EFFECTS OF CHELATION AND OF VARIOUS CATIONS ON THE MOBILITY OF FOLIAR-APPLIED ^{65}Zn IN SUBTERRANEAN CLOVER

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Summary

Injection of ^{65}Zn of high specific activity into old leaves of subterranean clover (*Trifolium subterraneum* L.) resulted in the virtual immobilization of the isotope in the laminae of the treated leaves. The addition of 30, 300, or 500 μg respectively of non-radioactive zinc to the dose resulted in progressively increased movement of ^{65}Zn into other parts of the plant.

Movement of ^{65}Zn from the injected leaf was also enhanced by the addition to the dose of either EDTA or one of various cations in amounts equivalent to 500 μg Zn. This enhancement was comparable with that induced by 500 μg Zn with EDTA, Cu^{2+}, Mn^{2+}, and Fe^{2+}; intermediate with Mg^{2+} and Ca^{2+}; and least with Na^{+} and K^{+}.

It is concluded that non-selective negatively charged sites of fixation occur in the leaf of subterranean clover.

I. INTRODUCTION

The use of zinc sprays applied to the aerial parts of plants has been recognized for approximately thirty years as an effective method of controlling zinc deficiency in plants (McWhorter 1936; Parker 1938).

However, from recent work in which ^{65}Zn was applied to leaves, it has been concluded that although the isotope may be absorbed by the leaf, its subsequent movement to other parts of the plant may be negligible or limited in extent (Leyden and Toth 1960; Bukovac and Wittwer 1961; Wittwer 1964).

Wallihan and Heymann-Herschberg (1956) found that the rate of zinc absorption into citrus leaves was directly related to the concentration of the applied zinc solution. Lingle (1960) reported that after spraying tomato plants with various zinc salts tagged with ^{65}Zn, he found the isotope localized in young tissue at the top of the plants. Also Sudia and Linck (1963), using large doses of ^{65}Zn (100–150 μc per leaf), demonstrated movement of the isotope from the treated leaf.

The possibility that the extent of the movement of ^{65}Zn from leaves was governed by the total amount of zinc in the applied dose was indicated by two unpublished experiments by the present authors in which subterranean clover and two different consignments of ^{65}Zn with widely different specific activities were used. Injection of the dose with a low specific activity caused marked movement of ^{65}Zn throughout the plants, whereas use of the high specific activity dose resulted in immobilization of the ^{65}Zn in the injected lamina.

The experiments described below were therefore done to determine whether ^{65}Zn of high specific activity could be made to move from an injected leaf either by neutralizing its charge by a chelating agent or by adding sufficient non-radioactive

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zinc to saturate exchange sites in the leaf. The effectiveness of other mono- and divalent cations added to the dose in facilitating the movement of the ^{65}Zn out of the injected leaf was also studied.

II. Method

Two experiments were conducted in which subterranean clover (*Trifolium subterraneum* L.) plants were grown in non-radioactive complete nutrient solution, the composition of which was identical to that described previously (Millikan 1961).

Table 1

Experiment 1: results of radioassays for ^{65}Zn

Results expressed as counts/min/mg dry matter on selected tissues from injected plants

Tissue	Injection Treatment			
	No Additive	Zn, 30 μg	Zn, 300 μg	EDTA, 0·01M
Injected leaf				
Petiole distal	6800	18600	10600	1150
Petiole centre	310	2200	—	160
Petiole proximal	25	260	10350	70
Old leaf				
Lamina	0	4	—	—
Edge	—	—	25	10650
Centre	—	—	80	1900
Petiole distal	0	0	570	160
Petiole proximal	—	—	2950	110
Young leaf				
Lamina	0	1	140	3450
Petiole	0	0	330	820
Hypocotyl	0	0	420	50
Roots	0	0	5	70

The plants were allowed to grow until they had at least seven trifoliate leaves, when the various radioactive doses as $^{65}ZnSO_4.7H_2O$ detailed below were injected into old leaves by the method described by Millikan and Hanger (1965).

In all cases the doses were taken up within 12 hr and the plants were harvested for radioautography approximately 24 hr after the commencement of the injections.

Each plant was then radioautographed by the method described by Millikan and Hanger (1964). Before this was done, the remains of each injected lamina was removed to prevent possible extensive fogging of the X-ray film in the vicinity of such highly reactive tissue.

After radioautography, selected tissues were dried, weighed to the nearest 0·005 mg, and then radioassayed for ^{65}Zn by means of a scintillation counter fitted with a large, deep-welled sodium iodide crystal and associated scaling equipment. The results were adjusted to allow for the decay of the isotope and were finally expressed as counts per minute per milligram of dry matter. Details of each experiment follow.

(i) *Experiment 1.*—Seedlings of the cultivar Clare were set out in the water cultures on November 5, 1964, and the injections were made on December 9, 1964. Each radioactive dose consisted of 2·5 μc ^{65}Zn (original activity) in 0·025 ml water, and contained a total of 3 μg Zn. The additions to these doses were:

(1) No additive (distilled water, two drops each of 0·08 ml);

(2) EDTA, disodium salt, 0·01M, one drop (0·08 ml);

(3) Zn^{2+}, 30 μg (0·92 μ-equiv.);

(4) Zn^{2+}, 300 μg (9·2 μ-equiv.).

The non-radioactive zinc was applied as a solution of $ZnSO_4.7H_2O$ in 0·1 ml water, and treatments (2)–(4) received in addition one drop (0·08 ml) of distilled water. Each treatment was duplicated.

(ii) *Experiment 2.*—Seedlings of the cultivar Dwalganup were set out in the water cultures on January 14, 1965, and the injections were made on February 12, 1965. The radioactive dose was identical to that used in experiment 1. The additions to the dose were:

(1) No additive (distilled water, two drops each of 0·08 ml);

(2) EDTA, 0·01M (one drop of 0·08 ml);

(3) Zn^{2+}, 300 μg (9·2 μ-equiv.);

(4) Zn^{2+}, 500 μg (15·3 μ-equiv.);

(5) Cu^{2+}, 486 μg (15·3 μ-equiv.);

(6) Mn^{2+}, 420 μg (15·3 μ-equiv.);

(7) Fe^{2+}, 427 μg (15·3 μ-equiv.);

(8) Mg^{2+}, 186 μg (15·3 μ-equiv.);

(9) Ca^{2+}, 307 μg (15·3 μ-equiv.);

(10) Na^{+}, 176 μg (7·65 μ-equiv.);

(11) K^{+}, 299 μg (7·65 μ-equiv.).

In treatments (5)–(11) the number of cations in each dose was constant and equivalent to the number present in the 500 μg Zn treatment.

With the exception of calcium each cation was applied as the sulphate. Calcium was added as $CaCl_2$. The volume of each cation solution added to the dose was 0·1 ml and treatments (2)–(11) received, in addition, one drop (0·08 ml) of distilled water. Each treatment was duplicated.

III. Results

(i) *Experiment 1.*—Because the duplicated radioautographs of ^{65}Zn distribution in plants from each treatment were in close agreement, only one radioautograph per treatment is presented in Plate 1, Figures 1–4. However, selected plant tissues from the other duplicate were radioassayed, and these radioassays are presented in Table 1.

Where the dose contained no additive, the ^{65}Zn was virtually immobilized in the lamina and distal portion of the petiole of the injected leaf (Plate 1, Fig. 1; Table 1). The addition of 30 μg Zn to the dose caused a limited movement of ^{65}Zn down the petiole of the injected leaf but only trace amounts of ^{65}Zn were detected in

other leaves of the plant (Plate 1, Fig. 3). With 300 μg Zn added to the dose, there was very marked movement of ^{65}Zn out of the injected leaf into the other leaves of the plant (Plate 1, Fig. 4). However, in each case, the petiole contained a much higher concentration of ^{65}Zn than the lamina. There was only limited movement of the isotope into the hypocotyl and roots (Table 1).

Greatest translocation of ^{65}Zn resulted from the addition of EDTA to the dose (Plate 1, Fig. 2). All the leaves of the plant contained appreciable amounts of ^{65}Zn which was higher in concentration in the edge of the lamina than its centre, while the petiole was much lower in ^{65}Zn concentration than its associated lamina. The root tissues had a much lower concentration of ^{65}Zn than the aerial parts.

(ii) *Experiment 2.*—The radioautographs and radioassays for ^{65}Zn of both plants receiving the no-additive treatment, and of one replicate of each of the other injection treatments, are presented in Plates 1 and 2. In the case of each treatment the radioautograph obtained from the duplicate was in full agreement with that presented herein.

With no additive in the dose there was very little movement of ^{65}Zn out of the injected lamina into its petiole and only trace amounts of ^{65}Zn moved into the rest of the plants (Plate 1, Fig. 5; Plate 2, Fig. 3).

The radioautographs and radioassays of ^{65}Zn obtained from the plants injected with EDTA and 300 μg Zn respectively fully confirmed the results obtained with these treatments in experiment 1 [Plate 1, Figs. 2 (cf. Fig. 6) and 4 (cf. Fig. 7)]. Where 500 μg Zn was added to the dose, there was evidence of more movement of ^{65}Zn into the lamina of the leaves and the hypocotyl than resulted from the 300 μg Zn treatment. However, some of the petioles of the plants receiving the 500 μg Zn treatment were still higher in ^{65}Zn concentration than their associated laminae (Plate 1, Fig. 8).

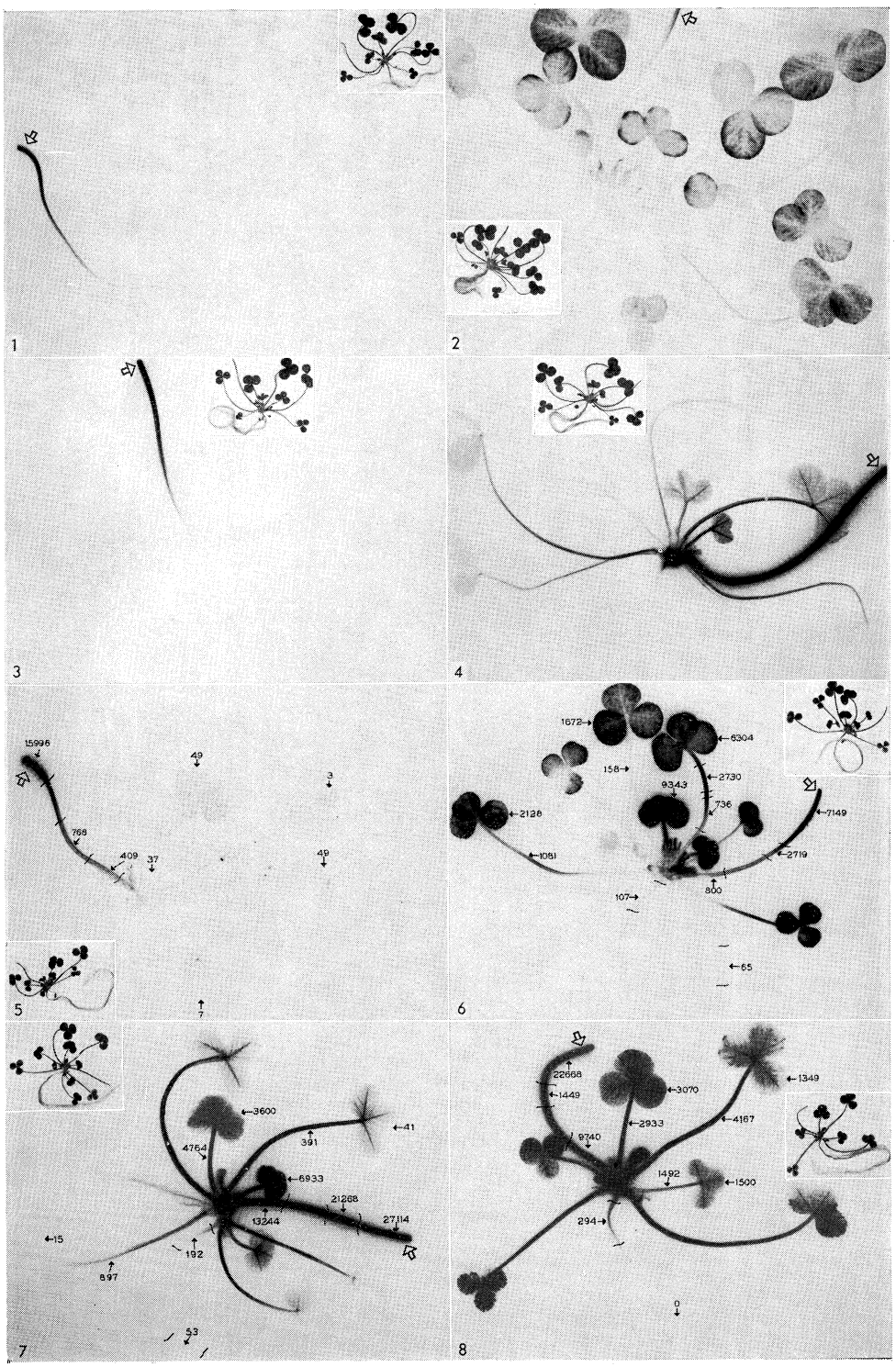
When compared with the result of the no-additive treatment, each cation addition other than zinc to the dose enhanced the movement of ^{65}Zn down the petiole of the injected leaf and into other leaves of the plant (Plates 1 and 2). However, in this regard, the cations Cu^{2+}, Fe^{2+}, and Mn^{2+} were more effective than was either Mg^{2+} or Ca^{2+}, but the effects of all these divalent cations were superior to that of either of the monovalent cations Na^{+} or K^{+}.

In some instances the radioassays showed the presence of ^{65}Zn in tissues which did not record on the radioautograph of the plant concerned. This was due to the relatively short exposure time of 3 days for these radioautographs.

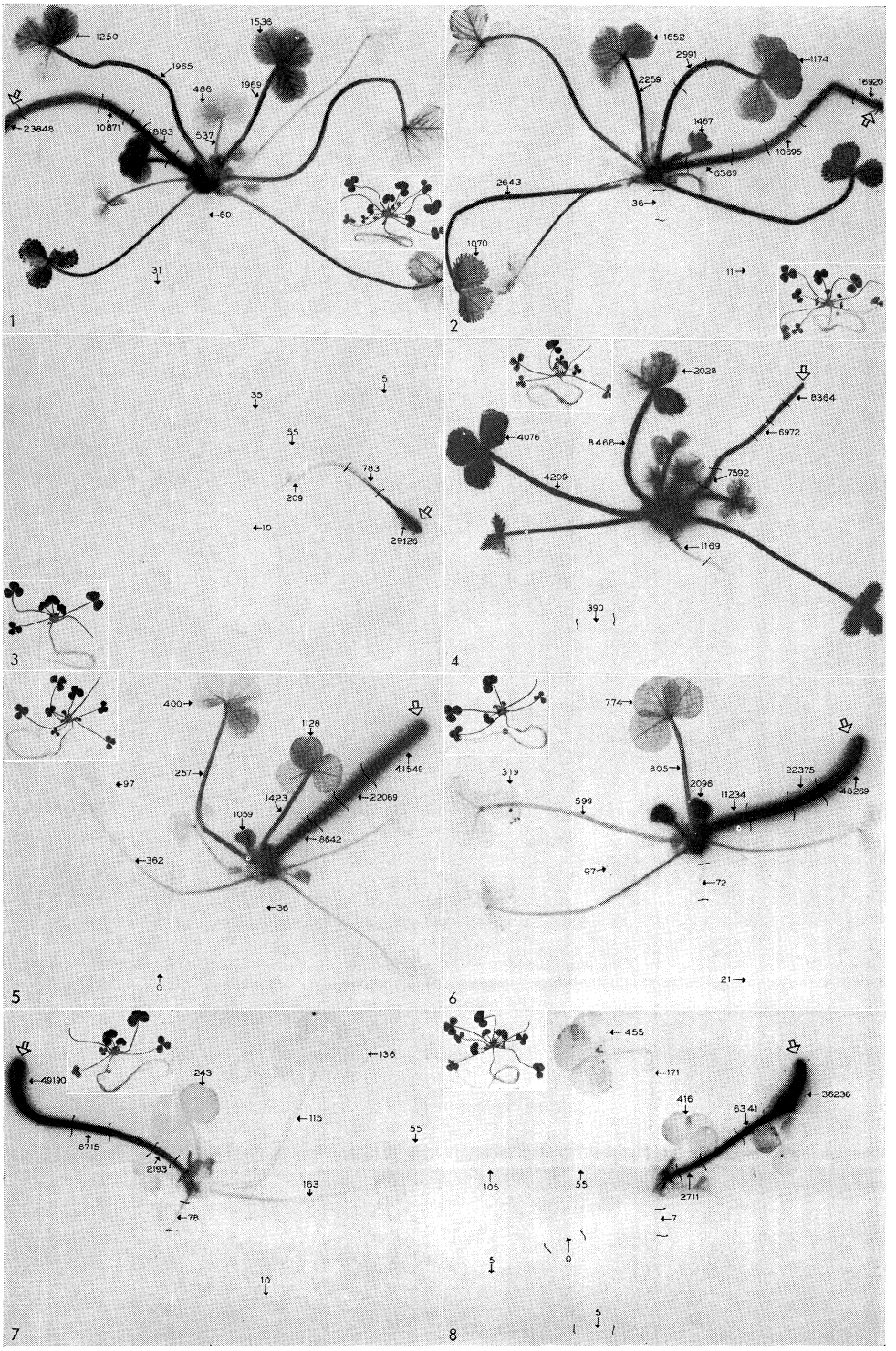
IV. Discussion

From the results of the above experiments it is concluded that non-selective negatively charged sites of fixation of ^{65}Zn occur in leaves of subterranean clover. The movement of ^{65}Zn was accelerated by competition for these sites by the divalent cations Zn^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+}, Mg^{2+}, and Ca^{2+}, and to a lesser degree by the monovalent cations Na^{+} and K^{+}. Thus it follows that although all of these cations were able to compete with the ^{65}Zn for the exchange sites in the leaf and also in the vessels of the petiole, it was probably due to their relative ability to saturate the charge upon these sites which finally influenced the degree of ^{65}Zn mobility.

MOBILITY OF FOLIAR-APPLIED ^{65}Zn



MOBILITY OF FOLIAR-APPLIED ^{65}Zn



The most rapid movement of the ^{65}Zn into the laminae of non-injected leaves resulted from the reduction of its charge by the chelating agent EDTA, thus allowing it to by-pass the exchange sites in the vessels.

Hewitt and Gardner (1956) have also shown that the movement of radioactive zinc in grapevine canes was by a process of cation exchange, whereby the adsorbed ^{65}Zn was displaced from the exchange sites by non-radioactive zinc or by hydrogen ions and thereby made to move along the vessels.

It is concluded from the above experiments that the specific activity of the ^{65}Zn applied to foliage or otherwise injected into plants must be recognized as a factor of prime importance in interpreting the resultant degree of mobility of the isotope obtained.

V. Acknowledgments

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VI. References

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Explanation of Plates 1 and 2

Plate 1

Radioautographs (experiments 1 and 2) and radioassays (experiment 2 only and expressed in counts/min/mg dry matter) of subterranean clover plants, grown in complete nutrient solutions into which doses of radioactive zinc had been injected into the leaves indicated by large arrowheads. In experiment 1, the plants were harvested 22 hr after treatment and exposed for 3 days, while in experiment 2 they were harvested after 24 hr and exposed 3 days. The non-radioactive additive to each dose is as follows:

Fig. 1.—No additive
Fig. 2.—EDTA, 0·01M
Fig. 3.—30 μg Zn^{2+}
Fig. 4.—300 μg Zn^{2+}
} Experiment 1.

Fig. 5.—No additive
Fig. 6.—EDTA, 0·01M
Fig. 7.—300 μg Zn^{2+}
Fig. 8.—500 μg Zn^{2+}
} Experiment 2.

Plate 2

Experiment 2: radioautographs and radioassays (expressed in counts/min/mg dry matter) of subterranean clover plants, grown in complete nutrient solution, into which doses of radioactive zinc had been injected into the leaves indicated by large arrowheads. The plants were harvested 24 hr after treatment and exposed for 3 days. The non-radioactive additions to each dose were:

Fig. 1.—420 μg Mn^{2+}. Fig. 3.—No additive. Fig. 5.—186 μg Mg^{2+}. Fig. 7.—176 μg Na^{+}.
Fig. 2.—427 μg Fe^{2+}. Fig. 4.—486 μg Cu^{2+}. Fig. 6.—307 μg Ca^{2+}. Fig. 8.—299 μg K^{+}.