COMPARATIVE EFFECTS OF AMMONIUM OR SODIUM ADDED TO SUBSTRATES LOW IN CALCIUM, ON THE DISTRIBUTION OF ^{45}Ca IN SUBTERRANEAN CLOVER

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Summary

^{45}Ca was used in order to study the relative effects of adding either Na^+ or NH_4^+ to nutrient solutions which were either very deficient (Ca1) or deficient (Ca2) in calcium on the distribution of calcium in subterranean clover grown therein. ^{45}Ca was added to the solutions at the commencement of the experiment, and plants were harvested 20, 30, and 40 days later. The distribution of ^{45}Ca was studied by means of radioautographs and radioassays of selected tissues.

A comparison of the effects of adding either Na^+ or NH_4^+ to the very calcium-deficient (Ca1) nutrient solution showed that the plants grown in the solution with NH_4^+ had the lowest overall ^{45}Ca concentration in the trifoliate leaves at each time of sampling. This was principally due to decreases in ^{45}Ca concentration in the lamina centres. As a result, the NH_4^+-treated plants had the highest ratio of ^{45}Ca concentration in the lamina edge to that in the lamina centre.

In the calcium-deficient (Ca2) series, similar differences in calcium distribution between the plants receiving the Na^+ and NH_4^+ treatments, respectively, were not recorded until the second and third samplings at 30 and 40 days.

The addition of NH_4^+ instead of Na^+ also caused a decrease in the ratio of ^{45}Ca concentration in the lamina to that in the petiole in plants grown in the very calcium-deficient (Ca1) solutions, but caused an increase in this ratio in the calcium-deficient (Ca2) series.

I. INTRODUCTION

It has been reported that the severity of disorders in plants induced by calcium deficiency may be increased by increasing the ratio of certain cations to calcium in the substrate. Thus Foster (1934) found that the incidence of celery black-heart was increased by excessive manuring and Spencer (1949) and Money (1962) observed severe blossom-end rot in tomatoes in nitrogen fertilizer plots. Taylor and Smith (1957) also obtained a significant increase in blossom-end rot in tomatoes in sand culture at high nitrogen levels.

The relation of such effects to the calcium nutrition of the plants was studied by Geraldson (1957) who partially replaced calcium in the nutrient solution by either NH_4^+, Mg^{2+}, K^+, or Na^+. The highest incidence of blossom-end rot of tomato and pepper and of black-heart of celery was associated with a high ammonium/calcium ratio, whereas a high sodium/calcium ratio was found to be not so important.

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In sand- and water-culture experiments calcium is normally supplied as $Ca(NO_3)_2.4H_2O$. Where a low-calcium solution is required the omitted calcium is usually replaced by an equivalent amount of $NaNO_3$ to ensure a constant nitrogen level in the solution. This procedure was adopted by Millikan and Hanger (1964) in their study of the effect of calcium level in the substrate on the distribution of ^{45}Ca in subterranean clover. However, in view of the reported effects of NH_4^+ on the severity of calcium-deficiency disorders in plants, the experiment described below was made to determine whether the replacement of $Ca(NO_3)_2.4H_2O$ by NH_4NO_3 instead of $NaNO_3$ had any effect on the distribution of ^{45}Ca in subterranean clover.

TABLE 1

DETAILS OF THE COMPOSITION OF THE NUTRIENT SOLUTIONS USED

The table gives the number of millilitres of a molar solution of each salt added per 2 litres of nutrient solution. In addition each pot received 2 ml of a modified A5 solution (Millikan 1961)* and 1·5 ml of a 0·5% FeEDTA solution

Salt	Ca1 Series		Ca2 Series		
	$+Na^+$	$+NH_4^+$	$+Na^+$	$+NH_4^+$	$+2NH_4^+$
KH_2PO_4	1	1	1	1	1
KNO_3	12	12	12	12	12
$Ca(NO_3)_2.4H_2O$	0·16†	0·16†	1	1	1
$MgSO_4.7H_2O$	2	2	2	2	2
NaCl	0·5	0·5	0·5	0·5	0·5
NH_4NO_3	—	16	—	14	14
$NaNO_3$	16	—	14	—	—
NH_4Cl	—	—	—	2	18

* The A5 solution consisted of:

H_3BO_3	0·358 g	$CuSO_4.5H_2O$	0·080 g
$MnSO_4.4H_2O$	1·020 g	$(NH_4)_6Mo_7O_{24}.4H_2O$	0·020 g
$ZnSO_4.7H_2O$	0·220 g	Deionized water	1000 ml

† Two drops.

II. METHOD

Subterranean clover (*Trifolium subterraneum* L.) cv. Dwalganup was grown in nutrient solutions in 2-litre pots. The compositions of the various solutions used are given in Table 1. A dose of 4·9 μc ^{45}Ca (as $^{45}CaCl_2$) and containing a total of 6·6 μg Ca^{2+} was added to each pot in which six seedlings were established. All treatments were duplicated.

A plant was sampled from each of the duplicate pots of each treatment on days 20, 30, and 40 respectively after the commencement of the experiment, and were immediately radioautographed by the method described by Millikan and Hanger (1964). During each exposure period, the samples being radioautographed were held in a cold room at 30°F to stabilize the isotope in the tissues.

After radioautography, tissues were selected for radioassay as follows:

Sample 1 (20 days): Lamina and petioles, separately, of the cotyledons, unifoliate leaf, and each of the first two trifoliate leaves.

Sample 2 (30 days): Leaf edge (outer third of leaflet), leaf centre, proximal and distal petiole of each trifoliate leaf.

Sample 3 (40 days): Leaf edge (outer third of leaflet), leaf centre, whole petiole of each trifoliate leaf.

Each sample was dried, weighed to the nearest 0·005 mg, and then radioassayed. An automatic sample changer and associated scaler and recorder was used for the radioassays which were corrected for the decay of the isotope and were finally expressed as the mean count/min/mg dry matter. All samples were counted at least twice. The mean ^{45}Ca concentration in each entire lamina or petiole was calculated for samples 2 and 3.

The ratios between the ^{45}Ca concentrations in lamina and petiole (samples 1, 2, and 3) and between leaf edge and leaf centre (samples 2 and 3) were also calculated, and the results analysed statistically.

III. Results

The results are presented in Table 2 and in Plates 1 and 2. At no time up to 40 days, when the experiment was terminated, were any symptoms of calcium deficiency observed in any of the plants. No differences were noted between the NH_4^+- and Na^+-treated plants apart from the ^{45}Ca distribution. The apparent greater size of the $2NH_4^+$-treated plants as compared with the corresponding Na^+-treated plants in Plate 1, Figures 1–4, is due to the fact that the radioautographs of the former plants (see Figs. 2 and 4) were made on smaller-sized X-ray film and were not reduced to the same extent as the radioautographs shown in Figures 1 and 3. The relative reduction of each figure is indicated by an appropriate scale.

The difference in the specific activities of the calcium available to the plants growing in the very low-calcium (Ca1) and low-calcium (Ca2) series, respectively, accounts for the lower ^{45}Ca concentrations recorded in Table 2 for the Ca2 series plants when compared with the Ca1 series plants.

(*a*) *Sample 1*

By day 20 certain changes in the relative ^{45}Ca content of various tissues were already apparent. Thus in both the Ca1 and Ca2 series plants a trend towards a higher ratio of ^{45}Ca concentration between lamina and petiole was recorded for the cotyledons and unifoliate leaves of the plants grown in the solutions receiving NH_4^+ than between similar tissues of comparable plants receiving Na^+ (Table 2). Although the trend was adjudged significant only for the $2NH_4^+$ treatment in the Ca2 series, it is also apparent between the radioautographs shown in Plate 1, Figures 1 and 2.

The radioautographs of the Ca1 series plants (Plate 1, Fig. 1) show the much higher concentration of ^{45}Ca in the proximal than the distal halves of the petioles of the trifoliate leaves. This is characteristic of such acutely calcium-deficient plants (Millikan and Hanger 1964). However, in the experiment described herein only the entire petiole of each leaf was assayed for ^{45}Ca.

In conformity with the results of the subsequent 30- and 40-day samples from the Ca1 series, the NH_4^+ treatment, when compared with the Na^+ treatment, caused

TABLE 2

MEAN RESULTS OF RADIOASSAYS FOR ^{45}Ca (EXPRESSED AS COUNTS/MIN/MG DRY MATTER) IN SELECTED TISSUES OF SUBTERRANEAN CLOVER PLANTS GROWN FOR 20, 30, AND 40 DAYS, RESPECTIVELY, IN VERY LOW (Ca1) AND LOW (Ca2) CALCIUM NUTRIENT SOLUTIONS IN WHICH OMITTED CALCIUM WAS REPLACED BY SODIUM OR AMMONIUM

Tissue	Ca1 Series				Ca2 Series				
	$+Na^+$	$+NH_4^+$	Significant Difference		$+Na^+$	$+NH_4^+$	$+2NH_4^+$	Significant Difference	
			5% Level	1% Level				5% Level	1% Level
	Sample 1 (20 days)								
Cotyledons									
Laminae	72	110			63	118	84		
Petioles	216	206			72	79	57		
Laminae/petioles	0·34	0·54	0·48	0·66	0·88	1·49*	1·53	0·48	0·66
Unifoliate leaf									
Lamina	80	118			72	102	94		
Petiole	205	185			63	50	59		
Lamina/petiole	0·38	0·67	0·48	0·66	1·15	2·04*	1·61	0·48	0·66
Trifoliate leaf 1									
Lamina	175	141			102	146	104		
Petiole	170	181			73	96	55		
Lamina/petiole	1·06	0·78	0·48	0·66	1·42	1·52*	1·92	0·48	0·66
Trifoliate leaf 2									
Lamina	163	139			87	102	86		
Petiole	105	106			69	55	49		
Lamina/petiole	1·56	1·39	0·48	0·66	1·30	1·89	1·77	0·48	0·66

* These results not included in the statistical analysis as one replicate was lost.

TABLE 2 (*Continued*)

Tissue	Ca1 Series				Ca2 Series				
	$+Na^+$	$+NH_4^+$	Significant Difference		$+Na^+$	$+NH_4^+$	$+2NH_4^+$	Significant Difference	
			5% Level	1% Level				5% Level	1% Level
	Sample 2 (30 days)								
Trifoliate leaves									
Whole lamina	199	157			150	158	144		
Lamina centre	176	133	23	31	133	130	107	23	31
Lamina edge/lamina centre	1·43	1·81	0·27	0·37	1·33	1·51	1·97	0·26	0·34
Petiole	186	196			91	70	58		
Lamina/petiole	1·31	0·95	0·68	0·88	1·74	2·32	2·58	0·43	0·60
	Sample 3 (40 days)								
Trifoliate leaves									
Whole lamina	263	206			200	164	160		
Lamina centre	215	148	23	31	183	153	124	23	31
Lamina edge/lamina centre	1·66	2·38	0·17	0·24	1·15	1·29	1·71	0·18	0·26
Petiole	188	177			101	64	55		
Lamina/petiole	1·59	1·20	0·31	0·40	2·02	2·72	2·97	0·56	0·73

a trend (non-significant for sample 1) towards a reduction in ^{45}Ca concentration in the whole lamina and in the ratio of ^{45}Ca concentration of the lamina to that of the petiole of the trifoliate leaves.

However, the radioautographs give the first indication that the addition of NH_4^+, when compared with the addition of Na^+, resulted in a relatively greater concentration of the isotope in the edge than in the centre of trifoliate leaf 1 of each plant (Plate 1, Fig. 1). This effect of NH_4^+ is more clearly evident in the radioautographs of the Ca2 series plants (Plate 1, Figs. 1 and 2). The radioassays of trifoliate leaves 1 and 2 of these plants (Table 2) show that this effect of NH_4^+ did not result in a consistent overall increase in the ^{45}Ca concentration in the lamina, but did result in significant increases in the ratio of ^{45}Ca concentration in the lamina to that in the petiole when compared with the results obtained from comparable leaves of the plants receiving Na^+.

(*b*) *Samples 2 and 3*

The relative differences between treatments shown by the results of the radioautographs and radioassays of sample 2 (30 days) and sample 3 (40 days) were in close agreement. However, the ^{45}Ca concentration was consistently higher for the laminae but not the petioles of sample 3 when compared with sample 2.

With each sample, addition of NH_4^+ at the Ca1 level resulted in a significant increase in the ratio of the concentration of ^{45}Ca at the edge to that at the centre of the leaf when compared with the effect of added Na^+. The increase was greatest for sample 3. At the Ca2 level there was a non-significant trend to a similar increase with NH_4^+ treatment, but with the $2NH_4^+$ treatment the increase in the ratio was highly significant.

These significant effects were principally due to significant reductions in ^{45}Ca concentrations in the leaf centres. These reductions, which increased in magnitude between days 30 and 40, were reflected in reductions in the concentration of ^{45}Ca in the whole laminae of the NH_4^+-treated plants as compared with that in the Na^+-treated plants at both Ca1 and Ca2 levels.

On the other hand, the radioassays (Table 2) show that for both samples 2 and 3 ^{45}Ca activity in the petioles of the NH_4^+-treated plants was comparable with that in the Na^+-treated plants at the Ca1 level but lower for the Ca2 series. These changes resulted in reverse effects on the ratio of ^{45}Ca concentration in the lamina to that in the petiole—NH_4^+ significantly reduced this ratio at the Ca1 level (sample 3) but increased it at the Ca2 level (samples 2 and 3).

IV. Discussion

It is known that sites of initial accumulation of ^{45}Ca in calcium-deficient subterranean clover plants grown in a substrate in which Ca^{2+} was replaced by Na^+ are the proximal half of the petiole, the veins (and in particular their endings), and the adjacent marginal interveinal tissues of the lamina. Provided the deficiency of calcium is not too acute the ^{45}Ca may later increase in concentration in the distal part of the petiole and in the centre of the lamina (Millikan and Hanger 1964).

It has now been further demonstrated that the distribution of ^{45}Ca in calcium-deficient plants is altered to a significant degree by the presence of NH_4^+ instead of Na^+ as a substitute for Ca^{2+} in the substrate. This substitution resulted in consistent reductions in the overall concentrations of ^{45}Ca in the trifoliate leaves at each time of sampling of the Ca1 series plants, and at the third sampling, in particular, of the Ca2 series plants (Table 2). These effects were principally due to significant decreases in ^{45}Ca concentration in the laminae centres, which in turn were reflected in significant increases in the ratio of ^{45}Ca concentration in the lamina edge to that in the lamina centre. There was an interaction effect resulting from the replacement of Na^+ by NH_4^+ in the substrate, as indicated by a decrease in the ratio of ^{45}Ca concentration in the lamina to that in the petiole of the Ca1 series plants, and an increase in this ratio in those of the Ca2 series (Table 2). However, in the Ca2 series decreases in ^{45}Ca concentration occurred in both laminae and petioles of the NH_4^+-treated plants but were relatively greater in the latter, hence the increases in this ratio.

A possible explanation for this interaction effect is provided by the radioautographs of the plants (Plates 1 and 2). From these it is evident that ^{45}Ca concentration was more uniform along the length of petioles of all plants of the Ca2 series when compared with the Ca1 series plants. This indicates that, in contrast with the Ca1 level, the Ca2 level provided sufficient calcium to saturate the sites of initial accumulations in the petioles, whereas the lamina edges were still unsaturated. Thus any restriction in ^{45}Ca supply induced by NH_4^+ would be expected to affect the petioles more than the laminae of the Ca2 plants.

These differences in the effects of NH_4^+ when compared with Na^+ on the ^{45}Ca concentration are consistent with the results of workers cited in the Introduction, which have shown that the presence of NH_4^+ in the substrate aggravated the severity of calcium-deficiency symptoms in plants.

In addition, Arnon (1939), Sideris and Young (1946), Evans and Weeks (1948), and Gausman *et al.* (1959) have reported that where ammonium was the source of nitrogen, the concentration of calcium in plants was lower than where nitrate nitrogen was supplied.

V. Acknowledgments

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VI. References

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Explanation of Plates 1 and 2

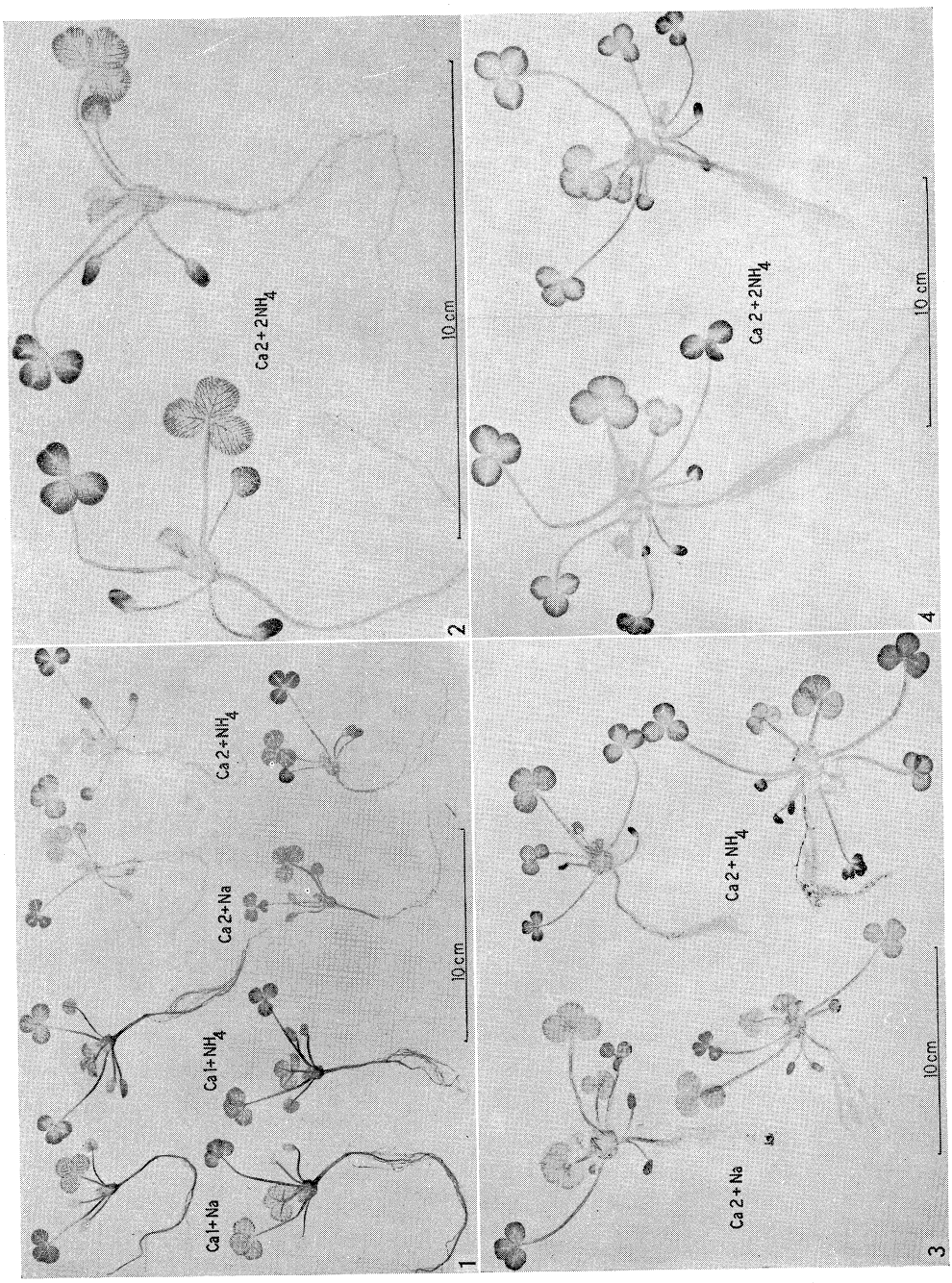
Plate 1

Radioautographs of ^{45}Ca in subterranean clover grown for 20 days (Figs. 1 and 2) or 30 days (Figs. 3 and 4) in very low (Ca1) or low (Ca2) calcium nutrient solutions in which omitted calcium was replaced by either Na^+ or NH_4^+. Time of each exposure 7 days.

Plate 2

Radioautographs of ^{45}Ca in subterranean clover grown for 40 days in very low (Ca1) or low (Ca2) calcium nutrient solutions in which omitted calcium was replaced by either Na^+ (Figs. 1 and 3) or NH_4^+ (Figs. 2 and 4). Time of each exposure 8 days.

DISTRIBUTION OF ^{45}Ca IN SUBTERRANEAN CLOVER



DISTRIBUTION OF ^{45}Ca IN SUBTERRANEAN CLOVER