THE MOBILITY OF ^{65}Zn IN *TRIFOLIUM SUBTERRANEUM* L. AND *ANTIRRHINUM MAJUS* L.

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Summary

T. subterraneum (cv. Clare) and *A. majus* were grown in nutrient cultures to which ^{65}Zn was added at two stages in the plants' development. It was found that the ^{65}Zn recently absorbed by the roots was preferentially routed to the youngest leaves. When the plants were transferred from radioactive to non-radioactive cultures, only a limited recirculation of ^{65}Zn took place. In *T. subterraneum* this was principally from the roots and hypocotyl, but also between and within some leaves, whilst in *A. majus* recirculation occurred along the stem, roots, and within some leaves.

Injection of 1000 μg of non-radioactive zinc into the third true leaf of *T. subterraneum* failed to alter the distribution of ^{65}Zn between leaves, although it did induce movement of ^{65}Zn along the petiole into the lamina of some individual leaves.

I. INTRODUCTION

The presence of free zinc ions in plant tissue has been investigated by a number of workers using dialysis, but with conflicting results. Both Day and Franklin (1946), using the common elderberry, and Wood and Sibly (1950), working with tomato and oats, failed to extract free zinc from leaves, whereas Hewitt and Todd (1952) and Johnson and Schrenk (1964) dialysed zinc from potato tubers and lucerne stem and leaves respectively.

In support of their results, Wood and Sibly (1950) reported that no zinc was transported from the oat leaves to other organs, the zinc present in the inflorescence coming directly from the roots and substrate. On the other hand, Williams and Moore (1952), Riceman and Jones (1958*a*), and Massey and Loeffel (1967) all concluded that zinc was translocated from the leaves and stem into the seeds of oats, subterranean clover, and corn respectively. Rinnie and Langston (1960) also produced autoradiographic evidence of zinc redistribution in peppermint.

The experiments described herein were designed to study the recirculation of ^{65}Zn within plants of *Trifolium subterraneum* L. and *Antirrhinum majus* L. during their vegetative stage in growth, and when grown in cultures with an adequate zinc supply. The mobility of zinc was assessed by determining changes in the distribution

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pattern of ^{65}Zn following the transfer of plants from radioactive to non-radioactive cultures, and also with *T. subterraneum* after the injection of non-radioactive zinc through a leaf.

II. Methods

Subterranean clover (*T. subterraneum*, cv. Clare) and tetraploid snapdragon (*A. majus*) were grown in water cultures. The composition of the complete nutrient culture and the procedure used for autoradiographing and radioassaying the plant tissue are identical to those described by Millikan and Hanger (1964, 1965*a*, 1965*b*).

With each species, two series of treatments were studied. In series A, the plants were grown for 20 days in non-radioactive cultures, and then transferred to fresh cultures containing 5 μCi ^{65}Zn (as $ZnSO_4$, containing 4·55 μg Zn) per pot. After 14 days in the radioactive cultures half the plants were harvested from each pot, and half were transferred to freshly prepared non-radioactive solutions and grown for a further 13 days. The treatment series B differed from A only in that during the first 20 days the plants were grown in radioactive cultures containing 5 μCi ^{65}Zn per pot.

For each series the following were established:

(1) Six pots, each with four *T. subterraneum* seedlings at the cotyledonary stage;

(2) Eight pots, each with three *A. majus* seedlings at the first true leaf stage.

On day 33, 0·2 ml of a $ZnSO_4$ solution containing 1000 μg Zn was injected into the third true leaf of one *T. subterraneum* plant per pot (Millikan and Hanger 1965*a*). Twenty-four hours later all the injected plants plus one non-injected plant from each pot of both series were harvested. The injection procedure was repeated on day 46, and all plants including those not injected were harvested 24 hr later.

When harvested, the plants were autoradiographed then subsampled into the following parts before radioassaying: root, hypocotyl, cotyledon, and unifoliate leaves. Each true leaf, where possible, was subdivided into distal and proximal halves of the petiole, and lamina edge and centre. The true leaves were also classified according to age, and numbered from the oldest.

In both series, one *A. majus* plant was harvested from each pot on days 33 and 46. Prior to autoradiography the plant stem was severed immediately beneath the node of each leaf pair. The following parts were later radioassayed: a portion of the root and hypocotyl, the internode region of the stem above the node of each leaf pair, and each leaf as petiole and proximal and distal halves of the lamina wherever possible. Mean values were calculated from the radioassays of the individual leaves of each pair.

In each plant part ^{65}Zn was presented as total content or as concentration or both, variations in dry matter production being taken into account in calculation of concentrations.

III. Results

(*a*) T. subterraneum

The radioassays of the various plant parts were statistically analysed in three main groups of tissue, namely root and hypocotyl, cotyledon and unifoliate leaf, and leaves 1–8.

Statistical analysis (not presented) showed that foliar injection of non-radioactive zinc into leaf 3 failed to induce any change in the concentration or absolute amount of ^{65}Zn in roots and hypocotyl.

The absolute amount of ^{65}Zn in root and hypocotyl fell between harvests, the fall being greatest in the roots (Table 1). The most probable reason for this change is that a portion of the ^{65}Zn was carried up the plant via the transpirational stream to the newly developing tissue between harvests. Autoradiographs of the

plants at harvest 2 (not presented) revealed the presence of ^{65}Zn in tissue developed between harvests 1 and 2 — a period when no ^{65}Zn absorption took place.

TABLE 1

ABSOLUTE AMOUNTS OF ^{65}Zn IN PARTS OF *T. SUBTERRANEUM* PLANTS

Plants were harvested on days 34 (harvest 1) and 47 (harvest 2), and values are means for series A and B. D_H and D_P refer to a difference of means of harvest and plant parts respectively, and D_D refers to a difference between any two such means

	Total ^{65}Zn Content [$\log_{10}$ (counts/min)] in:	
	Root	Hypocotyl
Harvest 1	3·28	2·53
Harvest 2	2·89	2·22
Difference	−0·39	−0·31
L.S.D. ($P = 0·05$)	$D_H = 0·07$; $D_P = 0·05$; $D_D = 0·07$	
L.S.D. ($P = 0·01$)	$D_H = 0·09$; $D_P = 0·07$; $D_D = 0·10$	

The absolute amount of ^{65}Zn in the cotyledons or unifoliate leaf (results not presented) was not changed by foliar injection or time of harvest.

In the time interval between harvests, some redistribution of ^{65}Zn took place in individual trifoliate leaves. There was a significant loss of ^{65}Zn from leaves 4 (series B) and 5 (series A and B), and a gain in leaf 6 (series B) (Table 2). During this period leaves 1 and 2 had died, yet during the senescent process no ^{65}Zn was translocated.

TABLE 2

DIFFERENCES IN TOTAL ^{65}Zn CONTENT OF INDIVIDUAL TRIFOLIATE LEAVES OF *T. SUBTERRANEUM* PLANTS AT HARVESTS 1 AND 2

Total ^{65}Zn contents were expressed as $\log_{10}$ (counts/min). Least significant differences ($P = 0·05$) were: individual differences 0·11; between differences 0·15

Leaf No.	Difference in Total ^{65}Zn Content (harvest 1 − harvest 2) in:	
	Series A Plants	Series B Plants
1	+0·07	+0·09
2	−0·02	−0·08
4	−0·07	−0·17
5	−0·11	−0·13
6	+0·09	+0·12

Statistical analysis (not presented) showed that the injection of 1000 μg of non-radioactive zinc into leaf 3 had no effect upon the total content of ^{65}Zn in any

leaves. However, by expressing the ^{65}Zn contents found in the lamina edge, lamina centre, distal petiole, and proximal petiole as percentages of the total ^{65}Zn content of the leaf, it was found that the foliar injection did induce some redistribution of the isotope within leaves, as shown in Table 3.

TABLE 3

DIFFERENCES IN ^{65}Zn CONTENTS OF PARTS OF LEAVES OF *T. SUBTERRANEUM* PLANTS WHICH HAD OR HAD NOT BEEN INJECTED WITH NON-RADIOACTIVE ZINC

^{65}Zn contents of leaf parts were expressed as a percentage of total ^{65}Zn content [$\log_{10}$ (counts/min)] of the leaf (lamina plus petiole). Values for series A and B are means for the two harvests, and those for harvests 1 and 2 are means for the two series

Series or Harvest	Leaf No.	Differences (injected − non-injected plants) in ^{65}Zn Contents of Leaf Parts:			
		Lamina Edge	Lamina Centre	Petiole (distal half)	Petiole (proximal half)
Series A	1	−0·07	+0·02	+0·04	−0·13
	2	−0·04	+0·10	−0·06	−0·12
	4	+0·11	0·00	−0·09	−0·17
	5	+0·09	−0·03	−0·04	−0·15
Series B	1	+0·09	+0·05	−0·17	−0·09
	2	+0·03	−0·01	0·00	−0·12
	4	+0·15	−0·02	−0·11	−0·24
	5	+0·09	0·00	−0·12	−0·18
L.S.D. ($P = 0·05$)					
Individual differences		0·09	0·05	0·08	0·10
Between differences		0·12	0·07	0·12	0·14
Harvest 1	1	+0·07	+0·12	−0·13	−0·32
(day 34)	2	−0·02	+0·13	−0·11	−0·22
	4	+0·10	+0·01	−0·10	−0·17
	5	+0·10	+0·01	−0·13	−0·20
L.S.D. ($P = 0·05$)					
Individual differences		0·04	0·05	0·08	0·10
Between differences		0·06	0·07	0·12	0·14
Harvest 2	1	−0·05	−0·04	0·00	−0·01
(day 47)	2	+0·01	−0·04	+0·04	0·00
	4	+0·16	−0·03	+0·09	−0·21
	5	+0·09	−0·04	−0·04	−0·13
	6	+0·09	−0·05	−0·02	−0·12
	7	+0·04	−0·02	+0·03	−0·10
	8	−0·03	+0·04	−0·08	−0·10
L.S.D. ($P = 0·05$)					
Individual differences		0·08	0·05	0·10	0·09
Between differences		0·11	0·07	0·14	0·12

It is evident from these results that the effect of the foliar injection was to move the ^{65}Zn already present in the leaf petiole into the lamina. However, in the

series A leaves the main efflux of ^{65}Zn was restricted to the proximal halves of the petioles, whereas in those from series B the whole length of the petiole lost ^{65}Zn to the lamina.

By studying the effect of foliar injection at each harvest, some assessment can be made as to the effect of leaf age upon the redistribution of ^{65}Zn within its tissues.

Table 3 shows that at each harvest, and with two exceptions, a foliar injection caused a reduction in the percentage of the total ^{65}Zn content of the leaf found in the petiole. In harvest 1 the reduction was along the whole length of the petiole, whereas in harvest 2 it was confined to the proximal portion.

Foliar injection induced an increase in the percentage of ^{65}Zn located in the central portion of the lamina of the two oldest leaves at larvest 1, but by harvest 2 these leaves had either died or were in an advanced stage of senescence, and no changes in ^{65}Zn distribution within them were recorded. In the younger leaves 4 and 5, at both harvests the foliar injection caused the percentage of ^{65}Zn in the lamina to rise. Of the youngest leaves developed after harvest 1, only in leaf 6 was any increase in ^{65}Zn in the lamina detected.

(*b*) A majus

In interpreting the results of the *A. majus* experiment, it should be borne in mind that the plants used were transplanted from seedling flats, and had one pair of fully expanded true leaves and a top rosette of progressively smaller leaves at the commencement of the experiment. These leaves would have contained non-radioactive zinc from the soil. Also, as the series A plants were initially set up in non-radioactive nutrient solutions for 20 days, leaf 2 of these plants was fully expanded and leaves 3, 4, and 5 were progressively less mature at the time of transfer to radioactive solutions.

TABLE 4

DIFFERENCES BETWEEN SERIES IN MEAN CONCENTRATIONS OF ^{65}Zn IN STEM INTERNODES AND PETIOLES AND LAMINAE OF INDIVIDUAL LEAF PAIRS OF *A. MAJUS* PLANTS

^{65}Zn concentrations were expressed as $\log_{10}$ (counts/min/mg dry matter). Stem internode refers to the internode immediately above the particular leaf pair. Least significant differences ($P = 0{\cdot}05$) were: individual differences 0·16; between differences 0·17

Leaf Pair No.	Difference in ^{65}Zn Concentration (series B − series A) in:		
	Stem Internode	Petiole	Lamina
1	+0·64	+0·64	+0·94
2	+0·52	+0·59	+0·93
3	+0·40	+0·50	+0·80
4	+0·39	+0·50	+0·62
5	+0·34	+0·41	+0·49

There is evidence from the results in Table 4 of preferential routing of recently absorbed ^{65}Zn to the younger tissues of the *A. majus* plants. Thus, although the ^{65}Zn concentration in stem, petiole, and lamina tissues of the series A plants were consis-

tently lower than that in comparable tissue of series B plants, this difference was significantly less between younger than between older tissues. Within each leaf pair this difference due to series was greater for the lamina than for stem internode or petiole, particularly in the older leaves.

Between harvests there were significant reductions in ^{65}Zn concentration in all stem internodes, some petioles, and the lamina of leaf pair 5 (Table 5). In the case of the lower stem internodes, where elongation between harvests was negligible,

TABLE 5

DIFFERENCES BETWEEN HARVESTS IN MEAN CONCENTRATIONS OF ^{65}Zn IN STEM INTERNODES AND PETIOLES AND LAMINAE OF INDIVIDUAL LEAF PAIRS OF *A. MAJUS*, AND WITHIN SERIES

^{65}Zn concentrations were expressed as $\log_{10}$ (counts/min/mg dry matter). Stem internode refers to the internode immediately above the particular leaf pair. Values for individual leaf pairs were averaged over both series, and those for series A and B were averaged over all leaves

Leaf Pair or Series	Difference in ^{65}Zn Concentration (harvest 2 − harvest 1) in:		
	Stem Internode	Petiole	Lamina
Leaf pair 1	−0·35	−0·18	−0·06
Leaf pair 2	−0·46	−0·11	−0·10
Leaf pair 3	−0·34	−0·01	−0·04
Leaf pair 4	−0·28	−0·13	−0·07
Leaf pair 5	−0·24	−0·49	−0·19
L.S.D. ($P = 0·05$)	Individual differences 0·15		
	Between differences 0·17		
Series A	−0·47	−0·34	−0·13
Series B	−0·15	−0·03	−0·04
L.S.D. ($P = 0·05$)	Individual differences 0·14		
	Between differences 0·20		

the reductions may be regarded as evidence of movement of ^{65}Zn out of them during the period of growth in non-radioactive solutions. Determinations of the absolute amounts of ^{65}Zn (as counts/min) in these internodes at each harvest are not presented as all tissue was not removed to ensure that no nodal tissue was included. However, the very small amount of internodal tissue discarded would have little, if any, effect on the mean ^{65}Zn concentration in the internode.

The fall in ^{65}Zn concentration between harvests was much greater in the stem internodes and petioles, but not the laminae, of the series A than of series B plants (Table 5), indicating that the more recently acquired ^{65}Zn in these tissues of the series A plants had not reached sites of permanent deposition at the time of transfer to the non-radioactive solutions.

Further evidence for the preferential movement of recently acquired ^{65}Zn into young rather than old leaves is afforded by the results in Table 6. At each harvest, total counts of ^{65}Zn in corresponding oldest leaf pairs were greater for series B than for series A, but the differences decreased progressively to the youngest leaf pairs.

TABLE 6

TOTAL ^{65}Zn CONTENTS OF LEAF PAIRS OF *A. MAJUS* PLANTS AT HARVESTS 1 (DAY 33) AND 2 (DAY 46)

Total ^{65}Zn contents are expressed as $\log_{10}$ (counts/min). D_H, D_S, and D_L refer to a difference between any two harvests, series, or leaf pair means respectively; D_SD_L, D_HD_L, and D_HD_S refer to a difference between two such differences

Leaf Pair No.	Total ^{65}Zn Content at Harvest 1			Total ^{65}Zn Content at Harvest 2		
	Series A	Series B	B − A	Series A	Series B	B − A
1	2·01	1·75	+0·74	1·91	2·83	+0·92
2	2·45	3·13	+0·68	2·35	3·27	+0·92
3	2·66	3·21	+0·55	2·66	3·35	+0·69
4	2·92	3·29	+0·37	2·91	3·36	+0·45
5	3·09	3·25	+0·16	2·95	3·21	+0·26
6	3·00	2·97	−0·03	2·88	2·99	+0·11
L.S.D. ($P = 0·05$)		$D_H = 0·22$; $D_S = 0·23$; $D_L = 0·17$; D_SD_L, $D_HD_L = 0·24$; $D_HD_S = 0·32$				

Also, at harvest 1 there were progressive increases in total counts between leaf pairs 1–5 of the series A plants, whereas in series B an increase occurred between leaf pairs 1 and 2, but not between 2 and 5.

Leaf pair 1 was the only fully expanded leaf pair when the experiment was commenced. It was evidently bypassed to a significant degree by the ^{65}Zn taken up by the roots of the series B plants between days 0 and 33.

It follows that any movement of ^{65}Zn up the stem between harvests (Table 5) must have been into leaf pairs younger than leaf pair 6. This conclusion is supported by autoradiographs (not presented), which show the occurrence of ^{65}Zn in the youngest leaves which developed between days 33 and 46.

The percentage of the absolute ^{65}Zn content of a leaf pair (lamina plus petiole) present in the petiole plus proximal half of the lamina is presented in Table 7. There appear to be two interacting factors involved in the distribution of ^{65}Zn in any leaf pair, namely the relative physiological age of the leaf pair and the duration of the period over which the plant was absorbing the isotope instead of non-radioactive zinc. For the shortest ^{65}Zn uptake period (series A), the younger the leaf pair the less was the proportion of the isotope found in the petiole and proximal half of the lamina (and conversely the greater the proportion in the distal half of the lamina). However, with a longer ^{65}Zn uptake period (series B), the proportion of the isotope in the petiole and proximal half of the lamina was considerably reduced and relative leaf age was much less important.

There was only a limited change in the ^{65}Zn distribution pattern within leaves between harvests. This only occurred in series B, and resulted in an increase in the percentage of the isotope in petiole plus proximal half of the lamina (Table 7).

TABLE 7

PERCENTAGE OF ^{65}Zn CONTENT OF LEAF PAIR PRESENT IN PETIOLE PLUS PROXIMAL HALF OF LAMINA IN *A. MAJUS* PLANTS

^{65}Zn contents of leaf pairs were expressed as counts/min. Explanation of symbols as for Table 6. Values for harvests 1 and 2 are averaged over all leaf pairs

Leaf Pair or Harvest	Percentage of Total ^{65}Zn Content of Leaf Pair in Petiole+Proximal Half of Lamina in:		
	Series A	Series B	B − A
Leaf pair 1	62·0	48·7	−13·8
Leaf pair 2	59·6	45·7	−13·9
Leaf pair 3	56·7	43·7	−13·0
Leaf pair 4	53·5	45·2	−8·3
Leaf pair 5	49·1	46·7	−2·4
L.S.D. ($P = 0·05$)	$D_S = 4·6$; $D_L = 3·5$; $D_SD_L = 4·9$		
Harvest 1	57·4	43·5	−13·9
Harvest 2	55·0	48·5	−6·5
L.S.D. ($P = 0·05$)	$D_S = 4·5$; $D_H = 4·1$; $D_SD_H = 5·8$		

The ^{65}Zn concentrations [expressed as log(counts/min/mg dry matter)] in the roots and hypocotyls of the plants at each harvest were found to be as follows:

	Roots	Hypocotyl
Harvest 1	2·68	1·77
Harvest 2	2·17	1·39
Difference	−0·51	−0·38

The least significant difference ($P = 0·01$) was 0·21, and there were no interactions. An appreciable fall in ^{65}Zn concentration in roots and hypocotyl occurred between harvests.

IV. DISCUSSION

Within the experimental limits, it was found in both *T. subterraneum* and *A. majus* that very little of the absorbed ^{65}Zn was recirculated from one plant part to another. This immobility of ^{65}Zn contrasts strongly with the considerable redistribution of ^{45}Ca within the same plant species (Millikan and Hanger 1967*b*).

Although the distribution pattern of ^{65}Zn in the plants remained virtually unchanged, the detection of the isotope in tissue formed during the period when there was no ^{65}Zn absorption by the roots indicates that some ^{65}Zn within the plant was still in a mobile form and capable of movement. A similar observation was made in peppermint plants by Rinnie and Langston (1960). The principal sources for this

mobile ^{65}Zn appears to be the roots and hypocotyl in *T. subterraneum* and *A. majus*. It must be stressed, however, that the roots of both species retained a substantial amount of their total ^{65}Zn after growth in non-radioactive cultures.

From the results presented here and by other workers, it is postulated that both the zinc status and the stage of development of the plant greatly affect the general mobility of zinc. In these experiments the plants had a continuous and ample supply of zinc. In both series the ^{65}Zn transported to the cotyledons of *T. subterraneum*, and to the old and middle-aged leaves of both species, was completely immobilized, even with the onset of senescence and death. Immobility of zinc in old leaves has also been shown by Wood and Sibly (1950) and Rinnie and Langston (1960). However, various workers using zinc-deficient plants have reported considerable zinc mobility. Riceman and Jones (1956) found that the cotyledons of *T. subterraneum* lost a considerable proportion of their zinc over a period of 31 days, during which time zinc-deficiency symptoms had developed. Later, Riceman and Jones (1958*b*) showed the transport of zinc out of the cotyledons and primary leaves to be more rapid from zinc-deficient than from corresponding parts of the control plants. They further reported (Riceman and Jones 1960) that the zinc in fully expanded leaves was largely retained, and it was only when these leaves became prematurely senescent as a result of zinc deficiency that any of this zinc was retranslocated. Millikan, Hanger, and Bjarnason (1968) also observed a similar removal of ^{65}Zn from senescent old leaves in zinc-deficient *T. subterraneum*. The reason for the release of zinc from zinc-deficient senescing leaves is not known.

The processes of flowering and grain formation appears also to induce a general mobilization of zinc within plants. Wood and Sibly (1950) found that in oats the zinc content of roots increased until the time of grain development, after which there was a decrease following translocation to other parts of the plant. Riceman and Jones (1958*b*) reported the transfer of zinc out of the leaves of *T. subterraneum* during seed formation, and Massey and Loeffel (1967) also demonstrated a transfer of zinc from the stalk and leaves of maize at the stage of grain development.

The results also indicated a preferential routing of ^{65}Zn to the youngest tissue, where the demand for zinc was probably the greatest. Thus, the majority of the ^{65}Zn within the old tissue had been deposited for a considerable period of time. This time lapse could greatly influence the degree of mobility of zinc within the tissue and its subsequent redistribution to other parts of a plant. In *T. subterraneum* it was shown that some of the ^{65}Zn recently acquired by young leaves was rerouted into yet younger tissue.

The foliar injection of 1000 μg of non-radioactive zinc (an amount sufficient to ensure its movements throughout the plant; Millikan and Hanger 1965*b*) into *T. subterraneum* also failed to induce movement of ^{65}Zn out of any leaves. However, the injections did cause some recirculation of ^{65}Zn within leaves, but apparently only in an acropetal direction. Rinnie and Langston (1960) had also observed a predominance of acropetal movement in zinc recirculation in peppermint.

It is postulated that the recirculated zinc came from the conducting strands, and that movement was by a cation exchange process, similar to that demonstrated by Hewitt and Gardner (1956) in grapevines. The evidence obtained previously by Millikan and Hanger (1967*a*) on the recirculation of ^{65}Zn in young pear trees following bark injection also supports the work by Hewitt and Gardner (1956).

The failure to induce any appreciable redistribution of ^{65}Zn from tissues following its initial transport and deposition, suggest that the element, once it enters the cell, is either tightly bound to the cellular constituents or, if still in the ionic form, is incapable of cellular release. Wood and Sibly (1950) concluded that zinc binding was upon the colloidal constituents of the cells. Tanford (1952) and Johnson and Schrenk (1964) have both reported the presence of zinc–protein complexes, and Vennesland (1960) has stated that several pyridine nucleotide dehydrogenases have been shown to contain zinc tightly bound in the molecule. The occurrence of free zinc ions in plant tissue is supported by the work of Johnson and Schrenk (1964). They found that a considerable amount of the zinc in macerated lucerne leaves and stems was dialysable.

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