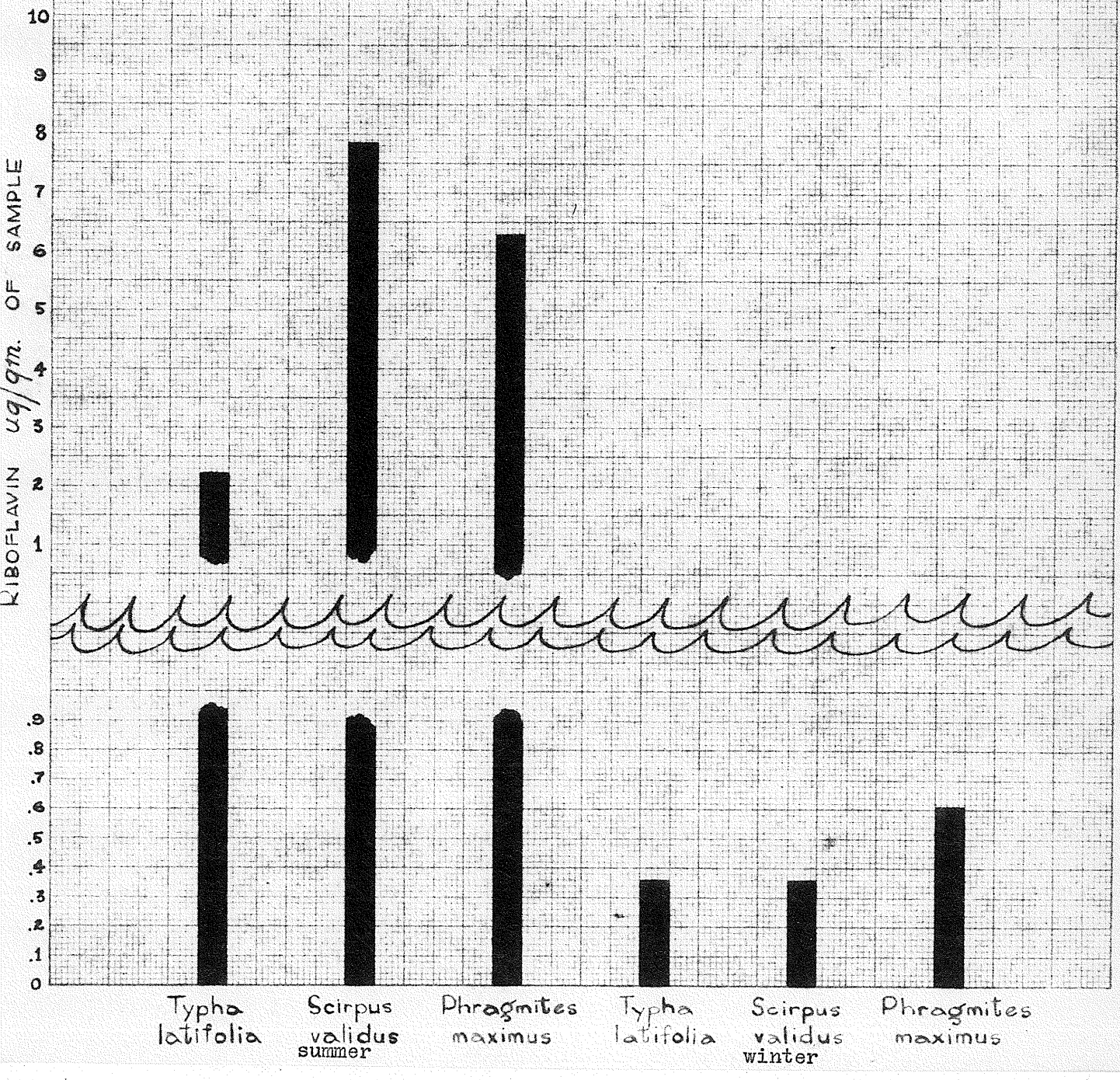
Vitamin B_2 (Riboflavin) Fall 1951 Coleman Photofluorometer

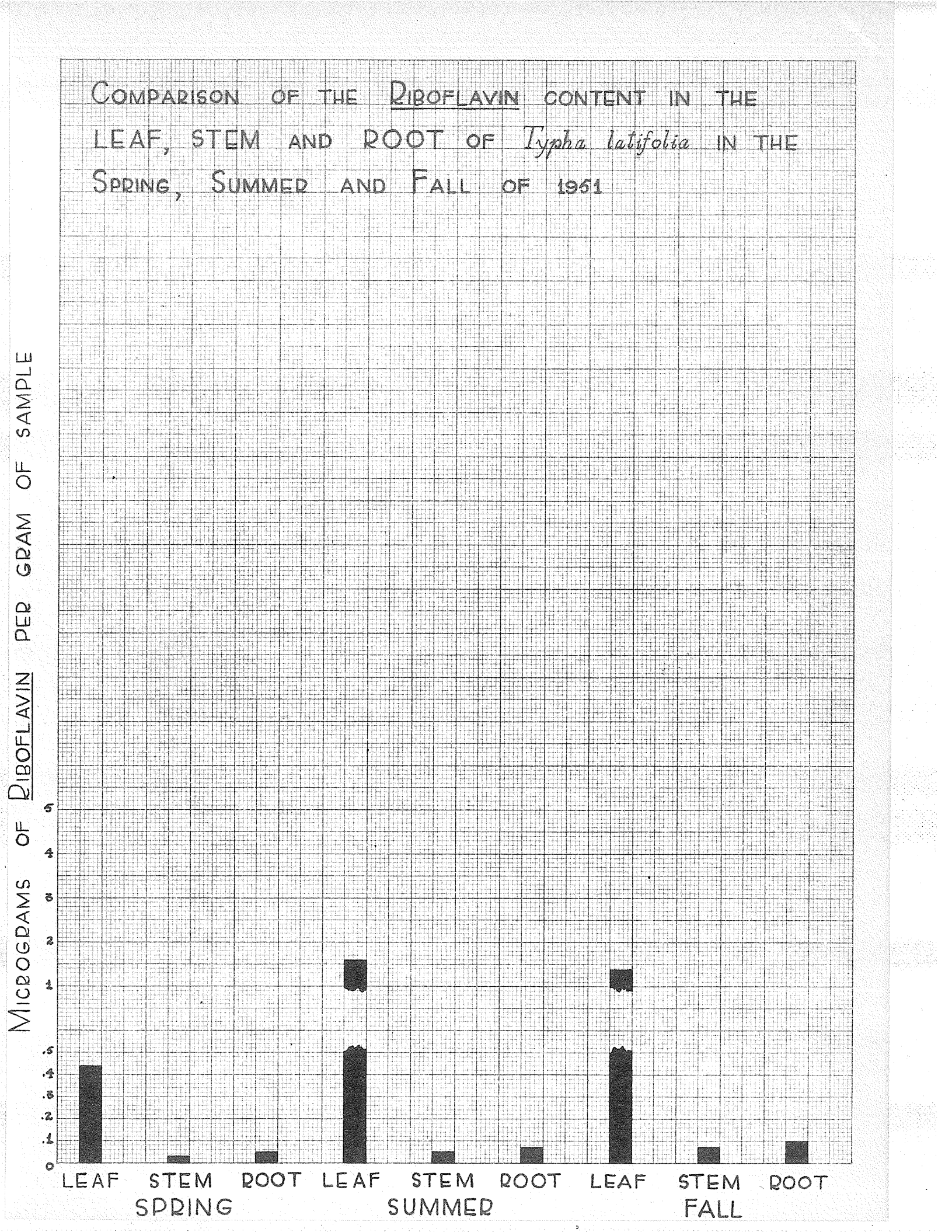
Plant	Sample Reading	Sample Reading - Sample Blank		Standard Reading	B_2 ug/gm.	% Moisture	Dry Basis
Phragmites maximus							
Leaf:							
1.	1 gm.	58 - 16	42	16	2.80	17.17	2.31
		60 - 17.5	42.5	16	2.83		
		57 - 16	41	16	2.74		
				mean -	2.79		
Stem:							
1.	3 gms.	30 - 15	15	16	0.62	58.24	0.27
		29 - 13	16	16	0.66		
		29 - 13	16	16	0.66		
				mean -	0.65		
Root:							
1.	3 gms.	39 - 15	24	17	0.94	68.05	0.32
		42 - 16	26	17	1.02		
		41 - 15	26	17	1.02		
				mean -	0.99		


COMPARISON OF RIBOFLAVIN (B_2) IN VARIOUS PLANTS IN THE SUMMER OF 1950
RIBOFLAVIN ug/gm. OF SAMPLE
0
1
2
3
4
5
6
7
8
9
10
Typha latifolia
Scirpus validus
Phragmites maximus
Scirpus fluviatiles
Spaganium eurycarpum

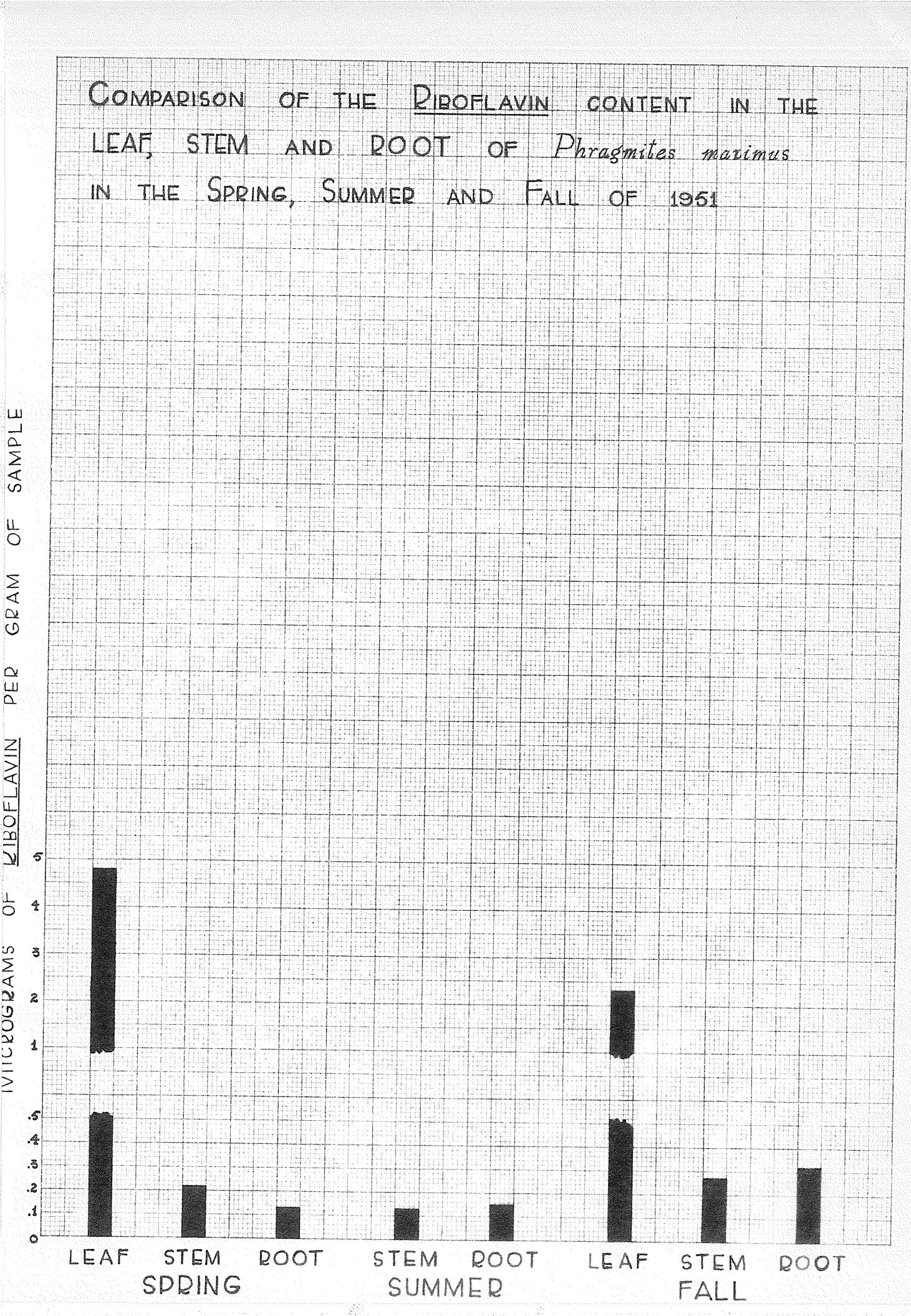

COMPARISON OF RIBOFLAVIN (B_2) CONTENT IN Typha latifolia LEAF, STEM AND ROOT IN THE WINTER
RIBOFLAVIN ug/gm. OF SAMPLE
0
.1
.2
.3
.4
.5
LEAF
STEM
ROOT
Typha latifolia

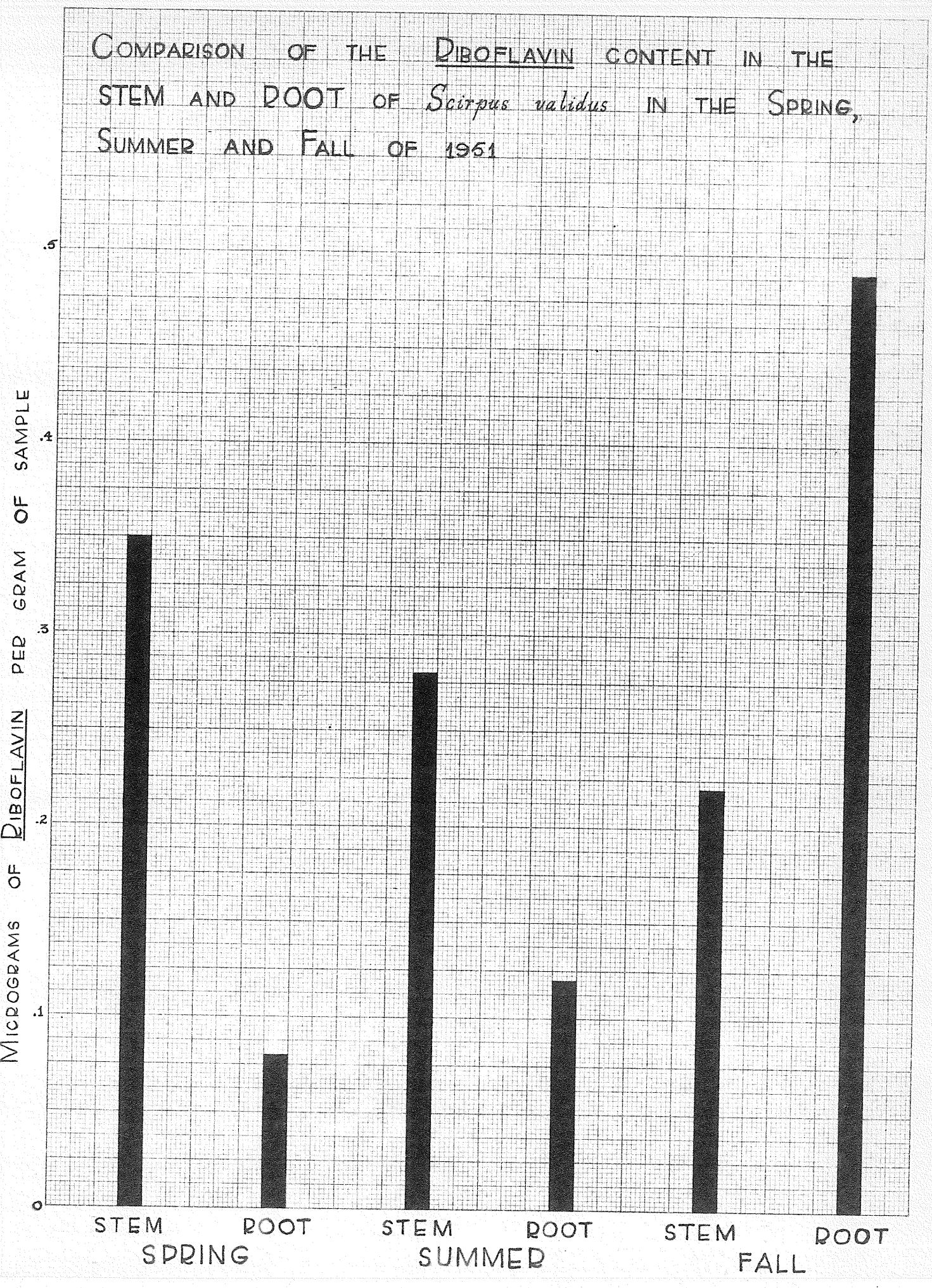
Comparison of Riboflavin (B_2) content found in *Typha latifolia*, *Scirpus validus* and *Phragmites maximus* in the Summer and Winter of 1950



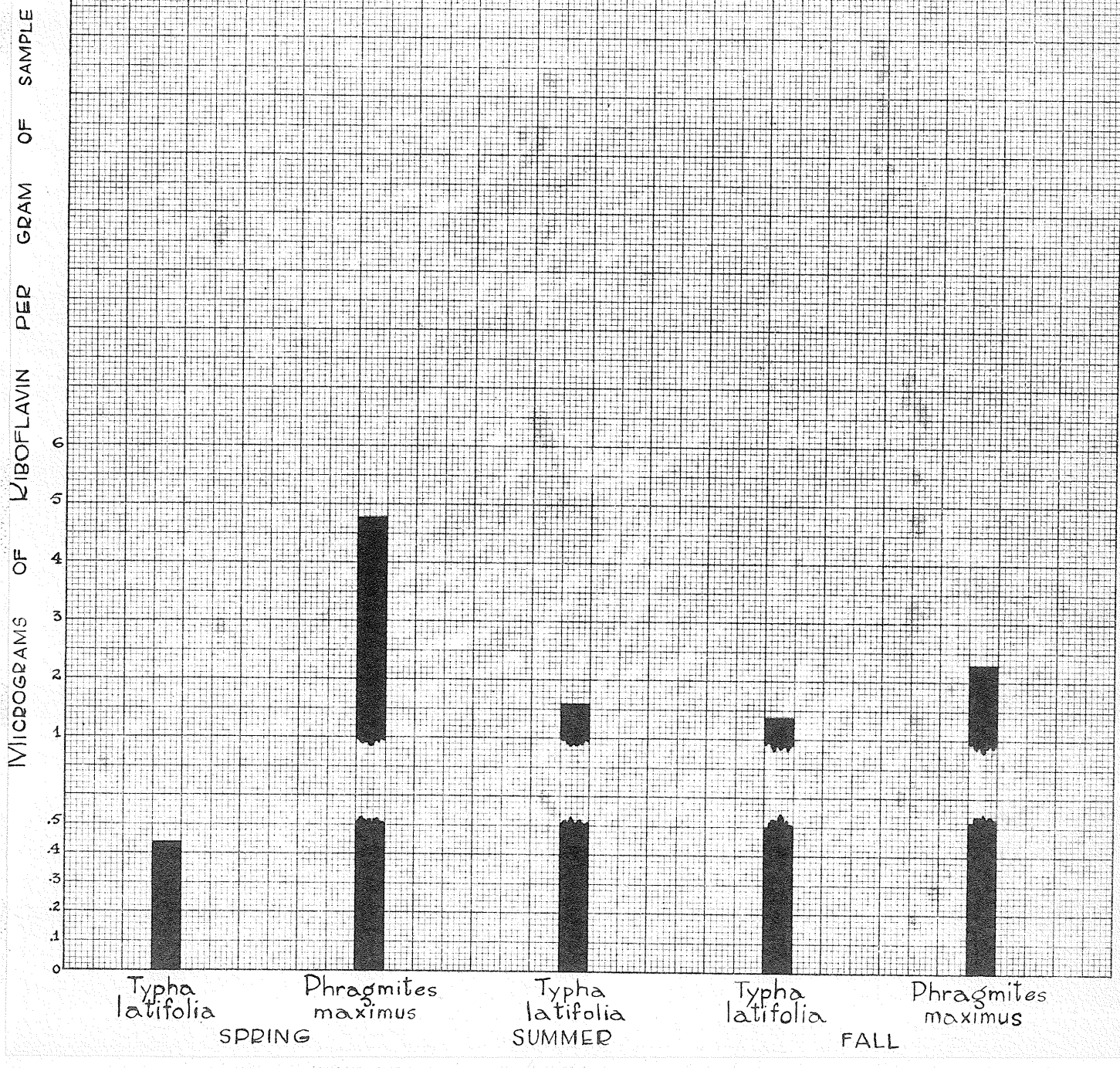

COMPARISON OF THE RIBOFLAVIN CONTENT IN THE LEAF, STEM AND ROOT OF Typha latifolia IN THE SPRING, SUMMER AND FALL OF 1961
MICROGRAMS OF RIBOFLAVIN PER GRAM OF SAMPLE
0
.1
.2
.3
.4
.5
1
2
3
4
5
LEAF
STEM
ROOT
LEAF
STEM
ROOT
LEAF
STEM
ROOT
SPRING
SUMMER
FALL

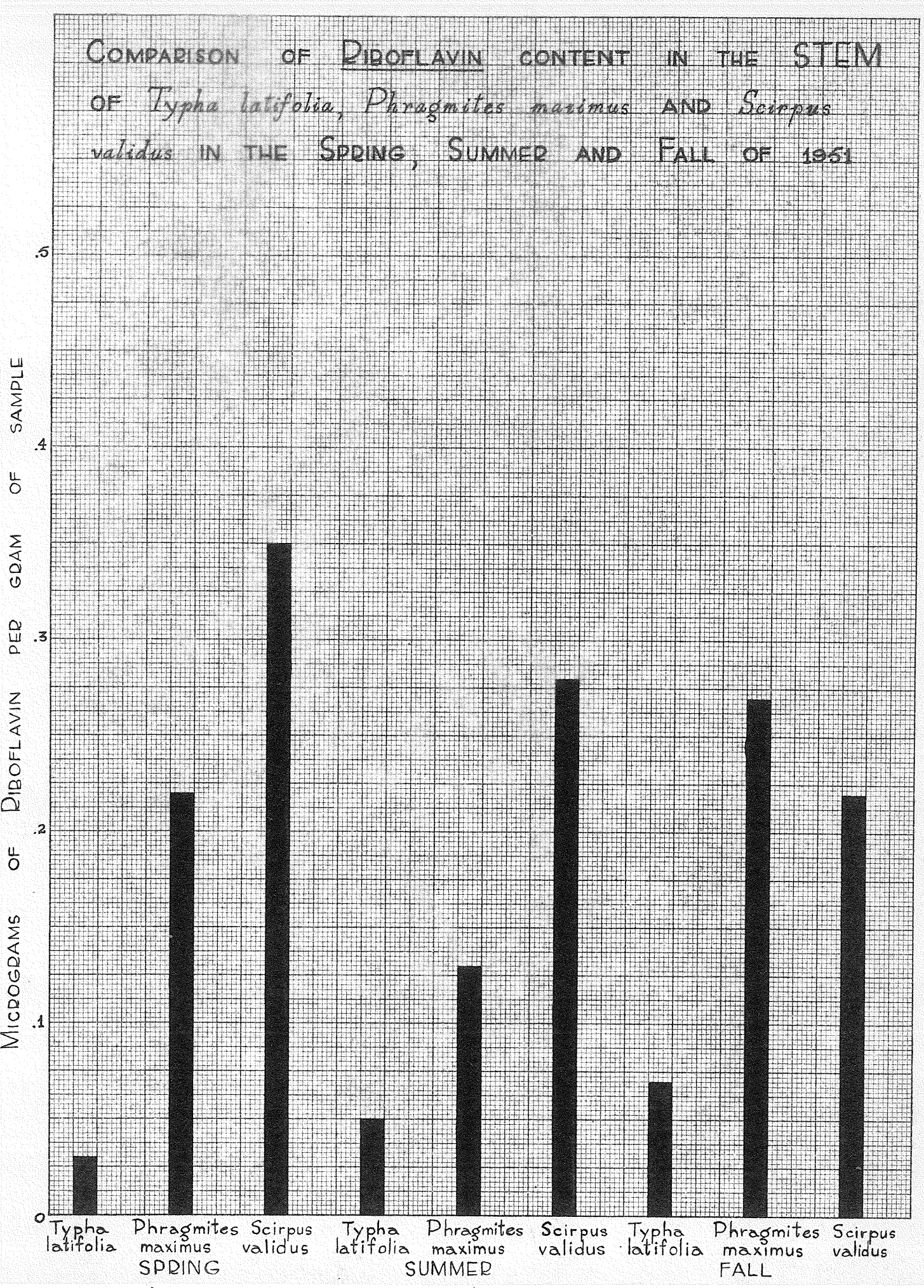
COMPARISON OF THE RIBOFLAVIN CONTENT IN THE LEAF, STEM AND ROOT OF *Phragmites maximus* IN THE SPRING, SUMMER AND FALL OF 1951

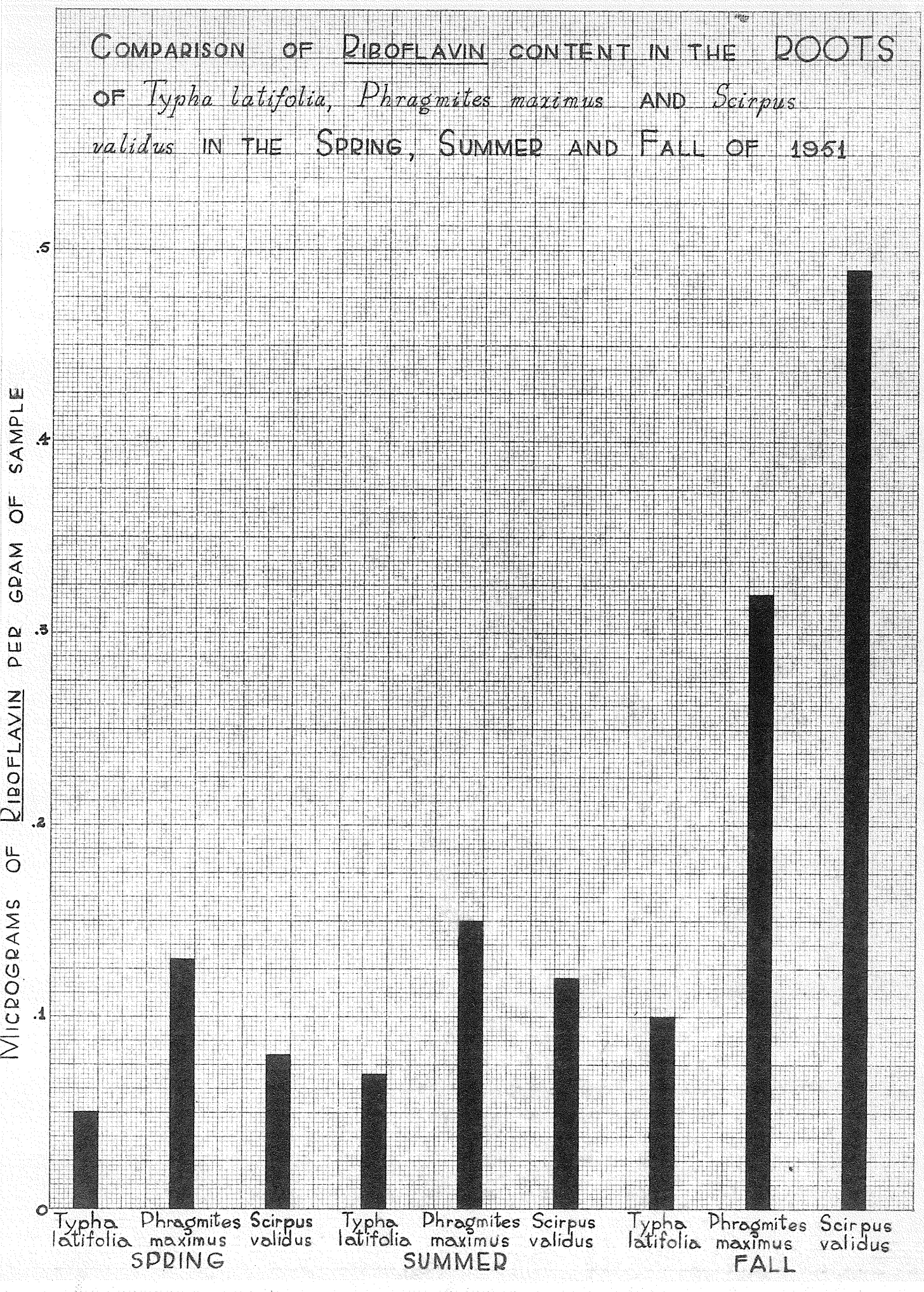


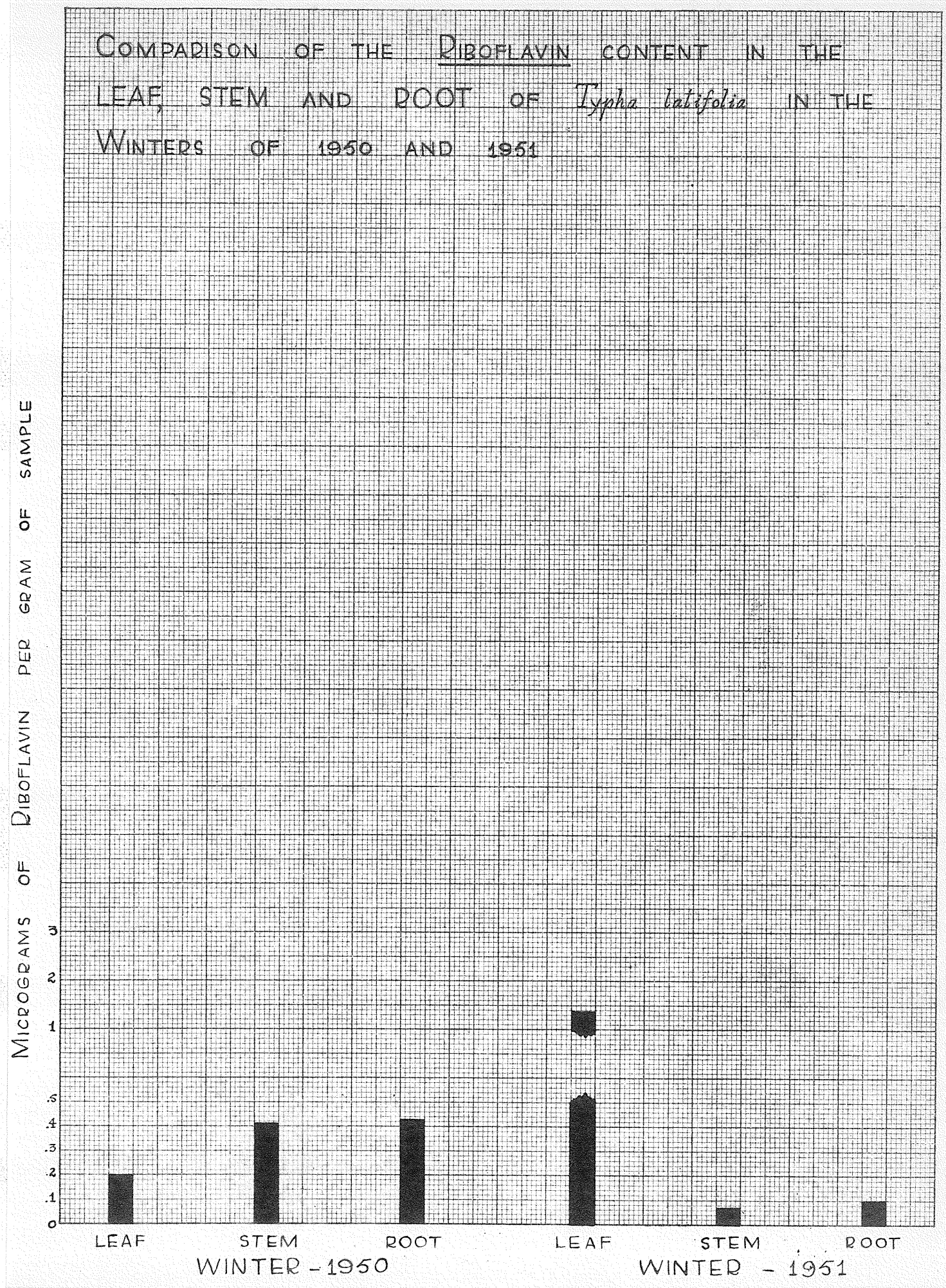

COMPARISON OF THE RIBOFLAVIN CONTENT IN THE STEM AND ROOT OF Scirpus validus IN THE SPRING, SUMMER AND FALL OF 1961
MICROGRAMS OF RIBOFLAVIN PER GRAM OF SAMPLE
0
.1
.2
.3
.4
.5
STEM
ROOT
STEM
ROOT
STEM
ROOT
SPRING
SUMMER
FALL

COMPARISON OF RIBOFLAVIN CONTENT IN THE LEAVES OF *Typha latifolia* AND *Phragmites maximus* IN THE SPRING, SUMMER AND FALL OF 1951




COMPARISON OF RIBOFLAVIN CONTENT IN THE STEM OF Typha latifolia, Phragmites maximus AND Scirpus validus IN THE SPRING, SUMMER AND FALL OF 1951
MICROGRAMS OF RIBOFLAVIN PER GRAM OF SAMPLE
0
.1
.2
.3
.4
.5
Typha latifolia
Phragmites maximus
Scirpus validus
Typha latifolia
Phragmites maximus
Scirpus validus
Typha latifolia
Phragmites maximus
Scirpus validus
SPRING
SUMMER
FALL


COMPARISON OF RIBOFLAVIN CONTENT IN THE ROOTS OF Typha latifolia, Phragmites maximus AND Scirpus validus IN THE SPRING, SUMMER AND FALL OF 1951
MICROGRAMS OF RIBOFLAVIN PER GRAM OF SAMPLE
.5
.4
.3
.2
.1
0
Typha latifolia
Phragmites maximus
Scirpus validus
Typha latifolia
Phragmites maximus
Scirpus validus
Typha latifolia
Phragmites maximus
Scirpus validus
SPRING
SUMMER
FALL


COMPARISON OF THE RIBOFLAVIN CONTENT IN THE LEAF, STEM AND ROOT OF Typha latifolia IN THE WINTERS OF 1950 AND 1951
MICROGRAMS OF RIBOFLAVIN PER GRAM OF SAMPLE
3
2
1
.5
.4
.3
.2
.1
0
LEAF
STEM
ROOT
LEAF
STEM
ROOT
WINTER - 1950
WINTER - 1951

DISCUSSION

RESULTS OF VITAMIN B_2 (RIBOFLAVIN) ANALYSES OF SUMMER AND WINTER PLANTS OF 1950

1. Scirpus validus tops the concentration with an 8.84 micrograms per gram of plant material. Sparganium was the lowest with a 2.62 micrograms per gram of material.
2. Comparison was restricted to three types: Typha latifolia, Scirpus validus and Phragmites maximus.
3. The vitamin content of these plants again are very high in the summer and extremely low in the winter. Scirpus validus contains the highest concentration in summer and Phragmites maximus contains the highest concentration in winter.
4. Comparing the riboflavin content in the leaf, stem and root of Typha latifolia in winter, the root contains over twice as much as the leaf.

DISCUSSION

RESULTS OF VITAMIN B_2 (RIBOFLAVIN) ANALYSES OF SPRING, SUMMER AND WINTER PLANTS OF 1951.

1. Typha latifolia

(i) Leaf - The concentration of riboflavin was comparatively low in the spring; and relatively high in the summer and fall, the summer being slightly higher than the fall.

(ii) Stem - Very little variation was observed in the amount of riboflavin in the spring, summer and fall.

(iii) Root - The concentration of the vitamin was low and almost constant in spring and summer, while the concentration was almost twice as high in the winter.

2. Phragmites maximus

(i) Leaf - Highest in the spring, about two times that found in the fall.

(ii) Stem - Very little variation was observed in the amount of riboflavin in spring, summer and fall.

(iii) Root - The concentration of vitamin B_2 was twice as high in the fall as compared with that of the spring or summer.

3. Scirpus validus

(i) Stem - The concentration was fairly well constant in the three seasons.

(ii) Root - There was a gradual increase in the vitamin from spring to fall, with fall having a concentration of six times that of the spring.

COMPARISON

RIBOFLAVIN CONCENTRATION IN 1951 AND 1950

Comparisons were based on the highest concentrations obtained from the plants in 1950 and 1951.

(i) Typha latifolia

1. Summer - The concentration of riboflavin showed a decrease in 1951; two-thirds that of 1950.
2. Winter - The concentration of riboflavin showed an increase, three times that of 1950.

(ii) Phragmites maximus

1. Summer - The concentration of riboflavin showed a decrease in 1951; three-quarters that of 1950.
2. Winter - The concentration of riboflavin showed an increase of four times that of 1950.

(iii) Scirpus validus

1. Summer - The concentration of riboflavin in 1951 was a mere fraction - only one-twenty-eighth of that in 1950.
2. Winter - Very little variation was observed in the amount of riboflavin in 1951 and 1950.

METHOD

DETERMINATION OF VITAMIN C (ASCORBIC ACID) IN PLANT TISSUE

A rapid method of analysis is that suggested by Loeffler and Ponting (1942).

Quantities of 5 to 50 grams of plant material are used. This is placed in a Waring Blender with about seven parts of 1% metaphosphoric acid solution to one part of plant tissue. The solution is filtered through a coarse fluted filter paper. When nearly a 100 ml. of filtrate is obtained, three aliquots of 1 ml. are pipetted into three matched cuvettes. To one, 9 ml. of distilled water is added to serve as a blank. To each of the other two, 9 ml. of standardized dye is added from a rapid delivery pipette and the mixture quickly stirred. Readings must be taken within 15 seconds on the spectrophotometer. The sample reading is obtained by subtracting the log of the dye reading from the log of the sample reading. This value is referred to the standard curve and the concentration of ascorbic acid per milliliter of filtrate obtained. The machine is set at 250 to read cuvettes.

Calculations:

$$\frac{100 \times \text{micrograms ascorbic acid} \times 350 - (\% \text{ moisture} \times \text{wt. of sample})}{1000 \times \text{wt. of sample}} =$$

micrograms of vitamin C per 100 grams of plant material.

Ascorbic acid extract is usually extracted by a strong acid to inhibit enzyme action and autoxidation, and determined by oxidation with 2-6 dichlorophenol indophenol. Large proportions of 1% metaphosphoric acid yield a pH low enough to prevent losses during blending and high enough to prevent fading during the reaction with the dye.

Solutions:

1% metaphosphoric acid - 10 grams in 990 cc. dist. water.

2-6 dichlorophenol indophenol.

stock - 800 mg. of stock solution per liter.

work solution - 16 cc. mgm. of stock solution per liter of distilled water prepared and standardized daily.

Standard Curve:

Dissolve 125 mgms. of pure ascorbic acid in 250 ml. of 1% metaphosphoric acid. Add 1 to 10 ml. amounts by 1 ml. increments to 10, 100 ml. volumetric flasks. Make to volume with metaphosphoric. These contain 5 to 50 mgm. ascorbic acid per ml. Dye solution, 9 ml. portions are added to each of a series of matched cuvettes. 1 ml. from each solution added and readings taken at 520 immediately. The instrument is set at a 100 ml. of standardized dye solution and 1 ml. of metaphosphoric acid. Log of readings minus log of standard dye solution are plotted against ascorbic acid content in micrograms per milliliter.

Coleman Spectrophotometer

x	X(mcg/ml).	log Yo	X log Y	X^2	Yc	log Yc - log Yo
-4	5	.05331	.26655	25	.04967	-.00364
-3	10	.09431	.94310	100	.09707	.00276
-2	15	.14358	2.15370	225	.14447	.00089
-1	20	.19821	3.96420	400	.19187	-.00634
0	25	.23463	5.86575	625	.23927	.00464
1	30	.28199	8.45970	900	.28667	.00468
2	35	.33530	11.73550	1225	.33407	-.00123
3	40	.37805	15.12200	1600	.38147	.00342
4	45	.42920	19.31400	2025	.42887	-.00033
5	50	.48035	24.01750	2500	.47627	-.00408
	275	2.62893	91.84200	9625		-.00077

$$\Sigma \log Y = m\Sigma X + 10b$$

$$\Sigma X \log Y = m\Sigma X^2 + b\Sigma X$$

$$2.62893 = m275 + 10b$$

$$91.84200 = m9625 + 275b$$

Solve for m.

$$m = \frac{\begin{matrix} 2.62893 & 10 \\ 91.84200 & 275 \end{matrix}}{\begin{matrix} 275 & 10 \\ 9625 & 275 \end{matrix}}$$

$$m = \frac{722.9558 - 918.4200}{75625 - 96250}$$

$$m = \frac{195.4642}{20625}$$

$$m = .00948$$

Solve for b.

$$b = \frac{\begin{matrix} 275 & 2.62893 \\ 9625 & 91.84200 \end{matrix}}{-20625}$$

$$b = \frac{25256.5500 - 25303.4513}{-20625}$$

$$b = .00227$$

$$\log Y = mX + b = \log Y = .00948X + .00227$$

STANDARD CURVE ASCORBIC ACID SPECTROPHOTOMETER SUMMER AND WINTER 1950

LOG y = .00948 + .00227

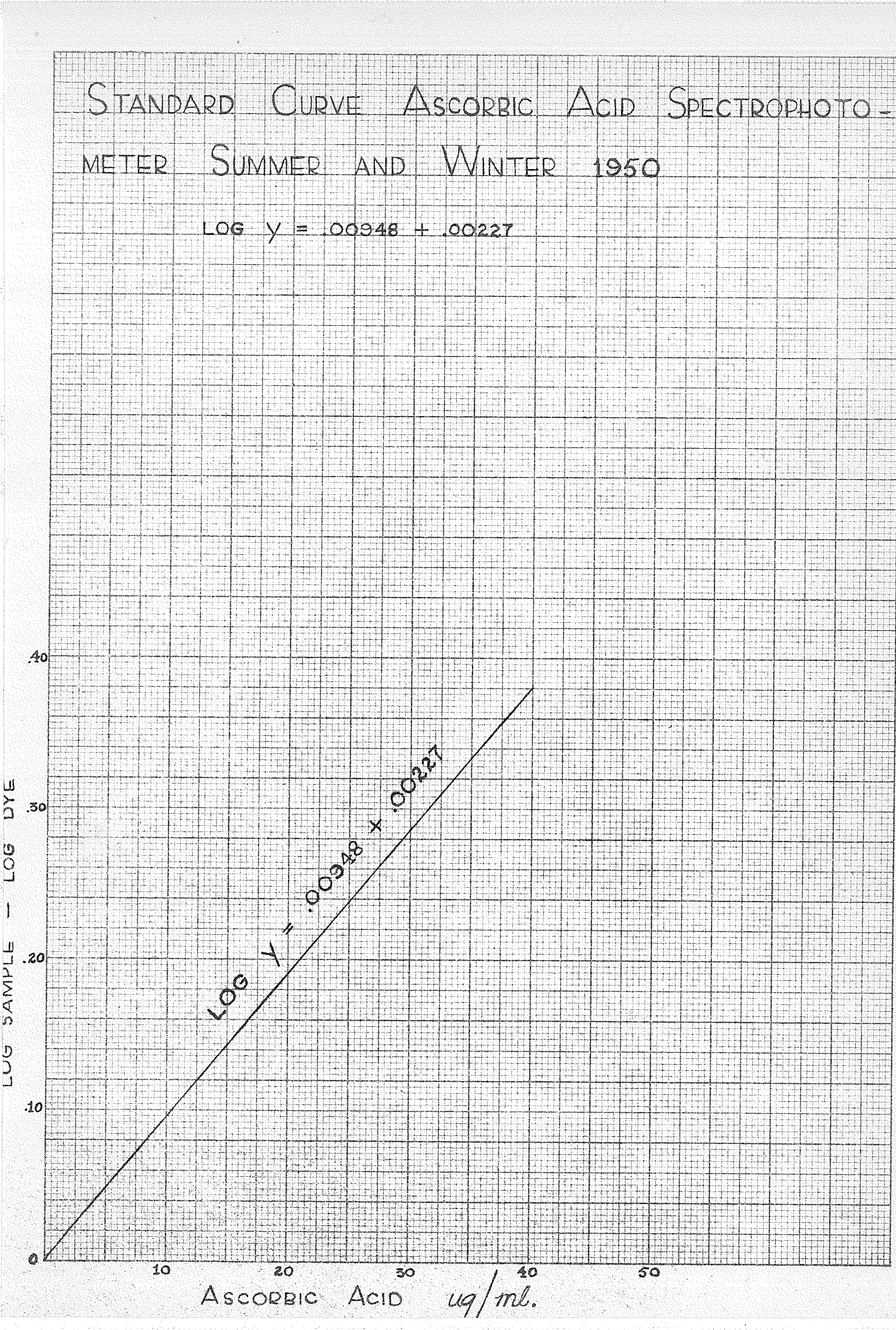


Table XI

Vitamin C (Ascorbic acid) Summer 1950 Coleman Spectrophotometer

Plant	Sample Reading	Average Reading	Log S	Dye & Log Dye	Log S - Log D	% Moisture	ug C /ml.	mg C 100 gms.
Typha latifolia								
1.	34.5 35.0	34.8	1.5414	26.8 1.4281	0.1134	45.5	17.3	90.62
2.	33.0 33.0	33.0	1.5250	26.8 1.4281	0.0969	48.5	14.9	82.29
3.	34.0 36.0	35.0	1.5440	26.8 1.4281	0.1159	44.2	17.8	91.13
								88.01
Scirpus validus								
1.	36.0 36.5	36.3	1.5599	26.8 1.4281	0.1318	38.7	20.3	92.77
2.	37.5 37.5	37.5	1.5740	26.8 1.4281	0.1459	38.0	23.2	104.40
3.	36.8 37.5	37.1	1.5693	26.8 1.4281	0.1412	38.5	21.7	98.73
4.	38.0 40.0	39.0	1.5987	26.8 1.4281	0.1706	35.5	26.8	113.90
5.	36.0 36.0	36.0	1.5563	26.8 1.4281	0.1282	40.1	19.6	94.00
								100.72
Phragmites maximus								
1.	36.0 36.0	36.0	1.5563	26.8 1.42813	0.1282	32.5	19.6	77.4
2.	35.0 35.0	35.0	1.5440	26.8 1.42813	0.1159	34.0	17.8	73.1
3.	36.0 36.0	36.0	1.5563	26.8 1.42813	0.1282	33.1	19.6	78.5
4.	35.0 35.0	35.0	1.5440	26.8 1.42813	0.1159	34.0	17.8	73.1
5.	35.0 35.0	35.0	1.5440	26.8 1.42813	0.1159	35.5	17.8	75.2
								75.4

Table XI (cont'd)

Vitamin C (Ascorbic acid) Summer 1950 Coleman Spectrophotometer

Plant	Sample Reading	Average Reading	Log S	Dye & Log Dye	Log S - Log Dye	% Moisture	ug C /ml.	mg C 100 gms.
Carex								
1.	35.5 36.5	36.0	1.5563	26.8 1.42813	0.1282	30.2	19.6	72.9
2.	35.5 35.5	35.5	1.5502	26.8 1.42813	0.1221	31.5	18.7	71.9
3.	36.0 36.5	36.3	1.5599	26.8 1.42813	0.1318	30.0	20.3	75.1
4.	36.0 35.0	35.5	1.5502	26.8 1.42813	0.1221	31.6	31.6	71.9
								72.9
Scirpus fluviatilus								
1.	37.0 37.0	37.0	1.5682	26.8 1.42813	0.1401	35.6	21.5	91.5
2.	37.0 37.0	37.0	1.5682	26.8 1.42813	0.1401	35.5	21.5	91.5
								91.5

Table XII

Vitamin C (Ascorbic acid) Winter 1950-51 Coleman Spectrophotometer

Plant	Sample Reading	Average Reading	Log S	Dye & Log Dye	Log S - Log Dye	% Moisture	ug C /ml.	mg C 100 gms.
Typha latifolia								
Leaf:								
1.	44.5 44.0	44.3	1.64640	42.5 1.6283	0.0181	2.08	3.0	8.99
2.	44.5 44.5	44.5	1.64836	42.5 1.6283	0.0200	1.92	3.1	9.25
3.	44.5 45.0	44.8	1.65128	42.5 1.6283	0.0229	1.33	3.5	9.83
4.	45.0 45.0	45.0	1.65321	42.5 1.6283	0.0249	1.34	3.8	8.19
5.	44.0 44.5	44.3	1.64640	42.5 1.6283	0.0181	1.49	3.0	8.60
6.	44.0 44.5	44.3	1.64640	42.5 1.6283	0.0181	1.29	3.0	8.92
7.	45.0 44.5	44.8	1.65128	42.5 1.6283	0.0229	2.66	3.5	10.73
								9.21
Stem:								
1.	45.0 47.0	46.0	1.6627	42.5 1.6283	0.0344	3.05	5.2	7.21
2.	44.5 44.0	44.3	1.6464	42.5 1.6283	0.0181	3.58	3.0	4.07
3.	45.5 45.0	45.3	1.6561	42.5 1.6283	0.0278	0.19	3.0	2.90
4.	44.5 44.0	44.3	1.6464	42.5 1.6283	0.0181	0.13	3.0	2.90
5.	43.0 44.5	43.7	1.6404	42.5 1.6283	0.0121	5.14	1.9	6.29
6.	44.0 44.5	44.3	1.6464	42.5 1.6283	0.0181	4.95	3.0	4.44
7.	46.0 44.5	45.2	1.6551	42.5 1.6283	0.0268	4.22	4.2	5.96
								4.74

Table XII (cont'd)

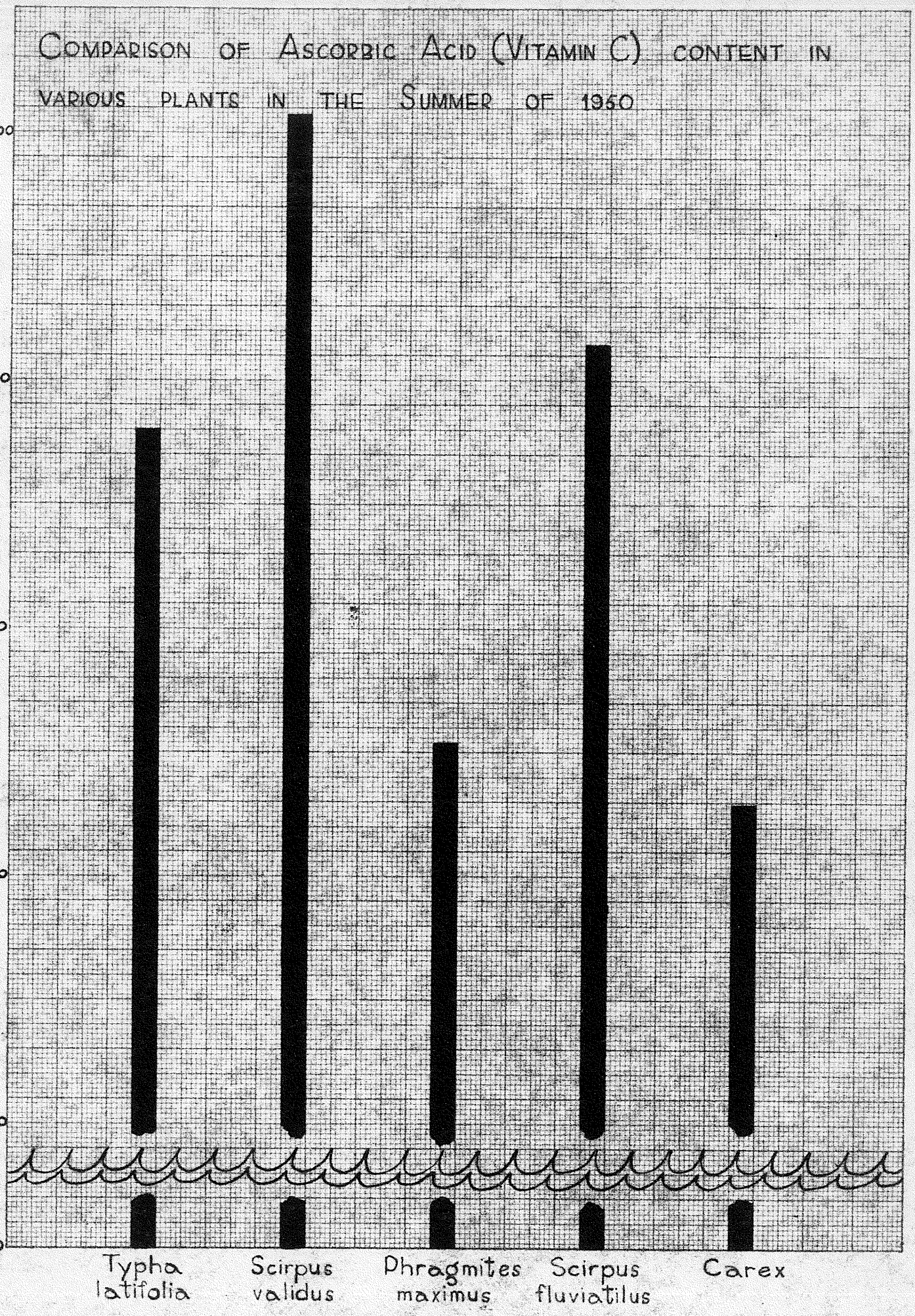
Vitamin C (Ascorbic acid) Winter 1950-51 Coleman Spectrophotometer

Plant	Sample Reading	Average Reading	Log S	Dye & Log Dye	Log S - Log Dye	% Moisture	ug C /ml.	mg C 100 gms.
Typha latifolia								
Root:								
1.	45.0 46.0	45.5	1.6580	42.5 1.6283	0.0297	9.65	4.6	7.62
2.	44.0 45.0	44.5	1.6483	42.5 1.6283	0.0200	16.81	3.1	8.50
3.	44.0 44.0	44.0	1.6434	42.5 1.6283	0.0151	13.13	2.3	5.20
4.	46.5 46.0	46.3	1.6655	42.5 1.6283	0.0372	2.88	5.6	7.21
5.	44.5 44.0	44.3	1.6464	42.5 1.6283	0.0181	8.04	3.0	4.50
6.	45.5 45.0	45.3	1.6561	42.5 1.6283	0.0278	1.51	4.5	5.16
7.	45.0 45.5	45.3	1.6561	42.5 1.6283	0.0278	7.64	4.4	6.20
								6.34
Scirpus validus								
1.	46.5 47.5	47.0	1.6721	43.0 1.6334	0.0387	0.53	5.8	19.05
2.	48.0 47.5	47.8	1.6794	43.0 1.6334	0.0460	0.25	7.1	10.12
3.	48.0 47.5	47.8	1.6794	43.0 1.6334	0.0460	0.41	7.1	10.23
4.	49.0 49.0	49.0	1.6902	43.0 1.6334	0.0568	2.28	8.8	14.32
5.	48.5 48.5	48.5	1.6857	43.0 1.6334	0.0523	1.59	8.0	12.45
6.	50.5 50.5	50.3	1.7015	43.0 1.6334	0.0681	1.01	10.5	15.78
7.	51.5 51.5	51.5	1.7118	43.0 1.6334	0.0784	0.43	12.0	17.26
								14.17

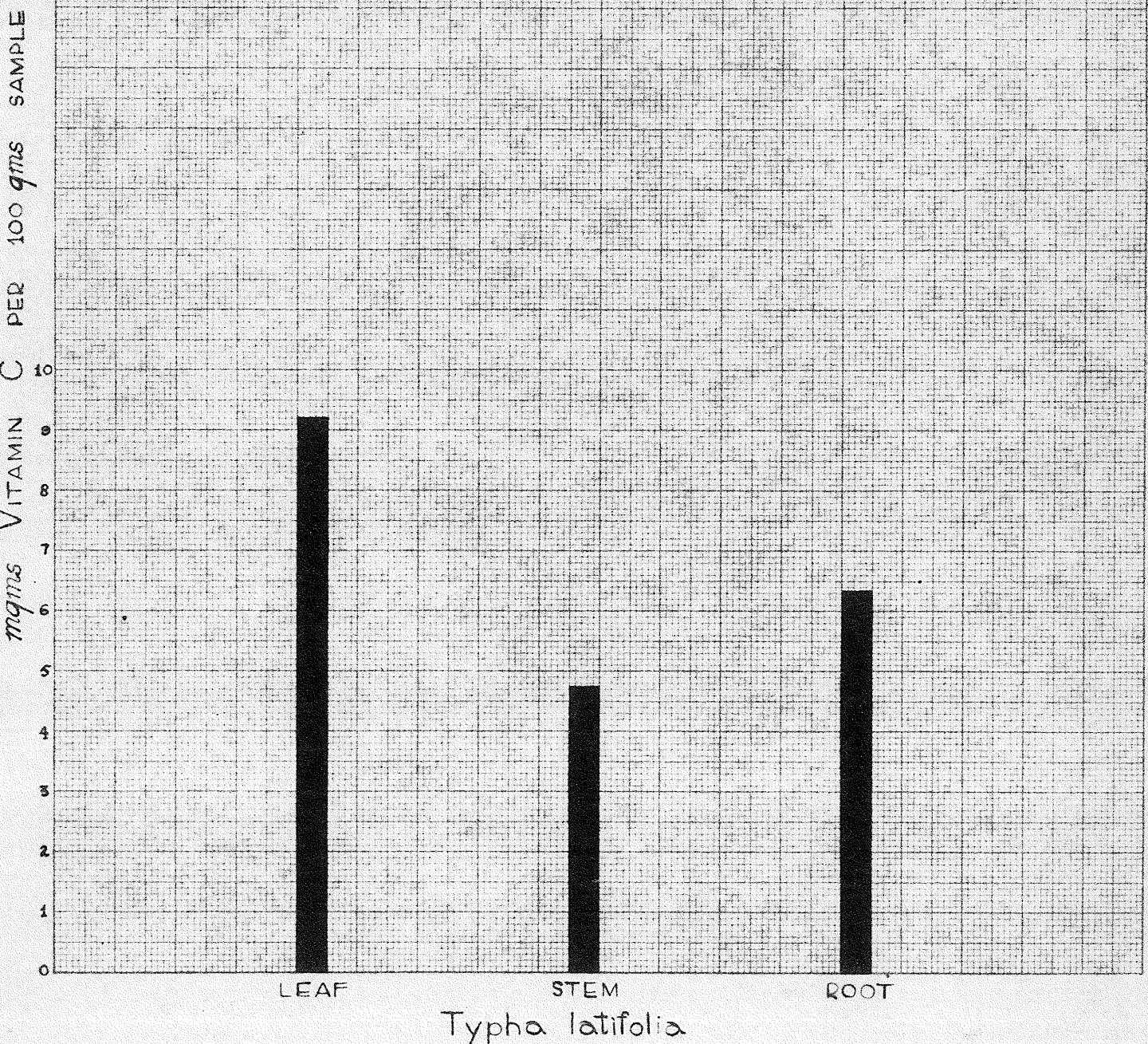
Table XII (cont'd)

Vitamin C (Ascorbic acid) Winter 1950-51 Coleman Spectrophotometer

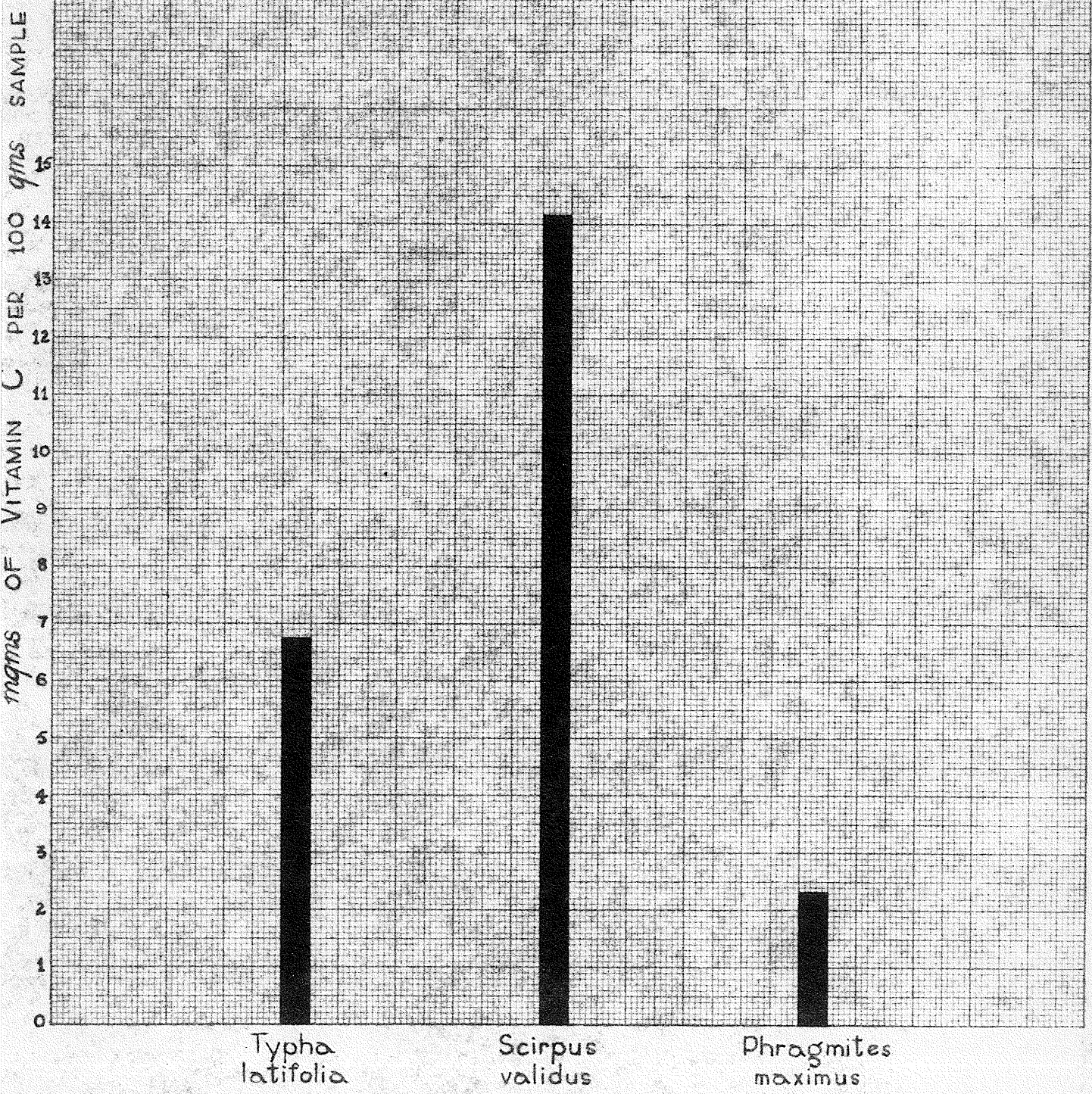
Plant	Sample Reading	Average Reading	Log S	Dye & Log Dye	Log S - Log Dye	% Moisture	ug C /ml.	mg C 100 gms.
Phragmites maximus								
1.	43.5 44.0	43.8	1.6414	42.5 1.6283	0.0131	6.04	2.0	2.60
2.	42.5 43.5	43.0	1.6334	42.5 1.6283	0.0050	11.37	0.8	1.46
3.	43.0 43.0	43.0	1.6334	42.5 1.6283	0.0050	8.50	0.8	1.23
4.	44.0 44.5	44.3	1.6464	42.5 1.6283	0.0181	5.47	3.0	3.73
5.	44.0 44.0	44.0	1.6434	42.5 1.6283	0.0151	4.37	2.3	2.61
								2.32

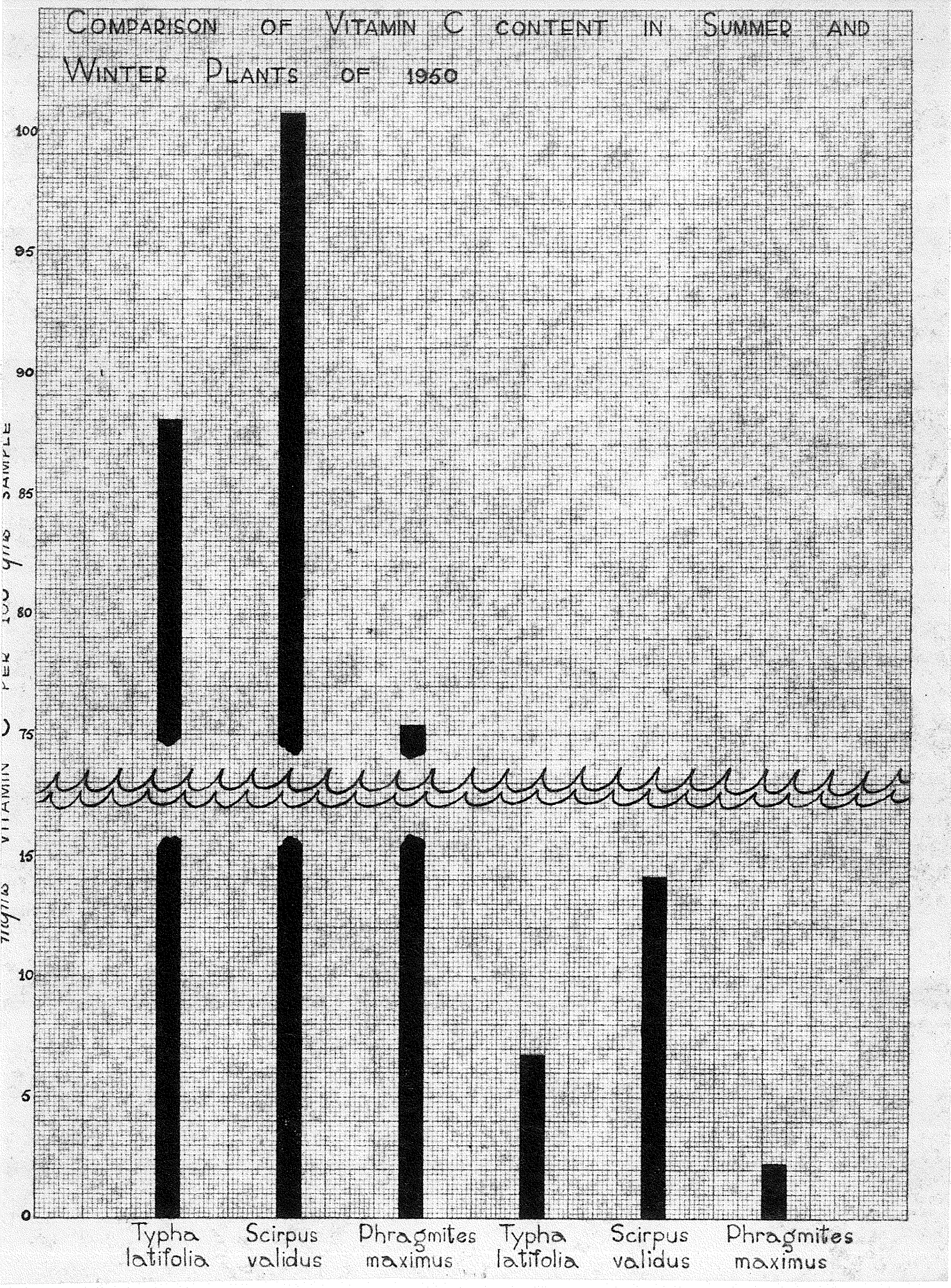

COMPARISON OF ASCORBIC ACID (VITAMIN C) CONTENT IN VARIOUS PLANTS IN THE SUMMER OF 1950
mgms VITAMIN C PER 100 gms SAMPLE
100
90
80
70
60
0
Typha latifolia
Scirpus validus
Phragmites maximus
Scirpus fluviatilus
Carex

Comparison of Ascorbic Acid (Vitamin C) Content in Typha latifolia Leaf, Stem and Root in the Winter of 1950



Comparison of Ascorbic Acid (Vitamin C) content in Typha latifolia, Scirpus validus and Phragmites maximus in the Winter of 1950




COMPARISON OF VITAMIN C CONTENT IN SUMMER AND WINTER PLANTS OF 1950
100
95
90
85
80
75
15
10
5
0
Typha latifolia
Scirpus validus
Phragmites maximus
Typha latifolia
Scirpus validus
Phragmites maximus

DISCUSSION

RESULTS OF VITAMIN C (ASCORBIC ACID) ANALYSES OF SUMMER AND WINTER PLANTS OF 1950

1. Comparing Typha latifolia , Scirpus validus, Phragmites maximus, Scirpus fluviatilus and Carex sp.it was found that Scirpus validus contained 100.72 mgms. per gram of sample; this being the highest. The lowest is Carex sp.
2. Scirpus validus contained the highest concentration of Vitamin C both in summer and winter.
3. In winter Typha latifolia leaf contains almost twice the vitamin content of the stem. The stem is the lowest with a 4.74 mgms. per gram, the root 6.34 and the leaf 9.21 mgm. per gram.
4. There is a drop in the vitamin C content in the winter.

GENERAL COMPARISON

PRO-VITAMIN A (CAROTENE)

	Summer		Winter	
Plant	1950	1951	1950	1951
Typha latifolia	7.58 mcg/gm.	2.30	1.60	19.52
Phragmites maximus	17.19	12.14 (spring)	1.24	95.25
Scirpus validus	30.08	5.00	11.30	18.89

VITAMIN B_2 (RIBOFLAVIN)

Typha latifolia	2.23	1.59	0.41	1.38
Phragmites maximus	6.30	4.79 (spring)	0.61	2.31
Scirpus validus	7.85	0.28	0.36	0.22

VITAMIN C (ASCORBIC ACID)

Typha latifolia	88.01 milligms. /100 gms.	-	4.74	-
Phragmites maximus	75.40	-	2.32	-
Scirpus validus	100.72	-	14.17	-

SUMMARY

PRO-VITAMIN A (CAROTENE)

Scirpus validus in the spring contained the highest concentration of carotene per gram of dry plant material, Typha latifolia the lowest. This, however, was not the case in the spring of 1951, where Scirpus validus stood second. Phragmites maximus appeared to be the best in the amount of carotene during the summer and winter of 1951. In surveying the Delta marsh during the summer of 1951 and comparing the observations of the surveys during the summer of 1950, certainly, the appearance of the Phragmites would indicate that there was a great improvement in the quality of the plant. Typha latifolia might be considered to be the poorest throughout the seasons of 1950 and 1951 as far as Pro-vitamin A is concerned.

VITAMIN B_2 (RIBOFLAVIN)

Scirpus validus was slightly higher than Phragmites maximus in 1950. Phragmites maximus contained the most riboflavin in the summer and winter of 1951, and also the winter of 1950. Thus we might state that the Phragmites is one of the best plants as far as the concentration of riboflavin is concerned.

VITAMIN C (ASCORBIC ACID)

Scirpus validus was the plant that contained the most ascorbic acid, both in summer and in winter. Typha latifolia came second and Phragmites maximus last. Since all animals with the exception of a few can synthesize their own vitamin C, it was decided unnecessary to analyze the 1951 plants.

GENERAL DISCUSSION

The lack of vitamins in sufficient quantity from the diet of an animal may show itself in one or more of the following ways, according to the species and age of the animal, the amount of the deficiency and the particular vitamin or vitamins involved:

1. Failure of young individuals to grow and mature at the normal rate.
2. Failure of mature individuals to reproduce.
3. Lowered resistance to infection in individuals of all ages.
4. Physiological disease or lack of well-being characterized by clinical symptoms and generally referred to as an avitaminosis.

The potential reproductive rate and extent of resistance to disease are factors which are extremely difficult to measure with accuracy the vitamin intake of an animal so that, in experimental work in setting up standards, the appearance of physiological disease is usually taken as the point below which the vitamin intake is deficient. However, it is widely agreed that approximately 2 to 4 times as much vitamin is required to promote optimum conditions of the body as will give rise to the first symptoms of avitaminosis.

It has been found that there is a definite correlation between the vitamin requirements of an animal and the body weight, the rate of growth and reproduction, the quantity of food consumed and the general metabolic rate. Standard sustaining and therapeutic amounts of vitamins required per day have been worked out empirically for only a few species of animals, mainly man. However, from this information, the approximate requirements of other species can be calculated.

The amounts of the three vitamins involved in this study are given by Best and Taylor for a man weighing 70 kg. as follows:

Pro A.	(carotene)	3,000 µg.	or	42.8 µg. per kg. body weight
B_2	(riboflavin)	3,598 µg.	or	51.4 µg. per kg. body weight
C	(ascorbic acid)	75 mg.	or	1.07 mg. per kg. body weight.

The average adult Manitoba muskrat weighs about .750 kg. but its metabolic rate is estimated to be about four times that of man. Thus, if one disregards the relatively larger food intake of the muskrat, it would have vitamin requirements equivalent to a body weight of 3 kg. On this basis its minimum vitamin requirements per day would be as follows:

Pro-A	(carotene)	128.4 µg.
B_2	(riboflavin)	154.2 µg.
C	(ascorbic acid)	3.21 mg.

It must be borne in mind that the above amounts represent only the quantities necessary to prevent avitaminoses and that from two to four times these quantities are necessary to permit normal growth and high resistance to infection. Thus, in order to have a completely vigorous individual, the daily intake will have to be in the neighborhood of the following values:

Pro-A	(carotene)	385 µg.
B_2	(riboflavin)	462.6 µg.
C	(ascorbic acid)	9.64 mg.

According to the interim report to the Manitoba Government (1948), in the section "Food Preference and Food Quantity Required", an adult female muskrat with eight suckling young consumed 99.6 gms. of Typha latifolia daily. According to our analyses of the edible parts of winter and summer plants, and working on the assumption that fresh plants contain about 90% moisture, the daily intake would be approximately:

	summer	winter
Pro-A	75.5 µg.	15.9 µg.
B_2	22.2 µg.	4.0 µg.
C	8764.8 mg.	438.2 mg.

This specimen also consumed 22.05 gms. of Scirpus validus per day or the following additional vitamins:

	summer	winter
Pro-A	66.2 µg.	25.2 µg.
B_2	17.3 µg.	0.865 µg.
C	2215.8 mg.	310.2 mg.

The total consumption of the above vitamins per day during summer and winter would be as follows:

	summer	winter
Pro-A	141.7 µg.	41.1 µg.
B_2	39.5 µg.	4.9 µg.
C	10,980.6 mg.	784.4 mg.

II. Two young muskrats about 28 days old consumed 117.6 gms. of Typha latifolia per day; that is approximately:

	summer	winter
Pro-A	89.4 µg.	18.8 µg.
B_2	26.3 µg.	4.7 µg.
C	10,385.2 mg.	519.3 mg.

At the same time these specimens consumed 90.4 gms. of Scirpus validus per day or additional vitamins as follows:

	summer	winter
Pro-A	270.7 µg.	102.9 µg.
B_2	70.65 µg.	3.5 µg.
C	9,064.8 mg.	1,269.1 mg.

The total vitamin consumption would then be:

	summer	winter
Pro-A	360.1 µg.	121.7 µg.
B_2	96.9 µg.	8.2 µg.
C	19,450.0 mg.	1,788.4 mg.

III An adult male in captivity consumed 129.9 gms. of Typha latifolia per day, that is approximately:

	summer	winter
Pro-A	98.5 µg.	20.7 µg.
B_2	29.0 µg.	5.2 µg.
C	11,441.3 mg.	572.1 mg.

In addition it consumed 96.9 gms. of Scirpus validus per day; that is approximately:

	summer	winter
Pro-A	291.8 µg.	110.9 µg.
B_2	76.1 µg.	3.8 µg.
C	9,769.8 mg.	1,367.8 mg.

The total vitamin intake per day would then be approximately:

	summer	winter
Pro-A	390.3 µg.	110.9 µg.
B_2	105.1 µg.	9.0 µg.
C	21,211.1 mg.	1,939.9 mg.

A summary of the estimated vitamin intake of the four experimental specimens and their estimated requirements is as follows:

Specimen number	Intake/day		Requirements/day	
	summer	winter	minimum to prevent avitaminosis	optimum to provide normal vigor
CAROTENE				
I.	141.7 µg.	41.1 µg.	128.4 µg.	385 µg.
II.	360.1 µg.	121.7 µg.	128.4 µg.	385 µg.
III.	390.1 µg.	131.6 µg.	128.4 µg.	385 µg.
RIBOFLAVIN				
I.	39.5 µg.	4.9 µg.	154.2 µg.	462.6 µg.
II.	96.9 µg.	8.2 µg.	154.2 µg.	462.6 µg.
III.	105.1 µg.	9.0 µg.	154.2 µg.	462.6 µg.
ASCORBIC ACID				
I.	10.9 mg.	7.4 mg.	3.21 mg.	9.63 mg.
II.	19.4 mg.	1.7 mg.	3.21 mg.	9.63 mg.
III.	21.2 mg.	1.9 mg.	3.21 mg.	9.63 mg.

Vitamin C need not be dealt with here since, although a deficiency except ascorbic acid is shown by specimen I in summer and all three specimens in winter, it is assumed on considerable evidence that all animals with the exception of primates and guinea pigs can synthesize their own supplies of ascorbic acid.

From the above figures, it can only be assumed that the muskrats of Delta and Netley marshes suffered from deficiencies of vitamins A and B_2 during the winter of 1950-51. To what extent the vitamin content of the plants concerned varies from year to year can only be determined by continuing the analyses over an appropriate length of time. It must be assumed, of course, that the muskrat diet will vary from place to place and from summer

to winter. These points can only be cleared up by further study.

What connection the apparent vitamin deficiency has with the sudden and extensive die-offs in muskrats from haemorrhagic enteritis must remain a matter for speculation until more information comes to hand. It is important to note that the incidence of disease in Iowa and in Netley and Delta marshes during the past fall and winter of 1950-51 appears to have been close to one-hundred per-cent. However, the mortality in Iowa was estimated by Errington (1951) to be about 8 percent while our estimates for Netley were 20-30 percent and for Delta 80-90 percent.

Such figures have more than casual significance and direct attention to some variable such as host resistance. This in turn could readily be associated with adequacy of the diet from place to place. Since the disease organism appears to be present at all times it might seem logical to assume that there is a dietary deficiency in years of epizootics.

CONCLUSIONS AND RECOMMENDATIONS

1. Vitamin investigation should be carried out further in order to determine whether there is any great difference between that of one area and that of another for the same plant.
2. The analyses should not be restricted only to vitamins A, B_2 and C, but B_1 (Thiamin) and E (Tocopherol) are both very important. Thiamin is an important growth vitamin and Tocopherol is a reproductive vitamin.
3. The analyses of the plants should not be limited to only three plants, but a survey of the other important plants used as muskrat food should be made.
4. As the results show there is a drop in the vitamin content from summer to winter. This drop in the vitamin content in plants would cause a lowered resistance in the muskrat. This lowered resistance opens the way for an invasion of viruses or bacteria.
5. Shortly after the onset of anaerobic conditions, the quality of the vegetation is drastically altered with the respect to the various vitamins. Such alterations in the food plants must exert a detrimental influence on the dependent muskrat population.
6. When the population density is sufficient to facilitate transmission of the causative organism and during the winter, will the disease assume epizoatic proportions?
7. Since the litter production during the earlier period constituted the greatest portion of the yearly total and subsequent occurrence of litters revealed a progressive decrease in their comparative size, some relative significance should be established between the concentration of the vitamin in the plant and litter production.

LITERATURE CITED

Association of Vitamin Chemists. 1947. Interscience Publishers. N.Y. " Method of Vitamin Assay."

BEST,C.H. and TAYLOR,N.B. 1945. The Physiological Basis of Medical Practice.

ERRINGTON,P. 1951. Personal correspondence with the Game and Fisheries Branch, Winnipeg, Manitoba, Canada.

LOEFFLER,H.J. and PONTING,J.D. Ascorbic Acid-Rapid Determination in Fresh, Frozen or Dehydrated Fruits and Vegetables. Ind. Eng. Chem. Anal. Ed. V.14. 846-849.

McLeod,J.A. 1950. A Consideration of Muskrat Populations and Population Trends in Manitoba. Trans. of the Royal Society of Canada. Vol. XLIV: Series III.

MOORE,L.A. and ELY,R. Extraction of Carotene from Plant Material. Ind. Eng. Chem. Anal. Ed. V.13. 600-601.

WALL,M.E. and KELLY,E.G. Determination of Pure Carotene in Plant Tissue. Ind. Eng. Chem. Anal. Ed. V. 15. 18-20.

According to the authors knowledge there appears to be no available literature on muskrat food and its relationship to fertility and disease.