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STUDIES ON THE EFFECTS OF CATION RATIOS ON TOBACCO

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Studies on the Effects of Cation Ratios on Tobacco

by

Benjamin Isgur

Thesis submitted for the degree of Master of Science

Department of Agronomy

Massachusetts State College

Amherst, Massachusetts.

1935

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PART I

GROWTH STUDIES

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Introduction and Object of Experiment

The problem of physiological balance in the soil under agricultural practice is of vast importance if the application of fertilizers is to be placed upon a scientific basis.

There have been several papers written upon the necessity of a certain Ca/Mg ratio. Loew ('03) proposed the hypothesis that one of the principal functions of calcium in plant metabolism is to neutralize the toxic action of magnesium and that a certain calcium magnesium ratio varying with the type of plant is necessary for the proper growth and development of the plant. An enormous amount of experimental work was carried on by Loew and his pupils, and by many others in an attempt to prove this hypothesis and to find the proper proportion of calcium and magnesium for various crop plants.

Lipman ('17), however, after a thorough resume of the literature on the subject, concluded that the experimental work presents no evidence in support of the hypothesis that a specific lime-magnesium ratio exists for any plant or group of plants. He considered that there was no more reason to assume that there should be a proper ratio in the soil between calcium and magnesium than that there should be between calcium and potassium, or between calcium and any other essential element.

The present study was undertaken in order to discover whether there is a relationship between the calcium-magnesium ratio and growth in tobacco; and also to determine whether the calcium-potassium and magnesium-potassium ratios also exert some effects on growth.

An attempt was made, in Part II of this experiment, to determine what effects, if any, changes in cation ratios would make in the tissues and cells of the plant.

REVIEW OF LITERATURE

Sachs ('93) and later Sorp's ('13) found that within certain limits a fairly constant cell size occurred in each species.

Plants grown in different nutrient solutions, by a large number of investigators, as well as by the writer, showed enormous variations in size. These variations, obviously, can be caused either by a difference in the number or by a difference in the size of the cells. The question as to whether these differences were reflected in the nuclei, the chloroplasts, or the vacuoles, has also been studied in a few cases.

Kuster ('25), Volk and Fiemann (27) Kissir ('27), and Sinnott ('30) have recently taken up the question of differences in cell numbers and cell sizes. Kuster reported that cell sizes differed in plants of various sizes, the epidermis being least modified.

Volk and Fiemann found that the cells as well as the tissues varied in size especially in diversely fed plants. They report, in agreement with Kuster that the epidermis changes less than any other plant tissue. Another point, which is of particular importance is the observation that when an element is lacking in the soil in which a plant grows, it usually forms secondary tissue which does not fully develop.

Kisser looked at the problem from an interesting standpoint. He attempted to work from the standpoint of both physiology and anatomy - a combination which is not frequently found in plant studies. The root data were considered to be a measure of water absorption and the stomata data, a measure of transpiration.

Calcium was found to increase water losses which were compensated by a larger root system, magnesium lowered the amounts taken in and lost, potassium lessened water losses and considerably increased water absorption, and sodium induced both lower water absorption and water loss. The plant used was wheat.

Lagatu and Maume ('27-'34) claim that a leaf diagnosis will reveal the nutrient requirements of a plant, and that any inorganic deficiencies will make themselves known through the appearance of its leaves.

The effect of a lack of an element would necessarily become evident to the least extent in regions of embryonic tissue, and cells produced in regions of later growth would feel a shortage most acutely, if the element is necessary to their normal continuance. Damford ('31) studied the effect on the root-tip cells of wheat and corn of a lack of or a very low content of calcium in the nutrient solution. He describes the disintegration of the cells in the absence of Ca.

Lutman, working with several different types of plants, described the effect of the total lack of certain elements (Ca, Mg, K, P, N) on plant cells.

Deuber ('26) states that chlorsis may be induced in soybeans if iron or potassium are lacking. These results were confirmed by Burrell (26) who reported the reddening of soybean leaves and an apparent difficulty in carbohydrate removal.

Schurtz (29) believes potassium to be less important for the manufacture of chloroplast pigments than either nitrogen or phosphorus.

Jacob ('28) reports that photosynthesis can only occur in the presence of potassium.

Nightingale, Schermerhorn and Robbins ('30) point out the fact that potassium being water soluble when in limited supply, is transported freely from mature tissues to regions of active cell division, no meristematic region being found which does not contain it in abundance. They further report that plants supplied with limited amounts of potassium increase in length but not in diameter, since cambial activity is not stimulated. They also show that the water soluble and therefor easily transportable potassium is conveyed to the fruits. These observations were confirmed by those of Penston (31) who found that potassium concentrates in the apical region of the young potato root, 5 mm. long, where the new cells are being formed, indicating that it is essential for new cell formation.

Loew ('34) emphasizes the function of potassium in the cell nucleus, cytoplasm, leucoplasts and chloroplasts, organs in which it is combined in complex organic compounds in such a manner that the ordinary microchemical tests gave no reaction for it. He believes that potassium is a part of the protoplasmic matrix in the chloroplasts.

True ('22) suggested that Ca is necessary to the formation of the middle lamella, since the middle lamella is composed of calcium pectate. This suggestion was later apparently confirmed by the studied of Sorokin and Sommer ('29) who found two nuclei often appearing in a single root-tip cell, no cross-wall being laid down

(when in calcium deficient nutrients. Sanford ('31) however, was unable to confirm their observations.

Kisser ('27) using wheat recorded a proportionately larger root system in plants grown on calcium-rich solutions.

Burrell ('26) indicates that lack of Ca tends to stunt five-week old soybean and field pumpkin plants and to lead to an accumulation of nitrates in the leaves with a diminution of amino acids and insoluble nitrogen.

Jackovljevic ('29) using Anchusa italica seedlings, found that even with an abundance of iron but no calcium chlorsis occurred and the chloroplasts were often deformed and assimilated carbon poorly.

Burke and Morris ('33) found that the larger percentage of the calcium used in the new growth and leaves of young apple trees was derived from the roots and soil but that about 18% of it came from that stored in the older growth.

Loeu ('32) holds that the most favorable calcium-magnesium ratio for grasses is 1:1, but for legumes it is 1:3. He believes that calciums important function is that of a nuclear constituent, making nuclear functions possible, its special role ('34) being to maintain a definite amount of water in the nucleus, this water being anlagous to that of crystallization in some chemicals. If the cell (and its nucleus) is placed in a salt which precipitates the nuclear calcium, the combined water is lost and the nucleus and cell die.

Lightingale, et.al. ('31) reported that tomato plants grown with deficient Ca were stunted in vegetative growth and that the stems

were stiff and woody. At the same time few blossoms were formed and no fruit was set. The parenchymatous and meristematic tissues browned but the conducting elements of the phloem and sylem retained their white color.

Mason and Maskill (31) showed that in the cotton plant, at least, calcium was an immobile element.

Sayre and Nebel ('30) claim that calcium affects the cell contents and walls of the seed coats of peas and the contents of stem cells.

In studies by Lutman and Walbridge ('29), and in a previous paper by Lutman ('25), magnesium has a dual role in the formation of protoplasm and of chlorophyl. The relation of magnesium to chlorophyl formation and destruction, and to chlorsis has been extensively recognized. Loew and May ('01) first pointed out the injury magnesium may induce in unbalanced solutions, especially in the absence or the pre sence of little calcium. Magnesium is supposed to be an active constinent of all protoplasm.

The percentage of magnesium in the leaves of the pea were shown to increase as the plant grew older. The percentage present in these organs varies but apparently has little relationship to the amount present in the soil. The pea plant can evidently use a certain amount of this element and approximately this proportion is absorbed regardless of that which is available.

Saizeva ('29) found the addition of magnesium salts to a culture solution necessary for the formation of chlorophyl, the optimum amount being 0.04 to 0.06 molar. Larger percentages seemed to retard chlorophyl formation. If these percentages were present, chorophyl formed in the

presence of glucose in the dark as in the light.

Garner, McMurtrey and Bowling ('30) found that lack of magnesium causes a chlorotic disease of tobacco known as "Sand-drown". Jones found that magnesium deficiency caused chlorosis in corn. Similar effects have been described in other plants.

Trelease and Trelease ('31) state that magnesium injury in plants is mainly controlled by the magnesium-calcium ratio; but to a lesser extent is influenced also by the proportions and concentrations of other elements in the culture solution by the total concentration of the solution, and possibly, by climatic conditions. In this, and in the paper by Robinovitz, ('33) may be found a collection of the literature on magnesium injury. Carmin ('31) found that magnesium sulfate was more toxic to the roots than to the top.

PROCEDURE

The tobacco plants used in this experiment were grown in flats. When these plants were about 8 weeks old (measuring about 4-5 cm. in height) they were transplanted to water cultures, care being taken to remove as much of the adhering soil as possible. One plant only was used to each culture. Six plants were used for each treatment. Each seedling was supported by means of a non-absorbent cotton plug in a one-hole cork stopper which had been previously paraffined. Glass jars of 1-quart capacity were used in this experiment. The technique used in this work was essentially that developed by Beaumont and Larsinos ('29) for the study of nutrition problems by means of water cultures. In the following photographs, one may see the apparatus set up. The jars were covered by means of metal cylinders in order to keep out the light and in this way prevented the growth of algae.

The nutrient solutions were changed every three days at first, but as the plants grew larger and the transpiration rate increased, the solutions were renewed every other day.

This experiment was divided into three parts or series, each series containing nine groups (or treatments) of six plants each. These series were designated as Series A, Series B, and Series C. Series A consisted of a full nutrient solution with varying ~~amounts~~ concentrations of the calcium and potassium ions. Series B consisted of a full nutrient solution with varying ~~amounts~~ concentrations of the magnesium and potassium ions. Series C consisted of a full nutrient solution

with varying amounts of the calcium and magnesium ions. Below will be found the complete data for the nutrient solutions used in the above series.

Series A

Group I

100% Ca - 0% K

$Ca(NO_3)_2$ - - - - - - - - - - - - - - - .060M

KNO_3 - - - - - - - - - - - - - - - .000M

Group II

95% Ca - 5% K

$Ca(NO_3)_2$ - - - - - - - - - - - - - - - .057M

KNO_3 - - - - - - - - - - - - - - - .003M

Group III

85% Ca - 15% K

$Ca(NO_3)_2$ - - - - - - - - - - - - - - - .051M

KNO_3 - - - - - - - - - - - - - - - .009M

Group IV

70% Ca - 30% K

$Ca(NO_3)_2$ - - - - - - - - - - - - - - - .042M

KNO_3 - - - - - - - - - - - - - - - .018M

Group V

50% Ca - 50% K

$Ca(NO_3)_2$ - - - - - - - - - - - - - - - .030M

KNO_3 - - - - - - - - - - - - - - - .030M

Group VI

30% Ca - 70% K

$Ca(NO_3)_2$ - - - - - - - - - - - - - - - .018M

KNO_3 - - - - - - - - - - - - - - - .042M

Group VII

15% Ca - 85% K

$Ca(NO_3)_2$ - - - - - - - - - - - - - - - - .009M

KNO_3 - - - - - - - - - - - - - - - - .051M

Group VIII

5% Ca - 95% K

$Ca(NO_3)_2$ - - - - - - - - - - - - - - - - .003M

KNO_3 - - - - - - - - - - - - - - - - .057M

Group IX

0% Ca - 100% K

$Ca(NO_3)_2$ - - - - - - - - - - - - - - - - .000M

KNO_3 - - - - - - - - - - - - - - - - .060M

All these groups contained, in ad ition to the above salts, the following:

$Mg\ SO_4$ - - - - - - - - - - - - - - - - .03M

$Mg\ H_4\ (PO_4)_2$ - - - - - - - - - - - - - - .00027M

Fe ammonium tartrate - - - - - - .01 grams

Boric acid (H_3BO_3) - - - - - - - - - - - .003 grams

$Mn\ SO_4$ - - - - - - - - - - - - - - - - - - .003 grams

Series B

Group I

100% Mg - 0% K

$Mg(NO_3)_2$ - - - - - - - - - - - - - .060M

KNO_3 - - - - - - - - - - - - - .000M

Group II

$Mg(NO_3)_2$ - - - - - - - - - - - - - .057M

KNO_3 - - - - - - - - - - - - - .003M

Group III

$Mg(NO_3)_2$ - - - - - - - - - - - - - .051M

KNO_3 - - - - - - - - - - - - - .009M

Group IV

$Mg(NO_3)_2$ - - - - - - - - - - - - - .042M

KNO_3 - - - - - - - - - - - - - .018M

Group V

$Mg(NO_3)_2$ - - - - - - - - - - - - - .030M

KNO_3 - - - - - - - - - - - - - .030M

Group VI

$Mg(NO_3)_2$ - - - - - - - - - - - - - .018M

KNO_3 - - - - - - - - - - - - - .042M

Group VII

$Mg(NO_3)_2$ - - - - - - - - - - - - - .009M

KNO_3 - - - - - - - - - - - - - .051M

Group VIII

$Mg(NO_3)_2$ - - - - - - - - - - - - - .003M

KNO_3 - - - - - - - - - - - - - .057M

Group IX

Mg (NO_3) ----------.000M

K NO_3----------.060M

All these groups contained, in addition to the above salts, the following:

Ca $(NO_3)_2$ ----------.17M

Ca SO_4 ----------.0014M

Ca

Ca H PO_4 ----------.0001M

Mn SO_4 ----------.003 grams

Boric acid ----------.003 grams

Fe ammonium tartrate ----------.01 grams

Group I

100% Ca - 0% Mg

$Ca(NO_3)_2$ - - - - - - - - - - - - - - - .060M

$Mg(NO_3)_2$ - - - - - - - - - - - - - - - .000M

Group II

95% Ca - 5% Mg

$Ca(NO_3)_2$ - - - - - - - - - - - - - - - .057M

$Mg(NO_3)_2$ - - - - - - - - - - - - - - - .003M

Group III

85% Ca - 15% Mg

$Ca(NO_3)_2$ - - - - - - - - - - - - - - - .051M

$Mg(NO_3)_2$ - - - - - - - - - - - - - - - .009M

Group IV

70% Ca - 30% Mg

$Ca(NO_3)_2$ - - - - - - - - - - - - - - - .042M

$Mg(NO_3)_2$ - - - - - - - - - - - - - - - .018M

Group V

50% Ca - 50% Mg

$Ca(NO_3)_2$ - - - - - - - - - - - - - - - .030M

$Mg(NO_3)_2$ - - - - - - - - - - - - - - - .030M

Group VI

30% Ca - 70% Mg

$Ca(NO_3)_2$ - - - - - - - - - - - - - - - .018M

$Mg(NO_3)_2$ - - - - - - - - - - - - - - - .042M

Group VII

15% Ca - 85% Mg

$Ca(NO_3)_2$ - - - - - - - - - - - - - - - .009M

$Mg(NO_3)_2$ - - - - - - - - - - - - - - - .051M

Group VIII

5% Ca - 95% Mg

$Ca(NO_3)_2$ - - - - - - - - - - - - - - .003M

$Mg(NO_3)_2$ - - - - - - - - - - - - - - .057M

Group IX

0% Ca - 100% Mg

$Ca(NO_3)_2$ - - - - - - - - - - - - - - .000M

$Mg(NO_3)_2$ - - - - - - - - - - - - - - .060M

All these groups contained in addition to the above salts, the following:

KH_2PO_4 - - - - - - - - - - - - - .03M

K_2SO_4 - - - - - - - - - - - - - .03M K

Fe ammonium Tartrate - - - - - - - .01 grams

Boric Acid - - - - - - - - - - - - - .003 grams

$MnSO_4$ - - - - - - - - - - - - - - .003 grams

Series C was grown in nutrient solutions for 7 weeks, after which photographs were taken and data concerning dry eights, green weights, etc. were recorded.

Series A and Series B were grown in nutrient solutions for a period of three months, after which photographs were taken and dry and green weights recorded. (Nov. 3 - Feb. 4, 1935)

At the conclusion of the growth period, records of the appearance of the plants under each treatment were taken.

DISCUSSION OF RESULTS

In Table A, may be found the data pertaining to Series A, and in Chart A may be seen the curves resulting from some of this data. The groups or, in other words, the different ratios of calcium to potassium were used as the abscissor while dry weights were used as the ordinates. In this series, A, we find that the maximum total dry weight was obtained when the calcium-potassium ratio lay between 85/15 and 70/30. The striking characteristic of this curve lies in the steep or rapid increase in total dry weight as the calcium-potassium ratio decreases, then the rapid decline in total dry weight with a further decrease in the calcium-potassium ratio.

When the dry weights of the tops alone are used as the ordinates, we find that, except for a rapid rise and fall at the extremities of the curve (where there is a total lack of one of the essential elements) the curve is much more even throughout. The point of maximum growth of the tops does not correspond exactly with that of the total dry weight.

The curve representing the dry weights of the roots as ordinates is very similar to the one representing the total dry weights.

In table B, may be found the data pertaining to Series B in which the magnesium and potassium ions varied. The three curves represented in Chart B correspond to those of Chart A. The most solient feature in these curves is that all three curves are very similar and show a distinct maximum, the curves showing steep rises and falls on either side of this maximum.

Table A (Series A)

Group No.	Ca/K Ratios	Avg. Length of Tops (cm)	Avg. Green Weight of Tops (Grams)	Avg. Length of Roots (cm)	Avg. Green Weight of Roots (Grams)	Total Green Weight (Grams)	Avg. Dry Wt. of Tops (Grams)	Avg. Dry Wt. of Roots (Grams)	Total Dry Wt. (Gr.)
I	100/0	9.12	8.87	11.38			2.071	1.144	3.215
II	95/5	16.15	35.91	12.42	3.15	39.06	6.539	2.675	9.215
III	85/15	18.36	47.15	16.10	19.75	66.90	7.471	3.758	11.229
IV	70/30	20.82	59.19	13.36	20.85	80.04	7.826	3.480	11.306
V	50/50	20.18	[illegible]	14.11	14.84	69.24	8.010	2.058	10.068
VI	30/70	19.40	59.12	14.96	15.45	74.57	7.659	1.336	8.995
VII	15/85	21.10	55.32	15.20	15.66	71.48	7.324	1.484	8.808
VIII	5/95	17.22	39.60	14.76	10.89	50,49	6.229	1.124	7.353
IX	0/100	7.46	1.37	15.54	5.14	6.51	1.711	0.186	1.897

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Table B (Series B)

Group No.	Mg/K Ratios	Avg. Length of Tops (cm)	Avg. Green Weight of Tops (Grams)	Avg. Length of Roots (cm)	Avg. Green Weight of Roots (Grams)	Total Green Weight (Grams)	Avg. Dry Wt. of Tops (Grams)	Avg. Dry Wt. of Roots (Grams)	Total Dry Wt. (Gr.)
I	100/0	6.94	[illegible].13	13.14	1.84	[illegible].97	1.356	0.120	1.476
II	95/5	6.24	4.83	14.78	1.74	6.57	1.648	0.144	1.792
III	85/15	[illegible].04	13.21	14.06	3.17	16.38	2.704	0.288	2.992
IV	70/30	[illegible].36	17.[illegible]	14.82	4.46	22.39	[illegible].786	0.342	3.128
V	50/50	12.20	25.14	16.92	5.03	30.17	3.902	0.373	4.275
VI	[illegible]/70	13.26	40.[illegible]7	15.60	[illegible].89	48.96	5.062	0.824	5.886
VII	15/85	14.45	34/31	14.08	10.46	44.77	3.812	0.719	4.531
VIII	5/95	14.14	29.48	16.34	10.31	39.79	3.760	0.636	4.396
IX	0/100	7.80	13.62	15.06	3.63	17.25	2.550	0.305	2.855

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Table C (Series C)

Group No.	Ca/Mg Ratios	Avg. Length of Tops (cm)	Avg. Green Wt. of Tops (Grams)	Avg. Length of Roots (cm)	Avg. Green Weight of Roots (Grams)	Total Green Weight (Grams)	Avg. Dry Weight of Tops (Grams)	Avg. Dry Weight of Roots (Grams)	Total Dry Weight (Grams)
I	100/0	8.05	7.23	11.01	1.61	8.84	1.700	0.333	2.033
II	95/5	7.44	8.10	12.90	2.57	10.67	1.873	0.266	2.139
III	85/15	8.48	10.60	10.81	3.52	14.12	2.366	0.376	2.742
IV	70/30	21.18	47.61	12.70	10.15	57.76	7.033	1.390	8.423
V	50/50	26.02	55.99	12.91	11.98	67.97	8.566	1.627	10.193
VI	30/70	31.20	70.75	15.05	15.76	86.51	9.363	1.680	11.043
VII	15/85	34.42	85.36	12.22	17.31	102.67	11.993	1.917	13.910
VIII	5/95	19.18	52.57	11.23	13.24	65.81	7.133	1.323	8.456
IX	0/100	7.48	7.22	9.72	2.34	10.16	1.923	0.256	2.179

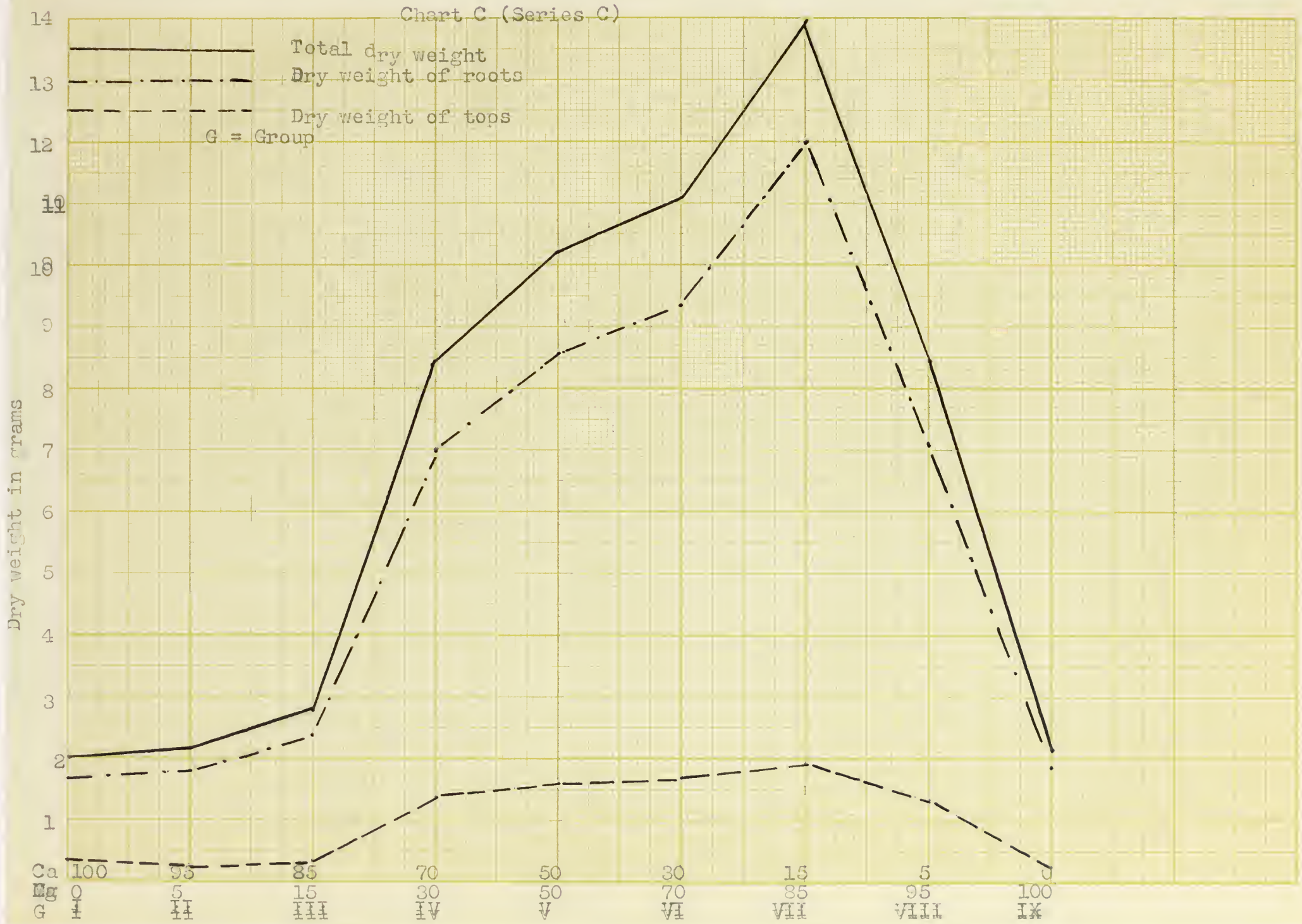


Chart C (Series C)
Total dry weight
Dry weight of roots
Dry weight of tops
G = Group
Dry weight in grams
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Ca 100 95 85 70 50 30 15 5 0
Mg 0 5 15 30 50 70 85 95 100
G I II III IV V VI VII VIII IX

Table C contains the data pertaining to Series C in which the calcium and magnesium ions varied. The three curves represented in Chart C correspond to those of Charts A and B. These curves all show a distinct maximum at the same calcium-magnesium ratio, in this respect being similar to Chart B and differing from Chart A.

The curves representing the total dry weights and the dry weights of the tops are similar and run parallel to each other, but the curve representing the dry weight of the roots, although showing a maximum at the same calcium-magnesium ratio, appears much more even and does not show the effect of changes in the calcium-magnesium ratio as much and to the same extent as do the other two curves.

In comparing the curves of the three charts the following facts may be noticed:

(1) That in charts B and C the maximum of the total dry weights are distinct points, whereas there is no such distinct maximum in Chart A representing varying amounts of calcium and potassium.

(2) That in these charts the same may be said of the dry weights of the tops.

(3) That the curves representing the dry weights of the tops in charts B and C run more or less parallel to the curves representing the total dry weights, whereas the similar curves in Chart A do not run parallel during a large portion of their paths.

(7) That the curves representing the dry weights of the roots do not show so distinct maximum points as do the curves representing the total dry weight and the dry weight of the tops.

(8) That the curves representing the dry weights of the roots are more smooth and do not show the effects of changes in the proportions of salts as do the other curves.

Charts A', B', and C' show graphically the manner in which the Ca/K Ca/Mg Mg/K ratios vary in each series and in relation to each other in the same series. In these graphs the group numbers are used as the abscissae and the ratios are used as the ordinates. Thus at a glance one can see how the Ca/K Ca/Mg and Mg/K ratios vary in the same series. In Series A (Chart A') we find that the curve representing the Ca/K ratio varies from infinity in group 1 to 0 in group IX. The curve is a smooth one. The curve representing the Mg/K ratio is somewhat similar to the former one. The Ca/Mg ratio here is almost a straight line and meets the other two lines at a point. Although in this series, only the Ca and K ions varied and the Mg ions remained at a constant value, yet since these former two ions did vary the Ca/Mg and the Mg/K ratios of necessity varied also. This same line of reasoning is followed out in the other series to show how all the ratios vary at the same time.

If the minimums of the dry weights of the tops are considered we find that they correspond very closely to the point where the three ratio lines meet in a point. Perhaps if the increments of the

ratios were narrowed down we would arrive closer to this meeting point when compared to the maximum growth point. If this is true, by knowing the concentration of the important cations (Ca, Mg, and K,) we could determine the point of maximum growth; and conversely, by varying the proportions of these salts we may determine or fix the point of maximum growth.

Charts I, II, III show the dry weights of the tops as ordinates plotted against the ratios of ions as the abscissae. In these charts, A represents the Ca/Mg ratios of all three series; B represents the Ca/K ratios of all three series; C represents the Mg/K ratios of all three series.

The most salient point of these charts lies in the fact that all the maximums range between the ratios of 0 and 1.

Evidently, when there is enough of an ion present to remove all question of deficiency - then the only other reason that can be attributed to any injurious fact can be attributed to the toxicity of the superabundance of a particular ion.

In the case of each of the three series in this experiment, it is clear that, except for Group I and IX in each series, the failure to attain maximum growth was not caused by deficiency but by a toxicity due to an excess of some particular ion or an unfavorable ratio of ions. Thus, we find that two ions may be toxic when used alone but when used together, they seem to neutralize each others toxicity. This type of is known as ion antagonism.

Series A

Group No.	Ca/K	Ca/Mg	Mg/K
I		2.00	
II	19.00	1.90	10.00
III	5.00	1.70	3.33
IV	2.33	1.40	1.67
V	1.00	1.00	1.00
VI	0.43	0.60	0.71
VII	0.18	0.30	0.59
VIII	0.05	0.10	0.53
IX	0.00	0.00	0.50

Ratios of the Ca, Mg, K, ions in series A

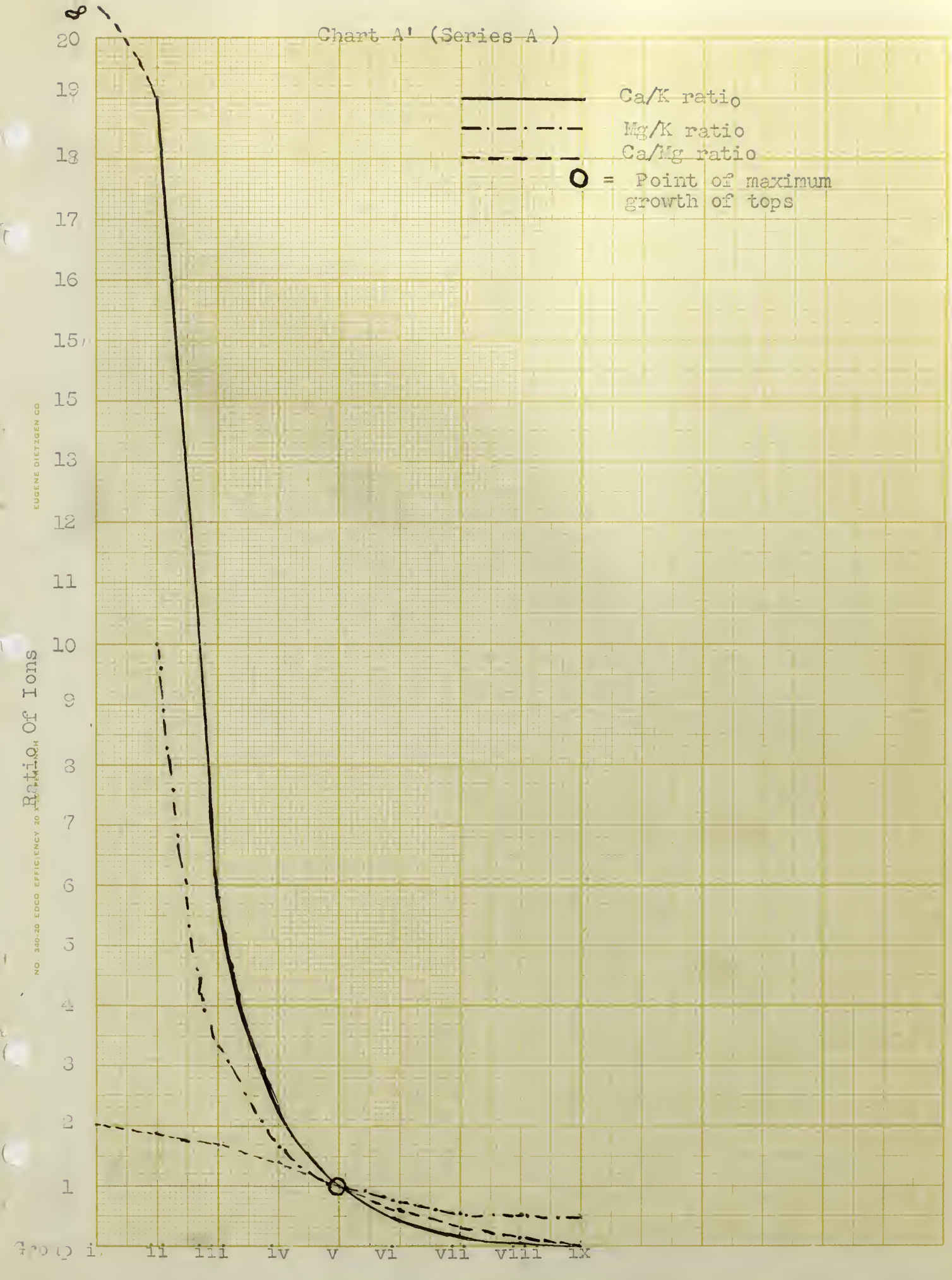


Chart A' (Series A)
Ca/K ratio
Mg/K ratio
Ca/Mg ratio
O = Point of maximum growth of tops
Ratio Of Ions
∞
20
19
18
17
16
15
15
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Group i
ii
iii
iv
v
vi
vii
viii
ix

Series B

Group No.	Ca/K	Ca/Mg	Mg/K
I		0.28	
II	5.67	0.30	19.00
III	1.89	0.33	5.66
IV	0.94	0.41	2.33
V	0.57	0.57	1.00
VI	0.41	0.94	0.43
VII	0.33	1.89	0.18
VIII	0.30	5.67	0.05
IX	0.28		0.00

Ratios of the Ca, Mg, and K, Ions in Series B

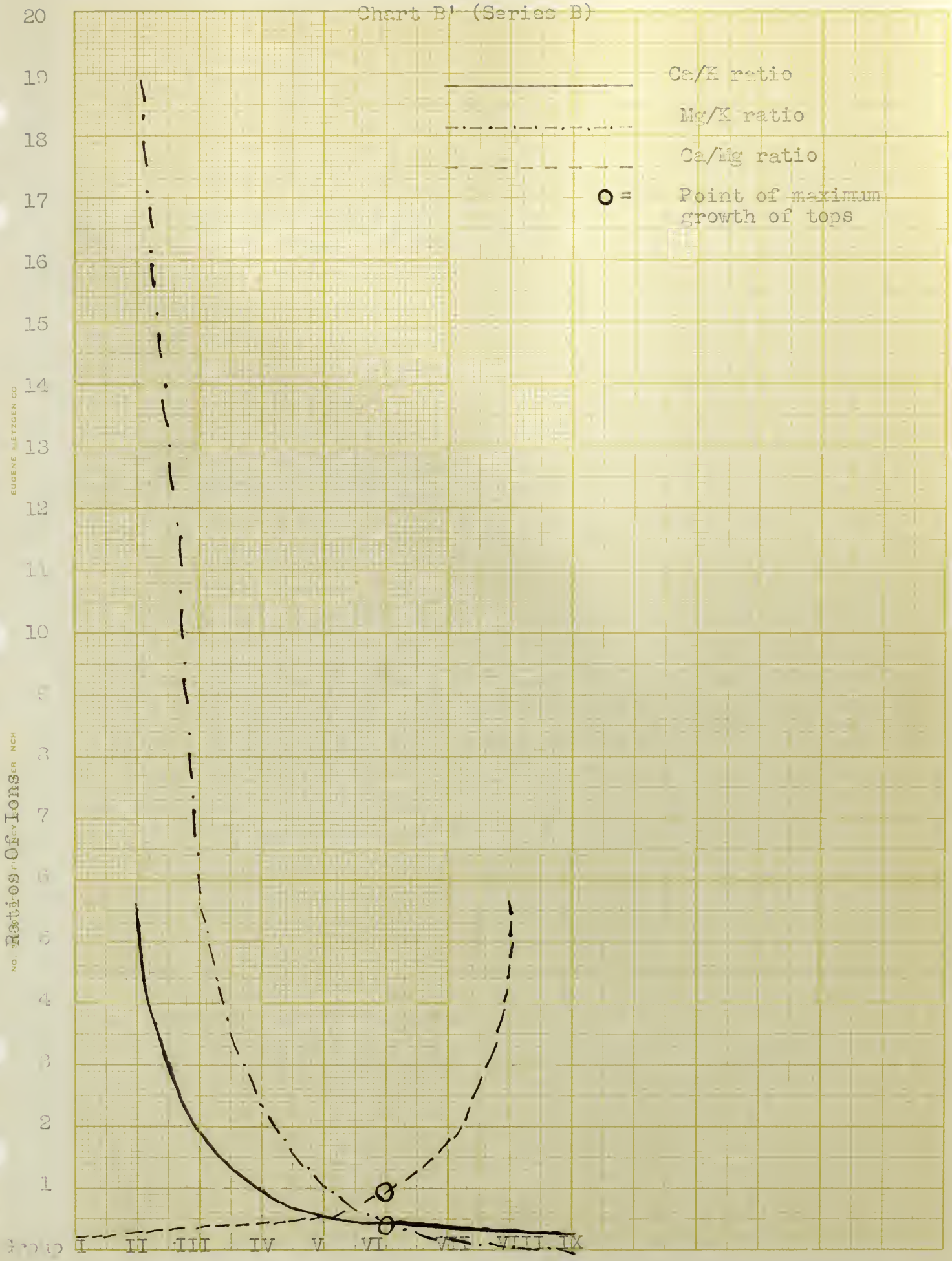


Chart B' (Series B)
Ca/K ratio
Mg/K ratio
Ca/Mg ratio
O = Point of maximum growth of tops
Ratios of Ions
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Frost
I
II
III
IV
V
VI
VII
VIII
IX

Series C

Group No.	Ca/K	Ca/Mg	Mg/K
I	1.00		0.00
II	0.95	19.00	0.05
III	0.85	5.66	0.15
IV	0.70	2.33	0.30
V	0.50	1.00	0.50
VI	0.30	0.43	0.70
VII	0.15	0.18	0.85
VIII	0.05	0.05	0.95
IX	0.00	0.00	1.00

Ratios of the Ca, Mg, K, ions in series C

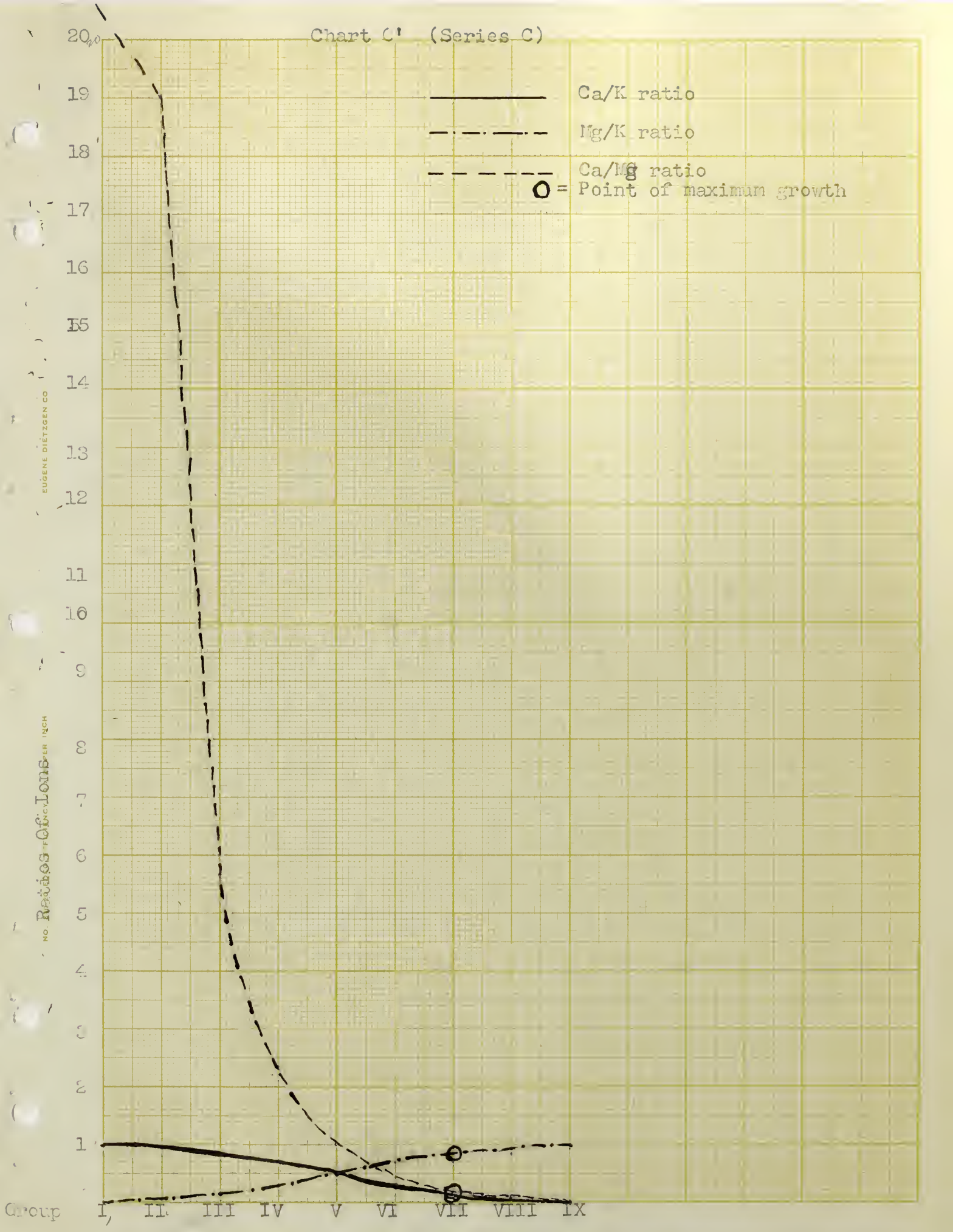
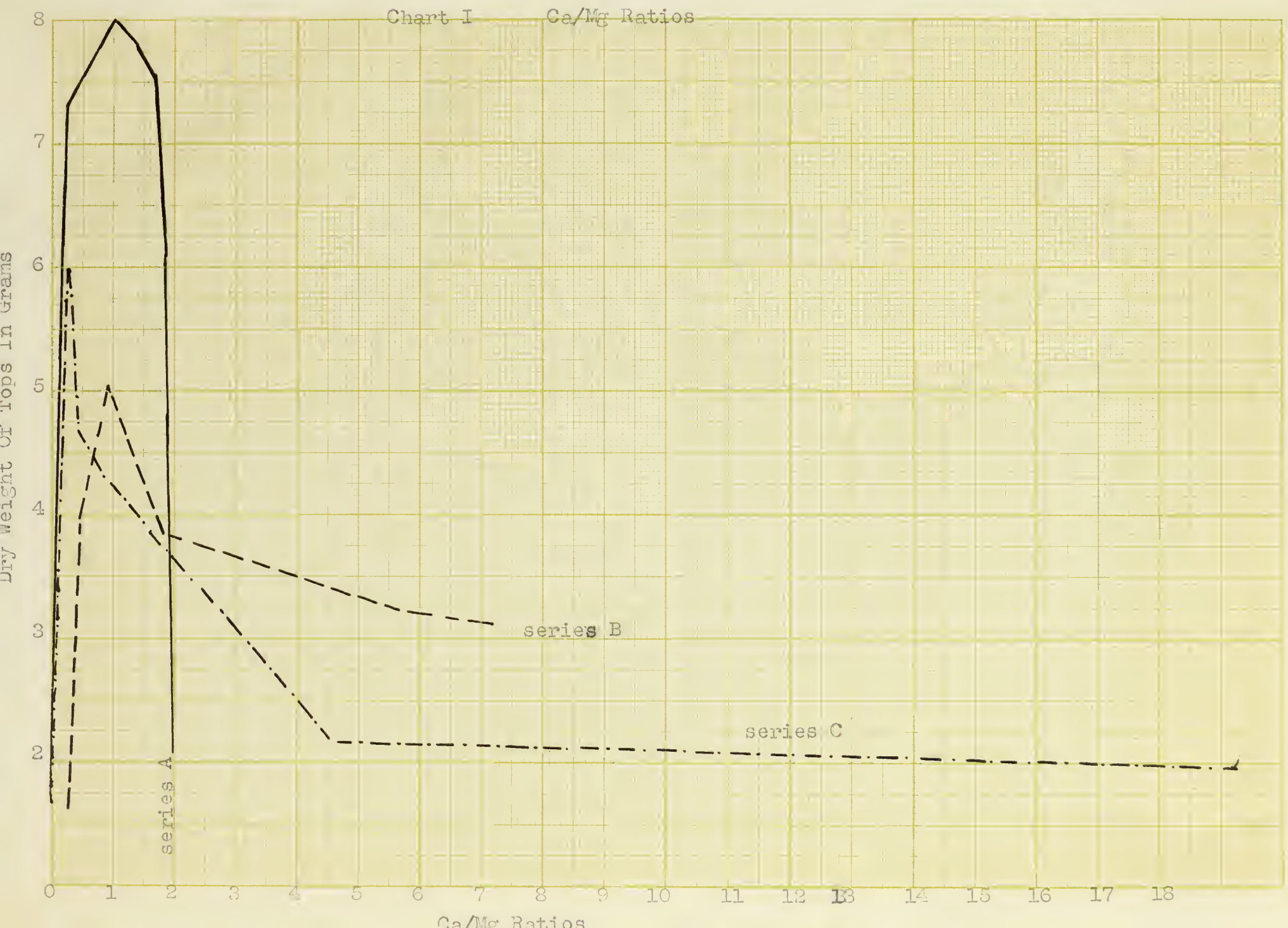


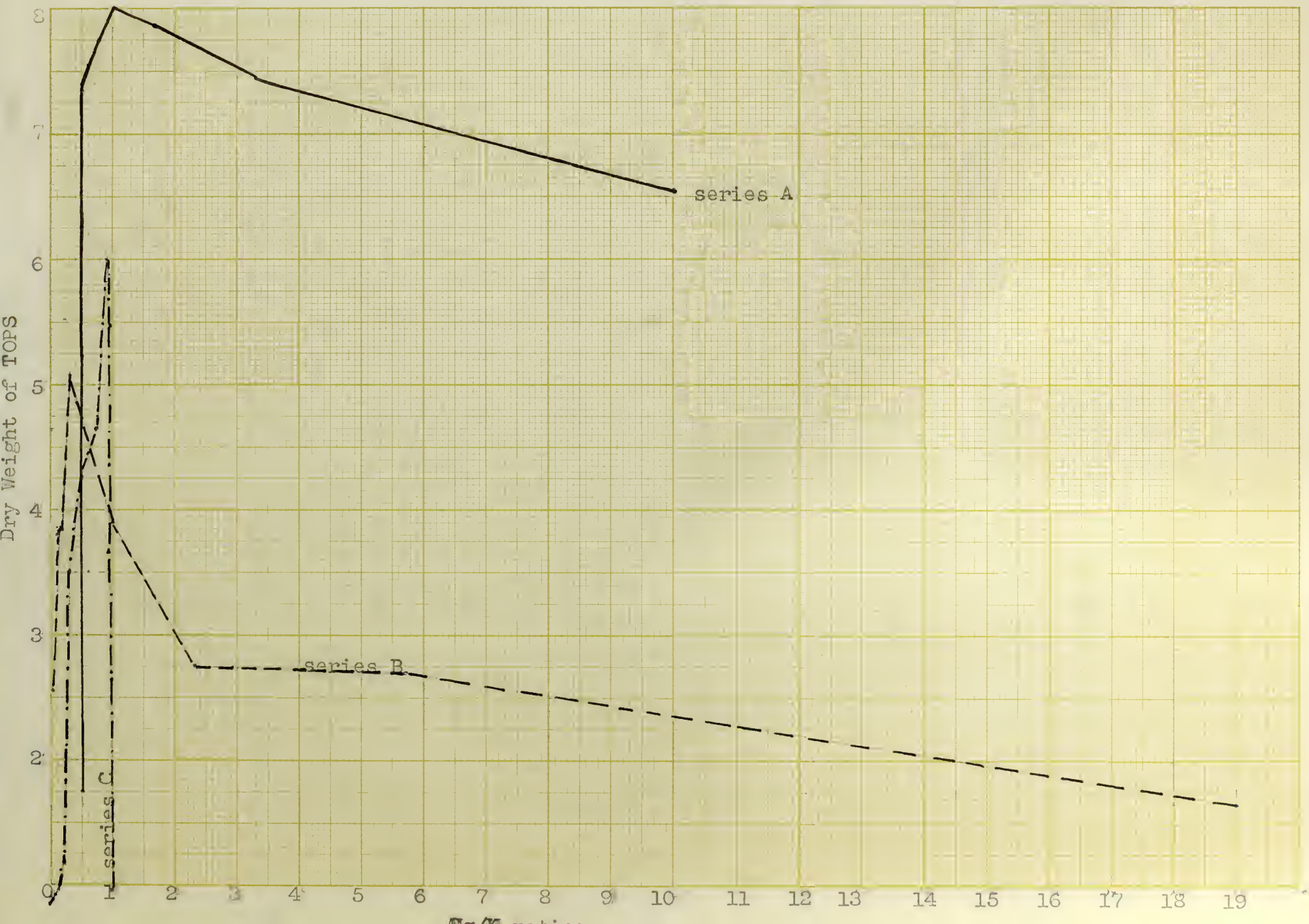
Chart C' (Series C)
Ca/K ratio
Mg/K ratio
Ca/Mg ratio
O = Point of maximum growth
Ratios of Ions
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Group
I
II
III
IV
V
VI
VII
VIII
IX

NO 340 0 EUGENE FFICIENCY X 20 PER INCH EUGENE FITZGEN

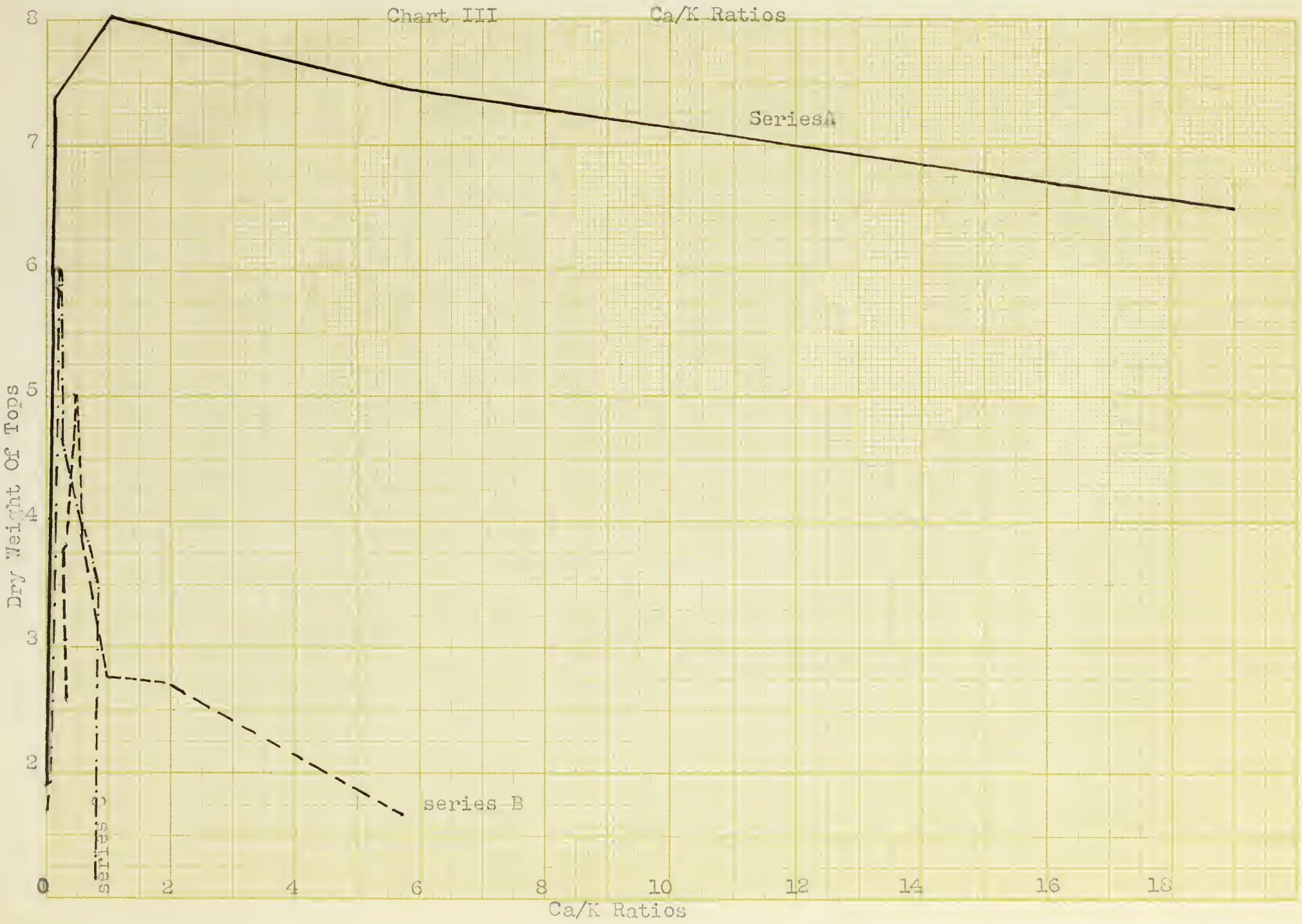


NO 340-20 EDCO EFFICIENCY 20 X 20 PER INCH EUGENE DIETZGEN CO.

Chart II Mg/K ratios



NO 340 .0 ED O EFFICIENCY .0 X 0 PER INCH EUGENE ETZGEN CO



In series B and C, which represent the Mg/K and Ca/Mg ratios respectively, we find that the dry weights of the tops are influenced by changes in the above mentioned ratios more than are the dry weights of the roots although the latter do show the influence of the changes to some extent. In series A, where the Ca/K ratio is varied, however, we find that the roots are influenced to a greater degree than are the tops. By "being influenced by change" is meant the degree of sensitivity to change in the ratios of ions as expressed by sudden changes in the direction of the growth curve.

In another way, also, do we find that Series A differs. Whereas in series B and C, the point of maximum growth or maximum dry weight of both the roots and the tops falls in the same group; in series A we find a marked difference. The maximum dry weight for the roots lies in Group III (Ca/K = 85/15), and the maximum for the tops lies in group V (Ca/K = 50/50 = 1). This point is an interesting one and should bear closer investigation.

Another outstanding feature of a comparison of the charts A, B, and C, lies in the fact that the sensitivity to change of ratios of the ions appears to lie in the order which follows the one of highest sensitivity first.

(a) Series C which represents the Ca/Mg ratios

(b) Series B " " " Mg/K "

(c) Series A " " " Ca/K "

The above observation seems to indicate, that for tobacco

under these conditions calcium in large amounts is more toxic than magnesium in like amounts, or in terms of antagonism the toxicity caused by calcium may be overcome only by a large amount of magnesium, an amount much above that of the former if both are quantities expressed in mols.

In the same manner it may be seen that the toxicity of an abundance of calcium may be overcome by a relatively small amount of potassium, and conversely, the toxicity caused by potassium can only be overcome by a relatively large amount of calcium.

If the toxicity caused by each ion were of the same kind, i.e., if these ions all effected the same process or processes then we should expect certain consequences to follow. To illustrate, since Potassium has a greater toxicity than a like amount (in mols) of calcium and since calcium has a greater toxicity than magnesium, it would be expected that potassium would be more toxic than magnesium. However, this is not the case and so we must conclude that the antagonism of each ion is of a different sort. Also, since the same ion may counteract more than one other ion we may further conclude that the ions may have more than one function and exhibit more than one type of antagonism.

It is my opinion, that the reason for the apparent lack of agreement among the workers on antagonism and also their disagreement as to the manner in which this supposed antagonism works is caused by their failure to recognize the important fact that, when two salts are varied the ratios of all the other salts are also varied. The problem is of extreme complexity when looked at from this angle. It is my opinion, further, that future work in this field

should limit ipsets to certain narrow ranges (and thus eliminate much useless work outside certain limits) and within these ranges manipulate the ionic ratios in such a manner that only one ratio be varied at a time. This is an entirely new approach to this subject and should yield fruitful results.

Description of Plants in Each Series

What follows is a description of the plants in each series, these being taken up by groups. Constant reference to the figures cited will give a better conception of the gross character of these plants.

Series A. (Figure I)

Varying Amounts of Calcium and Potassium

IA. 100% Ca - 0% K

Roots:

These roots were in good condition as far as general turgidity could show. They were dirty white in color with a slight tendency to brownishness showing that there was some trace of toxicity in this culture solution.

A peculiarity worthy of note is that there was a precipitation of the salts of the solution in large crystals on the surface of the rootlets. Although some of the other roots in this series were likewise affected by the salt precipitating on them, nowhere else was the amount as great as in this particular case.

These roots, although not as large as some of those which follow, were of fairly good size. New rootlets were continually being formed.

Tops:

The leaves, although relatively small in size, showed up better than was expected in the absence of what is considered such a necessary element as potassium. The dying leaves were characteristic in their symptoms before and during the period of time in which they turned brown. Browning appeared to take place first at the

tips of the leaf and from there worked backward to the petiole. Before beginning to brown the leaf took on a general chlorotic appearance in which the whole leaf participates.

The leaves in the midregion of the plant showed a chlorosis intervenously. The top leaves were very green in color.

The growing tip here was unaffected.

The rate of transpiration as seen by the amount of liquid transpired from the quart jar, may be considered fairly good.

IIA. 95% Ca - 5% K

Roots:

These roots appeared to be in excellent condition and of a good white color. The rootlets were in a high state of turgidity and showed that new rootlets were continually forming. As in IA, crystals of salt had formed on the rootlets although not to the same degree.

Tops:

These leaves were large and healthy in appearance. The process of browning of the dead and dying leaves was somewhat different from what it was in the preceding case. Here the discoloration (browning) started along the midrib and along the main veins and then spread over the entire leaf. Before the leaf began to turn brown, it presented a beautiful orange yellow color.

The transpiration rate in this group of plants at the end of the experiment was approximately twice that of the preceding group.

IIIA 85% Ca - 15% K.

Roots:

These roots were in excellent condition, of large size and of

a healthy normal color. In fact, in this particular culture the roots attained the maximum dry weight. New rootlets were continually being formed.

Tops:

The leaves in this series of plants were large and of a healthy appearance. They had an even green color throughout. The leaves that browned, did so in the same manner as did those of group IIA, but showed a marked tendency to start browning at the tip of the leaf. There may be some significance in the fact that as the proportion of Ca and Mg varies, the way in which browning sets in on the dead or dying leaves also varies. This phase may bear further investigation.

The transpiration rate showed a slight increase over that of IIA.

IVA 70% Ca - 30% K

Roots:

The roots in this series were in very good condition, but did not quite come up to IIIA in color, fulness, or in quality of turgidity. The dry weight of these roots approached that of IIIA more nearly than did any of the other groups as may be seen from a consideration of Table A.

Tops:

These leaves showed little difference in appearance from those of Group IIIA except that the tendency to begin browning from the tip backwards to the petiols was much more markedly exhibited than in the latter group. Here too we find that the leaves took on a

beautiful orange yellow appearance, before the process of browning took place.

The transpiration ratio was about the same as that of Group IIIA.

VA 50% Ca - 50% K

Roots:

The roots of this group were in very good condition as to color, turgidity, and general appearance. The dry weight of these roots was much lower than that of IVA.

Tops:

The leaves were in a condition which was very similar to that of IIIA and IVA. The browning process of the leaves was however, the same as was manifested in IIA and IIIA. The remaining groups do not show the tendency to turn brown from the tip backwards. However the same orange yellow was present here before browning took place.

VIA 30% Ca - 70% K

Roots:

The roots in this group were in good condition in all respects even though the dry weights were much less than in Va.

Tops:

These leaves were very similar to those of Group VA.

VIIA 15% Ca - 85% K

Roots:

These roots appeared normal in every way except that the dry weight was low.

Tops:

The leaves in this group resembled those of VA except in that the lower leaves began to show a tendency to an intervenous chlorotic condition.

VIIIA 5% Ca - 95% K

Roots:

In this group was found a marked change in the appearance of theroots. Approximately 50% of the rootlets appeared to be in a healthy and normal condition whereas the remaining 50% were brown and flaccid showing decided signs of toxicity.

Tops:

The lower leaves of this group showed a decided inclination to a chlorotic condition, these leaves being noticeably pale green in color. The leaves of the mid-portion of the plant showed a normal color, whereas the leaves at the top of the plant were coarse and dark green. The browning of leaves went on first as in VIIA, although the preceding yellowing of the leaves was not as rich and mellow as in the latter.

The transpiration rate was still comparatively good.

IXA 0% Ca - 100% K

Roots:

The roots of this group were brown in color, flaccid, and in generally poor condition. No new rootlets were evelopin and the plant showed signs of some toxic condition.

Tops:

The leaves in this group were small, unhealthy looking and

drooping as though from lack of water. In general, the plant seemed to find it difficult to take up water from the solution. The process of browning was merely preceded by a general chlorosis. All the leaves on the plant with the exception of the top two or three, showed a decided chlorosis. These top leaves although deep green in color were of very coarse texture and in general did not appear normal.

The transpiration rate was very small, indeed hardly noticeable.

Series B

Varying Amounts of Magnesium and Potassium

IB 100% Mg - 0% K

Roots:

These roots were in very poor condition, being brown in color and very flaccid. The roots were apparently dead and showed no evidence of having attempted to send out new rootlets after the first two weeks of the experiment.

Tops:

The plant as a whole was stunted in growth and showed no appreciable gain in height during the entire experiment. However, that some growth occurred is positive, for leaves were being formed at least during the early part of the experiment.

In browning the leaves showed the following symptoms: browning occurred from the midribs outwards towards the margins of the leaf and at the same time along the veins.

The top leaves were of a very dark green, much darker than one would expect to find in a normal plant, while the texture was extremely coarse.

The growing tips did not seem to be effected in any way by this treatment.

There was hardly any noticeable amount of water taken up by the plant from its nutrient solution, showing that there was very little water intake in the plant.

IIB 95% Mg - 5% K

Roots:

The roots of this group of plants resembled those of Group IB. The same brownish color and the same lack of turgidity was evidenced.

In this case, also, there was no evidence that any new rootlets were being formed after the first two weeks of growth.

Tops:

The tops in this group were bigger and much healthier in appearance than were those of Group IB. Whereas in Group IB only the top two leaves were green and alive, this group there were between 6 and 8 leaves in an apparently healthy condition. Here, the leaves were also larger than in the preceding group.

The dying leaves did not brown in the same manner as did those of IB, but began this process from the tip and gradually worked toward the petiole of the leaf, until it was entirely covered.

The lower leaves showed a general chlorotic condition (intravenously). The leaves in the mid-portion of the plant were of a very deep green, whereas the newer leaves seemed to be of a more normal green color.

Here also the growing tip remained unaffected.

The water intake in this group was just about twice that of Group IB at the conclusion of the experiment.

IIIB 85% Mg - 15% K

Roots:

The appearance of the roots in this group was decidedly better than that of the preceding two groups. Although for the most part brown in color there were white streaks present showing, superficially at least, that new rootlets were being formed to some extent. However, these roots showed very little or no turgidity which may be taken as a sign of some toxic action.

Tops:

The tops here showed a marked improvement over those of IIB

both in height and the size of the leaves. The dying leaves browned as did those of group IIB, i.e., from the tip towards the petiole.

The growing tips of these plants seemed to have some difficulty in developing in that the leaves arising in that region appeared to be incapable of unfolding, or unrolling.

The leaves were in general of a good normal green color.

The water uptake in this group was about the same as that for IIB.

IVB 70% Mg - 30% K

Roots:

In this group was seen a decided change in the appearance of the roots. These showed neither the dark brown color of the preceding groups, nor the flaccid condition. Rootlets were continually being produced and thus gave the general appearance of whiteness. All in all this group was the first to exhibit anything approaching what the normal root should look like.

Tops:

The tops of this group were much larger than those of any of the preceding ones. They exhibited a sturdier appearance in every respect. The color of the leaves was good with the exception that the lower leaves had a tendency towards chlorosis (intervenous). The top leaves here too showed a lighter green than did those of the mid-region. The process of browning in this group occurred in the same manner as in IIIB.

The growing tips showed the same type of injury that was present in those of group IIIB, though to a higher degree.

The water intake was slightly higher than that of Group IIIB.

VB 50% Mg - 50% K

Roots:

The roots in this group also show vast improvement over all the preceding groups. This improvement was manifested by an increase in size, improvement in color, and a higher state of turgidity. However there still were streaks of brown indicating rootlets in some state of decay. Of course, there was plenty of evidence of continual production of rootlets.

Tops:

The tops in this group were somewhat larger than those of the preceding group. There was very little difference in the appearance of these tops and those of IVB. The growing tip here was affected in the same manner as in IVB.

The water uptake was somewhat greater than in IVB.

VIB 30% Mg - 70% K

Roots:

The roots of this group were in decidedly better condition than were the roots of any of the other groups so far considered. They were of a healthy white color and in a very turgid condition. Although the actual length of these roots was smaller than the lengths of the preceding groups, they were, of a greater bulk and weight. New rootlets were continually being formed.

Tops:

The tops of this group were in excellent condition. They were larger and their leaves were of a good and healthy color throughout.

The growing tip was only slightly affected in the manner of IVB and VB.

The water uptake of this group was about twice that of VB.

VIIB 15% Mg - 85% K

This group of plants is by far the best appearing one in every respect.

Roots:

Old rootlets as well as new rootlets were in perfect condition of turgidity and color.

Tops:

The tops of this group were of about the same size as those of VIB. The leaves were all of a healthy green color and appeared normal in every respect.

The growing tips were in excellent condition, showing none of the unfavorable symptoms present in some groups.

The water uptake was about the same as for VIB.

VIIIB 5% Mg - 95% K

Roots:

The roots of this group were in just as good a condition as were those of VIIB although not quite as large as the latter.

Tops:

The tops of this group were not as large as those of VIIB although in just as good a condition.

The water uptake for this group was approximately the same as for group VIIB.

IXB 0% Mg - 100% K

Roots:

The roots of this group were in very good condition. In fact, they proved to be better in color and turgidity than were groups IB - VB inclusive, although they did not come up to the standard

of VIB, VIID, and VIIIB. There were new rootlets and the entire root gave the impression of being in a turgid and healthy condition.

Tops:

The tops of this group were fairly large and compared favorably with Group VB in size. There was a very marked chlorotic condition in all the leaves. Even the top two or three leaves did not escape this condition to any extent.

The leaves were fairly large and did not show a marked stunting as did those groups at the other end of this series.

The water uptake is approximately equal to that of VB.

Varying the Proportions of Calcium and Magnesium.

This series was begun and run almost a year before the other two series, and thus some data which was considered important for the latter through experience with the former was not gathered. Thus this series (which was first conceived as the total experiment in itself and from which followed an expansion into this entire project) lacks the completeness which experience gave to the last two series.

What is lacking in description, however, may be gathered both from the data in the corresponding table, and from the photographs obtained at the conclusion of the experiment.

Descriptive Matter

Four days after the experiment began, IC and IIC containing 100% Ca - 0% Mg and 95% Ca - 5% Mg respectively began to show symptoms of toxicity in their tops. The roots at this time still appeared normal. The leaves acted as though they were suffering from a lack of water.

One day later the roots began to brown in these two groups and the lower leaves began to show a yellowing.

The next day showed an increase in the above symptoms in Groups IC and IIC and Group IIIC (85% Ca - 15% Mg) began to show symptoms similar to the former two. The leaves of all these three groups appeared to be in a wilted condition as though they were finding difficulty in obtaining water from the nutrient solutions in which they were being grown. This fact was further borne out by the culture

solutions themselves, in that there appeared to be no appreciable change in the amount of solution in the jars. The roots in all three of these plants had lost their turgid condition and presented a flabby and decayed condition.

At about this time Group IXC (0% Ca - 100% Mg) began to show similar symptoms as those mentioned above for Groups IC, IIC, and IIIC. This group also seemed to find it difficult to obtain any water from its nutrient solution. From this time on, none of the above mentioned plants showed any increase in growth or tendency to recuperate.

Group IVC began to give the above toxic symptoms about two weeks after the experiment was begun.

Figure I

Showing Plants Of Series A (Calcium And Potassium Varying)

Ca	100	95	(85,70,50,30)	5	0
K	0	5	(15,30,50,70)	95	100

Figure II

Showing Plants Of Series B (Magnesium And Potassium Varying)

Mg	100	95	85	70	50	30	15	5	0
K	0	5	15	30	50	70	85	95	100

Figure III

Showing Plants Of Series C (Calcium And Magnesium Varying)

Ca	100	95	85	70	50	30	15	5	0
Mg	6	5	15	30	50	70	85	95	100

Summary

Part I

1. This experiment was divided into three sections designated as Series A, B, and C.

2. In series A, the cations calcium and potassium were varied from 100/0 to 0/100. It was found that the optimum calcium-potassium ratio lay between 85/15 and 70/30 for this nutrient solution.

3. In series B, the cations magnesium and potassium were varied from 100/0 to 0/100. It was found that the optimum magnesium-potassium ratio was 30/70 for this nutrient solution.

4. In series C, the cations calcium-magnesium were varied from 100/0 to 0/100. It was found that the optimum calcium-magnesium ratio was 15/85 for this nutrient solution.

5. Growth curves were compared.

6. Graphs were drawn for each series showing how the Ca/K, Mg/K, and Ca/Mg ratios varied for each series. It seems that there is some relationship between the point of maximum growth (of the tops) and the point at which these curves come close together or even cross.

7. Curves were drawn representing the Ca/Mg ratio against the growth. This brought out the interesting fact that in all three series the optimum growth was obtained when the ratio lay between 0.0 and 1.0.

8. Similar curves were drawn for the Ca/K and Mg/K ratios. These graphs showed that exactly the same thing was true for these ratios as for the Ca/Mg ratio, i.e., optimum growth was obtained when the ratio lay between 0.0 and 1.0.

9. It was observed that the roots were less sensitive than the tops to changes in cation ratios.

10. It was shown that the type of antagonism displayed by each of the cations must be of a different kind in its action, at least in part.

11. A new approach is suggested for future work in this field.

PART II

HISTOLOGICAL AND CYTOLOGICAL STUDIES

Part II
Histological and Cytological Studies
Introduction

Despite the fact that there is a large amount of literature dealing with the particular functions of the individual inorganic elements in plant nutrition, it is a deplorable fact that the hypothesis and so-called theories advanced concerning these functions are, at best, to be regarded only as intelligent guesses since the evidence upon which they are based is extremely meagre and in many cases quite obscure and contradictory.

It has long been recognized by plant scientists that a deficiency or a superabundance of one or more of the necessary inorganic elements will affect the growth of a plant especially as to size. Histologists and cytologists have not devoted much time to this subject even though it is clear that the direct effects of these inorganic elements must be visible in the plant cells and tissues. It is an obvious fact that not only chemical but also cytological and histological analysis must be brought into play before this complicated system of processes can be brought within the range of classified knowledge.

It is regrettable that most of the studies dealing with the effects of varying proportions of the inorganic elements on plant growth and nutrition were carried out without any consideration whatsoever to their cytological and histological effects. These studies have mainly concerned themselves with gross effects, as dry weights, and in some cases with chemical analysis, as well. It is regrettable that, in this way, much useful information is lacking and a well rounded view of the problem is thus made less possible.

It is the object of this paper to present certain observations on cell and tissue changes noted in tobacco plants grown in varying

ratios of some of the so-called necessary cations. The original scope of the study included only ratios of calcium and magnesium, but it was later extended to include ratios of calcium and potassium, and magnesium and potassium. It must be realized that the above mentioned cations, although considered as essential, are not the only essential cations known. It is becoming more and more evident that certain other cations, such as copper, long considered as unessential and even toxic, are actually necessary in minute quantities to the full development of the plant.

A chemical analysis of the plants has been deferred to a later date.

Procedure

In reference to the charts and tables in Part 1 of this paper, a comparison was to made between the microscopic and macroscopic effects of certain ratios of ions. The plants of series A, B, and C were here used.

The plant tissues were killed and fixed in chrom-acetic acid and then subjected to the usual xylol-paraffin method. Sections were cut 10 microns thick and stained with Heidenhains hematoxylin and counterstained with safranin.

Cross sections were made of the stem in the region of the crown and longitudinal sections of the root tips. In this way two types of tissues and cells were obtained: -

1. Root tips-an example of undifferentiated fundamental cells.

2. Stem cells and tissues-highly specialized cells with functions of support, of water transport, and of food and mineral transport.

Effects on Stems

Studies were made of the actual size of the several tissues and also of their relative amounts. The separate tissues may be crudely classified into two groups: (1) storage tissue, (2) conducting or transporting tissue. In the first class are the pith and cortex; in the second class, the xylem and phloem.

It seemed probable that the conducting tissues, especially the xylem, which conducts water and minerals would be the first tissues to be affected by changes in the ion ratios. These tissues were given especial study and results seem to have borne out this assumption.

The tables which follow, A''', B''', C''', correspond to series A, B, and C.

Table C should be considered first in that there appears to be a well defined correlation between the calcium-magnesium ratio and the phloem-xylem ratio. Comparison of table C and chart C indicates there is a correlation between dry weights phloem-xylem ratio$\left(\frac{\text{phloem}}{\text{xylem}}\right)$. It will be observed that in groups I (Ca/Mg = 100/0) II. (Ca/Mg = 95/5), and III (Ca/Mg = 85/15), where growth is extremely poor the phloem-xylem ratio lies between .64 and .76. With a sharp rise in the curve comes a sharp drop in the phloem-xylem ratio, which, in the region of good growth, varies between .27 and .31. As the "dry weight" curve takes a sharp drop, the phloem-xylem ratio again rises rapidly.

From the above fact it appears that under good growing conditions the phloem is approximately one-third the size of the xylem, but when the calcium-magnesium ratio becomes unfavorable the phloem tissue increases over the xylem to two-thirds or more. The difference

Series C

Table C'''

Group	Ca/Mg	xylem	phloem	cortex	pith	phloem/xylem
I	100/0	.170 mm.	.108 mm.	.935 mm		.64
II	95/5	.223	.151	1.66		.68
III	85/15	.162	.122	.969		.76
IV	70/30	.255	.083	.629		.31
V	50/50	.967	.289	1.598		.30
VI	30/70	.289	.079	.629		.27
VII	15/85	.510	.144	.901		.28
VIII	5/95	.374	.158	1.054		.42
IX	0/100	.198	.119	.782		.60

observable in the phloem-xylem ratio is caused, not so much by changes in the size of the phloem, as by changes taking place in the size of the xylem. Again this is rather to be expected if the xylem is the tissue which concerns itself with water and mineral transport. Then this tissue would probably be affected first by any change. Further mention will be made of this fact later.

Using Table A''' and comparing it with Chart A we find that there is apparently no correlation between the phloem-xylem ratio and the growth curve. However, there is a rise in the phloem-xylem ratio in group VIII (Ca/Mg = 5/95) where the calcium content was extremely low, and in IX (Ca/Mg = 0/100) where calcium was entirely absent. In both these cases the calcium-magnesium ratio is very small and this fact bears out what was found to be true in series C, that when the calcium-magnesium ratio is either very high or very low the phloem-xylem ratio increases. Thus, in groups VIII and IX, where the calcium-potassium ratios are 5/95 and 0/100 respectively, and the calcium-magnesium ratios are 10/100 and 0/100 respectively (of course, the absolute amount of magnesium remains constant) there is a rise in the phloem-xylem ratio. This fact is to be expected from the results obtained in series C. In series A, then, it appears that the calcium-magnesium ratio, in some way affects the phloem-xylem ratio, and that whatever effect the calcium-potassium ratio has is not evidenced in these tissues, at least as far as the phloem-xylem ratio is concerned. Even in group I where the Ca/K = 100/0, the phloem-xylem ratio remains at a numerical value not too much unlike the values in the groups of optimum growth.

Series A

Table A'''

Group	Ca/K	Xylem	Phloem	Cortex	Phloem/Xylem
I	100/0	.595	.136	.381	.228
II	95/5	1.360	.374	.765	.274
III	85/15	1.700	.340	.389	.200
IV	70/30	1.309	.221	1.190	.169
V	50/50	1.785	.408	1.088	.228
VI	30/70	1.844	.357	1.020	.191
VII	15/85	1.360	.340	.612	.250
VIII	5/95	1.955	.250	.555	.135
IX	0/100	.765	.34	1.02	.445

Series I

Table B'''

Group	Mg/K	Xylem	Phloem	Cortex	Phloem/Xylem
I	100/0	.425	.051	.335	.120
II	95/5	.595	.204	1.071	.343
III	85/15	.799	.306	.833	.384
IV	70/30	1.037	.34	.952	.328
V	50/50	1.105	.323	.935	.292
VI	30/70	1.445	.425	.782	.294
VII	15/85	1.190	.340	1.83	.286
VIII	5/95	.884	.255	.867	.288
IX	0/100	.850	.170	1.275	.200

In series [illegible], however, we find an obstacle to the theory that the calcium-magnesium ratio alone governs the phloem-xylem ratio. If table V''' is compared with Chart I of Part I, it may be seen that, although the curve is steep and sudden changes in the direction of its path many, there is no corresponding change in the phloem-xylem ratio. One might have expected to find, in series VIII and IX, where the Mg/K ratios are 5/95 and 0/100 respectively, and the Ca/Mg ratios are 5.67/1 and 100/0 = (∞) respectively, that the phloem-xylem ratio would change markedly because of the high Ca/Mg ratios. This, however, was not the case and at this time ~~it~~ these results cannot be explained by the writer. It is to be noticed however, that as far as the dry weight measurements are concerned, the plants of groups VIII and IX were in good condition (Mg/K ratios 5/95 and 0/100 respectively). The plants in group IX (Mg/K = 100/0), showed remarkable growth for a plant deficient in one of the essential elements. It appears that the plants in this group may either have taken up sufficient magnesium from the soil before they were transplanted or that the presence of a large amount of potassium in some way compensates for and acts as an antagonistic ion, in the same way as will magnesium, towards the toxicity caused by a large amount of calcium. This may be maintained because of the fact that in this group (Group IX, Mg/K = 0/100), there was no evidence of high toxicity, and also because the actual calcium ion concentration was much lower than its concentration in both series A and C.

Cytological Studies

Effect on Root Tip Cells

The root tip cells of any plant are embryonic in nature and undifferentiated. Studies were here made of longi-sections of the root tips in an effort to see what effect various ratios of specific cations would have upon these cells.

The roots used in all cases were functional secondary roots or at least roots which appeared to be functional because of their color and turgidity.

Series A

The root tip cells all presented the same appearance regardless of the group from which they were taken (Figure IV). However, in those groups where the ratios of ions were unfavorable, a difference became apparent in the after growth when the contents of the original cells were spread out over the larger volume to which these cells had grown.

The older cells in all cases, except where there is a deficiency in any particular cation, resemble the younger cells in respect to their contents and differ only in size. In groups I and IX, where the calcium-potassium ratios were 100/0 and 0/100 respectively, the original cell contents of these meristematic cells had been forced to fill a larger area without any apparent addition of cytoplasmic materials. Under these conditions the cells appear as in Figure VI with hardly any stainable cytoplasm and very few nuclei.

There are all stages of this process present and Figure V shows the intermediate stage between Figures IV and VI. Here the nucleus is beginning to dissociate and has spread its entire

contents throughout the cell.

These same photomicrographs may be used to demonstrate what takes place in series B and C.

From the above we can see that the roots seem to be less affected by changes in the cation ratios than do the tops and this fact is nowhere brought out so clearly as in a cytological study of the root tips.

Series B

The newer root tip cells here too were quite normal in all groups and as before in series A, where the nutrient solution was unfavorable to the roots we found that the same symptoms were observable in the older cells. First there was a loss of the cytoplasm and later, the disintegration of the nucleus. These latter changes occurred in the older cells in groups I, II, and IX where the magnesium-potassium ratios were 100/0, 95/5, and 0/100 respectively. Figures IV, V, and VI may be used here as illustrations, for there was little or no difference in the manner in which these cell contents became disorganized.

Series C

The same was true for this series as was true for series A and B. The new meristematic tissues of the root tips were the same for all groups. Here, however, groups I, II, III, and IX having calcium-magnesium ratios of 100/0, 95/5, 85/15, and 0/100 respectively, had their older cells in a condition, such that the cell contents were disintegrated in the same manner as described above in series A and B.

It may thus be seen that the roots are affected by changes in the cation ratios; and nowhere is this fact brought out so clearly as in a study of the root tip cells.

Cytological Studies

Effect on Stem Cells

The following are the results of a detailed study of the progressive changes in the cells of the different tissues which have been subjected to nutrient solutions containing varying ratios of certain cations. For the sake of clarity, it is best to discuss series C first.

Series C

The cells of the plants of groups I, II, and III (containing calcium-magnesium ratios of 100/0, 95/5, and 85/15 respectively) were similar in all respects. The xylem was reduced to only a few cells in thickness and contained no visible wood rays. The phloem was in a highly disorganized state most of the nuclei of its cells being dispersed over the whole cell and the cytoplasm plasmolyzed. The phloem cells had penetrated the region of the xylem and thus gave the appearance that the latter had been laid down in a bed of phloem tissue.

The storage tissues, both the cortex and the pith, appear to have become non-functional, in that the cells of both had lost their nuclei and cytoplasm completely. Even the cell walls were in such poor condition that they were crushed by the microtome in the process of sectioning. The reason for the disorganization of these storage tissues may be accounted for, perhaps, in the following manner. The plant, even if able to manufacture food materials, could not replenish the storage cells due to injured conductive tissues which could not transport these life giving materials to the cells. These cells during growth would consume

their own contents and thus autolysis would take place and the resulting cell, would be similar to those described above.

Figure VII shows the state of these tissues and their cells.

In group IV, where the calcium-magnesium ratio was 70/30, there was an increase in the amount of xylem present. The wood rays in the xylem were prominent and appeared functional in some cases where the cytoplasm and nuclei were present. In fact there was evident a great improvement in all the tissues and cells even though they were far from normal. The phloem showed some cells which were not plasmolyzed even though the majority of them were in that state.

In Figure VIII may be seen the state of the xylem, the phloem and the pith of group IV. It will be noticed that the pith cells here show well defined cell walls but that the cell contents were in a highly plasmolyzed condition.

Figure IX shows the cortex whose cells were in a state very much like that of the pith. These cell contents were plasmolyzed and we may thus assume that they were non functional.

The cells in groups V, VI, VII, and VIII, (Ca/Mg ratios 30/50, 30/70, 15/85, 5/95, respectively) were all in excellent condition. There were no signs of plasmolysis and the cell contents were well defined. The xylem of these groups was larger and contained more numerous wood rays than in the preceding groups. Figure X is a photomicrograph of a cross-section of group VII Ca/Mg = 85/15 which, when compared with Figures VII, VIII and IX shows plainly the differences between normal and non-normal tissues

[illegible] various proportions or ratios of cations.

In group IV, where the calcium-magnesium ratio is 0/100 a condition existed which is so like that of groups I, II, and III, that no differences could be established even after a detailed study. The xylem alone in some cases appeared to be somewhat better organized, but even this occurred so rarely that it was considered unimportant.

In this series, as in the other two series, it was found that the size of the plant cells varied with the size of the plant itself and that the size of the plant was a good indicator as to the cell size, especially those cells of the cortex and the pith.

Cytological Studies Continued

Series A

The following are the results of a detailed study of the progressive changes in the cells of the various tissues of plants which have been subjected to nutrient solutions containing varying ratios of calcium and potassium.

Constant reference to Chart A is desirable in order to gain a better understanding of the changes in the cells in their relation to the growth curves and to the ratios of cations.

In Group I (Ca/K = 100/0), we found that the xylem, although larger in amount than that of Groups I, II, and III of Series C, (Ca/Mg ratios 100/0, 95/5, 85/15, respectively) was in a highly disorganized condition in respect to the manner in which the xylem had been deposited. These cells of the plants in this group were not arranged in the orderly manner that the cells of more normal plants exhibit. This fact can be explained as caused by so high a calcium-magnesium ratio.

The phloem, pith, and cortex in this group showed a plasmolyzed condition. It must be remembered that in this group, no potassium was present in the nutrient solution.

Group II (Ca/K = 95/5) of this series exhibited a marked improvement in the cell structure of the xylem and in the number of wood rays. This tissue took on a normal appearance. The phloem was in good condition while the cortex and pith still showed signs of plasmolysis.

Groups III, IV, V, VI, and VII, (Ca/K ratios of 85/15, 70/30, 50/50, 30/70, 15/85, respectively) showed normal development of all cell tissues in respect to both their cytoplasmic and nucleic contents.

Cross-sections of the stems of these plants did not vary markedly from that shown in Figure X.

Group VIII (Ca/K = 5/95) showed a marked change in the cortical and pith cells. Most of these cells had become plasmolysed. The xylem had become somewhat darkened, although on the whole it had maintained a certain orderliness of arrangement.

Group IX (Ca/K = 0/100) carried to completion what was beginning to suggest itself in Group VIII (Ca/K = 5/95). The xylem was reduced and the cortex, pith and phloem completely plasmolyzed. Figure VII may be used to demonstrate the condition of the tissues of this group.

Cytological Studies

Series D

The following are the results of a detailed study of the progressive changes in the cells of the various tissues of plants which have been subjected to nutrient solutions containing varying ratios of magnesium and potassium.

Constant reference to chart 5 is desirable in order to gain a better understanding of the changes in the cells in their relation to the growth curves and to the different ratios of cations.

Much difficulty was experienced in attempting to correlate the results obtained in this series with the results obtained in series A and C. The same difficulties for this series were experienced in every attempt at histological and cytological correlation studies. At present, the writer is unable to explain these discrepancies and therefore must content himself with merely citing his observations. The only suggestion the writer is able to offer is that in the absence of magnesium large amounts of potassium may in some way compensate for and act as an antagonist to calcium.

The xylem of all the groups in this series was in good condition and seemed unaffected except in Group IX (Mg/K = 0/100) where the organization of the cells was somewhat disturbed. The wood rays did not appear to be too numerous in this region.

In Group I (Mg/K =100/0) the cortex, phloem and pith were in a plasmolyzed state showing few nuclei and almost no cells of normal cytoplasmic content.

Groups II and III (Mg/K ratios of 95/5 and 85/15 respectively)

were very similar in cytological effect to group I with the exception that the cortical cells of group III showed some improvement as evidenced by a lesser amount of plasmolysis and the presence of many more cells which still retained their nuclei.

Groups IV, V, VI, and VII (Mg/K ratios of 70/30, 30/30, 30/70, 15/100 respectively) showed the cells of the above mentioned tissues in excellent condition. Nuclei and cytoplasm were present with no evidence of plasmolysis.

In group VIII (Mg/K = 5/95) we find the xylem somewhat serpanted though not too apparently so. The cortex exhibits the beginning of a reaction towards unfavorable conditions. Most of the cells were plasmolyzed and only a few scattered nuclei were to be seen. The phloem, however, appeared to be in excellent condition although here and there in individual cells there was some evidence of plasmolysis.

In group IX (Mg/K =0/100) there was very little change from what was evidenced in group VIII. Although both cortex and phloem of this group were in a higher state of dissociation.

In this series, then, we find that the high calcium-magnesium ratio present in groups VIII and IX (Ca/Mg ratios 5.67/1 and 100/1 respectively) had some influence on the cell structure although not to the extent that might have been expected from the studies conducted in A and C.

Figure IV
Root Tip Showing New
Meristematic Tissue

Figure V
Region Just Behind
Meristematic Tissue
Showing A High State
Of Plasmolysis

Figure VI
Region Just Behind
That Of Fig. V-
Showing Cells Which
Have Lost Most Of
Their Stainable Con-
tents And Most Of
Their Nuclei.

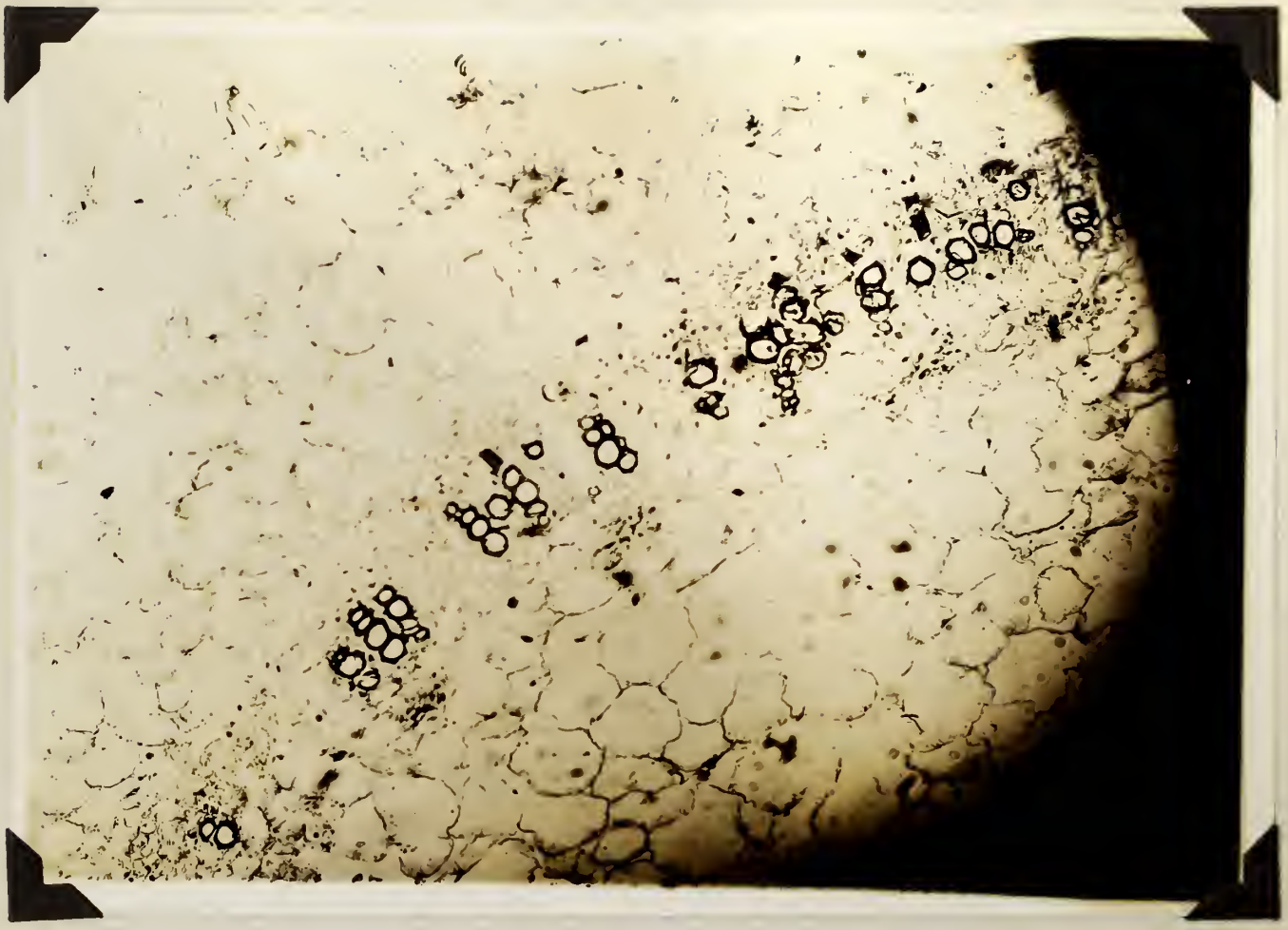


Figure VII
Figure Showing Disorganized Cells And
Degenerate Xylem Of Plant From Group I
(Series C)

Ca/Mg = 100/0

Figure VIII
Figure Showing Improvement IN Tissues Caused By A Decrease In The Calcium-Magnesium Ratio. Photomicrograph Taken From Group IV(Series C). These Tissues Show Distinct Improvement Over Those Of Groups I-III Although These Cells Are Highly Plasmolyzed.

Ca/Mg = 70/30

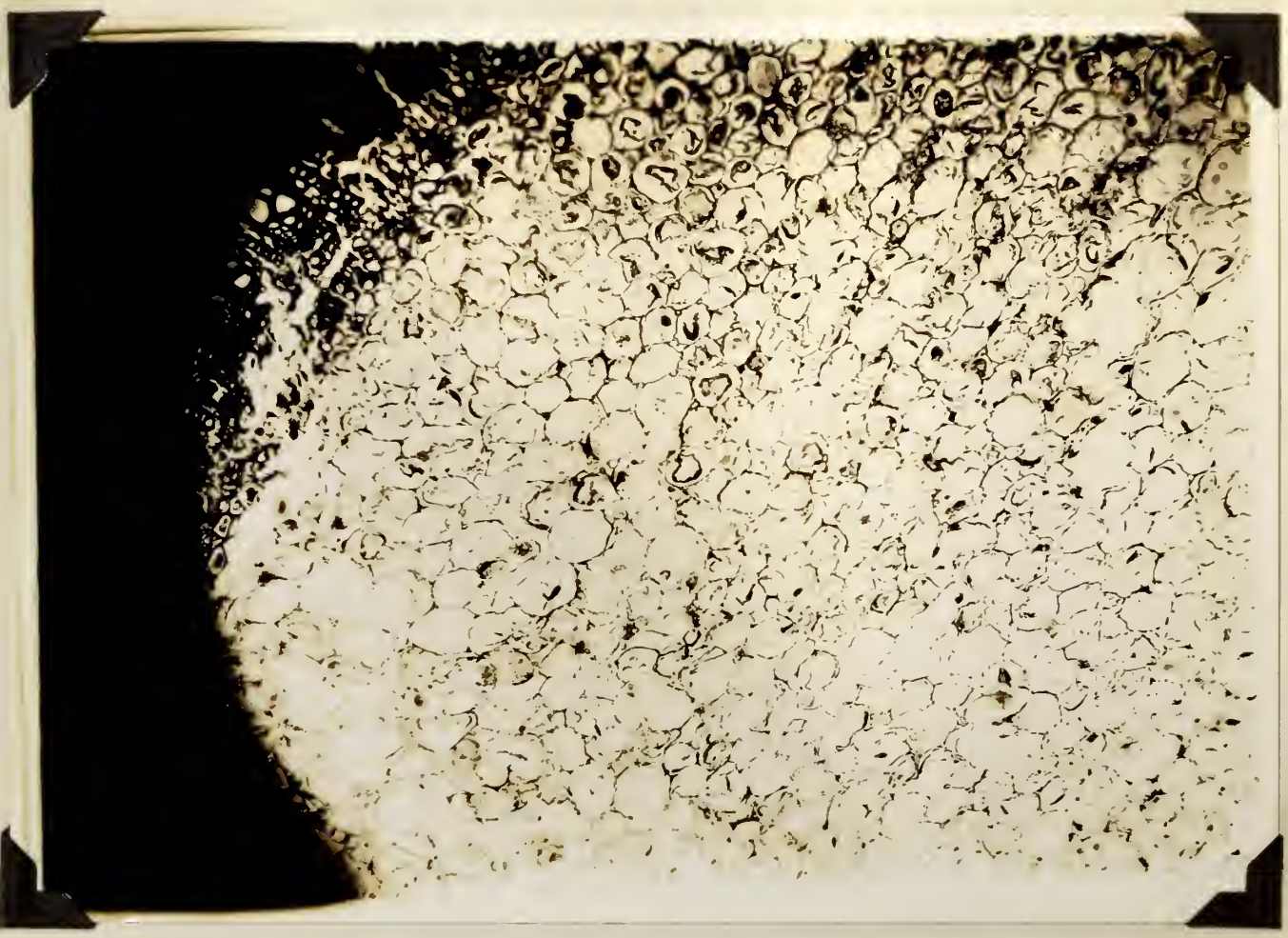


Figure IX
Figure Shows Plasmolyzed Cortical Cells. This Photomicrograph Was Taken From Group IV (Series C).

Ca/Mg = 70/30

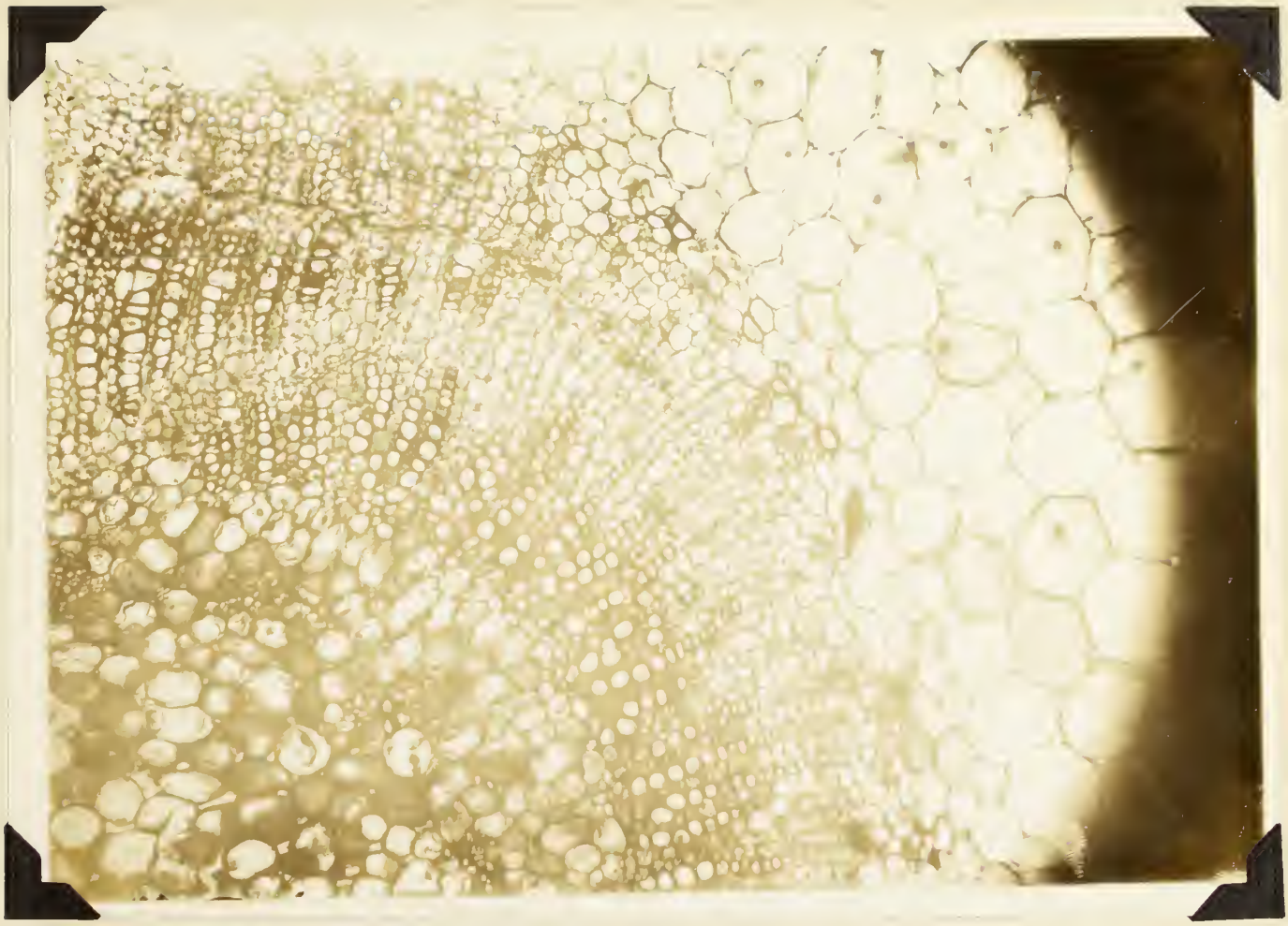


Figure X
Figure Showing A Photomicrograph Of A Cross-Section Of A Plant From Group VII (Series C). This Figure Shows All Cells In A Normal And Healthy Condition.

Ca/Mg = 15/85

Summary of Part II

1. Plant tissues were killed and fixed in chrom-acetic acid and then subjected to the usual wax-paraffin method prior to sectioning.
2. Sections were cut 10 μ thick and stained with Heidenhains haematoxylin and counterstained with safranin.
3. Two types of tissues were studied
   1. Root tips - an example of undifferentiated, fundamental cells.
   2. Stem cells and tissues - an example of highly specialized cells with functions of support, of water transport, and of food and mineral transport.
4. Studies were made of the actual size of the several tissues and also of their relative amounts.
5. The phloem-xylem ratio appeared to be the only one which bore any relationship to the proportions of ions present.
6. Detailed studies were made of the changes caused by various ratios of cations.
7. Detailed studies were made of the effect of various ratios of cations on the specialized tissues and cells of the stem.
8. Photomicographs were taken to bring out more clearly the cytological details of the above.

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