# EFFECT OF PHOSPHORUS AND ZINC LEVELS IN THE SUBSTRATE ON $^{65}$Zn DISTRIBUTION IN SUBTERRANEAN CLOVER AND FLAX

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*Summary*

Subterranean clover (*Trifolium subterraneum* L.) and flax (*Linum usitatissimum* L.) were grown in water cultures containing $^{65}$Zn and all combinations of deficient or normal zinc supply and low or excess phosphate levels. $^{65}$Zn distribution in the plants was determined by autoradiographs and radioassays.

Irrespective of phosphorus or zinc level in the substrate the overall trend was a fall in $^{65}$Zn concentration with time in all aerial tissues of both species. However, the variation in $^{65}$Zn concentration with zinc-deficient plants showed considerable relative differences from that within plants with a normal zinc supply, both between comparable tissues, and with time. In zinc-deficient subterranean clover a greater percentage than normal of the total zinc in the plant was located in the roots. With flax the $^{65}$Zn concentrations in leaves and stems varied independently with time. In this species $^{65}$Zn accumulated in the nodes and as "islands" of high concentration in the oldest leaves.

Within zinc-deficient plants of both species, $^{65}$Zn concentrations were highest in tissues actually involved in zinc-deficiency symptoms, i.e. "little" leaves of subterranean clover, or the apical tissues of flax affected with "dieback". There was considerable retranslocation of $^{65}$Zn from the oldest leaves of zinc-deficient subterranean clover.

Increase in phosphate supply caused increased $^{65}$Zn concentrations in the oldest leaves of subterranean clover at deficient zinc levels, and also had differential effects on the relative distribution of $^{65}$Zn within lamina and petiole depending on the level of zinc supply. Plants showing little leaf symptoms had P/Zn ratios greater than 400 in their tops. Difference in phosphate supply had no significant effect on $^{65}$Zn distribution in flax.

## I. Introduction

The results of earlier work on effects of phosphorus–zinc interactions in plant nutrition, which were reviewed by Millikan (1963), generally agreed that zinc-deficiency symptoms were aggravated by high phosphate treatment. However, while some workers attributed this aggravation of symptoms to decreased concentrations of zinc in the phosphate-treated plants, others observed no consistent differences in zinc content, but a positive correlation between the P/Zn ratio and the severity of zinc-deficiency symptoms. It was suggested that zinc is essential to phosphate utilization by the plant. These differing views were also expressed in recent literature on the subject.

Increased phosphate concentration in zinc-deficient plants has been reported by Fuehring (1960), Terman, Allen, and Bradford (1966), and Giordano, Mortvedt, and

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Papendick (1966) for maize; Ellis, Davis, and Thurlow (1964) for maize and beans; and Rosell and Ulrich (1964) for sugar beet. Paribok and Kuznetsova (1963) showed for tomatoes and Paribok and Alekseeva-Popova (1965) showed for tomatoes and peas that most of the phosphate accumulated by zinc-deficient plants was in the form of inorganic phosphate, whereas the acid phosphatase and apyrase activity in the leaves, and the amount of phospholipids, nucleotides with energy-rich bonds, and nucleic acids decreased. Kolev (1965) showed that phosphorus utilization in beans was increased by spraying the plants with zinc sulphate.

Zinc deficiency induced by phosphate applications has been reported by Boawn and Leggett (1963) for potatoes, and Alexander and Woodham (1964) for vines. Martin, McLean, and Quick (1965) showed that high phosphate concentrations induced zinc-deficiency symptoms in tomatoes grown at 50–60°F, but not in plants grown at 70–80°F, even though the zinc and phosphorus concentrations were similar in the plants of the two series. Ellis, Davis, and Thurlow (1964) found that a decrease in soil temperature from 75 to 55°F not only decreased the yield of corn, but also the zinc concentration in the plants and total zinc uptake by the plants.

Langin *et al.* (1962), Ward *et al.* (1963), and Stukenholtz *et al.* (1966) considered that the effect of high phosphate was physiological in that it depressed the uptake of zinc and its translocation from roots to tops of corn and sorghum. The latter found no critical P/Zn ratio in the tissues.

On the other hand, Boawn and Leggett (1964) observed for potatoes and Watanabe, Lindsay, and Olsen (1965) observed for corn and beans that the occurrence of zinc-deficiency symptoms was correlated with the P/Zn ratio, and not with the zinc concentration in the plants.

As far as the present authors are aware, there has been no report in the literature of any experiment in which zinc-deficiency symptoms have been produced in plants grown in a substrate containing $^{65}$Zn. The experiment described below was, therefore, designed to obtain information on the effects of all combinations of low and high levels of phosphate and deficient and normal levels of zinc in the nutrient solutions, by studying the distribution of $^{65}$Zn in subterranean clover (*Trifolium subterraneum* L.). Because flax (*Linum usitatissimum* L.) has a different habit of growth, and shows a distinctly different type of zinc-deficiency symptom to that exhibited by subterranean clover, namely "dieback" of the growing point (Millikan 1942), a limited experiment was also conducted with this species.

## II. Method

### (*a*) *General Procedure*

The water-culture method was adopted for these experiments. The general procedures regarding the composition of the nutrient solutions and the production of seedlings were as described by Millikan (1963), except as follows:

(1) The 2-litre pots used for the nutrient solutions were lined with disposable plastic bags to prevent their contamination with radioactive zinc.

(2) The contamination of the molar stock solutions of potassium and calcium nitrates and magnesium sulphate with heavy metal ions was reduced by co-precipitation with magnesium hydroxide as described by Munns and Johnson (1960), but sodium dihydrogen phosphate was eluted several times with a solution of dithizone in carbon

tetrachloride (Millikan 1963). These methods evidently reduced zinc contamination in the solutions to a satisfactory level, as their use resulted in the development of zinc-deficiency symptoms in plants, even though zinc was added to each pot in the radioactive dose.

### (*b*) *Plant Cultivars Used*

The subterranean clover cultivar Clare was used throughout as previous work (Millikan 1953) had shown that high phosphate levels affected it more than cv. Edenhope. Hazeldeane was the flax cultivar selected.

### (*c*) *Zinc and Phosphorus Treatment of Plants*

For the subterranean clover experiments all combinations of zinc treatments (supplied as $ZnSO_4.7H_2O$) at levels of 0, 0·005, and 0·05 p.p.m. (referred to as Zn1, Zn2, and Zn3 levels respectively) and of phosphorus treatments (supplied as $Na_2HPO_4$) at levels of 3, 30, and 60 p.p.m. (referred to as P1, P2, and P3 levels respectively) were included. In the flax experiment, zinc treatments were similar but the P2 treatment was omitted.

### (*d*) *Cultivation of Plants*

Each pot received a dose of 5 $\mu$Ci $^{65}Zn$ (as $^{65}ZnSO_4.7H_2O$) containing 2·275 $\mu$g Zn on day 0. However, because they contained different amounts of total zinc, the specific activity of each zinc treatment decreased proportionately from the Zn1 to the Zn3 level, viz. 2·2, 0·4, and 0·05 $\mu$Ci/$\mu$g Zn respectively. The specific activity of the $^{65}Zn$ used was sufficiently high to ensure that at the Zn1 and Zn2 levels radioactive plants showing zinc-deficiency symptoms could be produced.

For each species, two pots of each zinc–phosphorus combination were provided and six plants of the appropriate species were established per pot. A full set of nutrients was also added to each pot on day 0, and, with the exception of zinc and phosphorus, again on day 43.

### (*e*) *Harvesting and Radioassay of Plant Samples*

All plant samples were first autoradiographed before being subsampled for radioassay by the methods described by Millikan and Hanger (1964, 1965).

(i) *Subterranean Clover*

Two plants per treatment (one from each duplicate pot) were harvested on each of days 35, 44, and 51 (harvests 1–3 respectively) and five (Zn1 series) or six (Zn2 and Zn3 series) plants on day 63 (harvest 4). The whole of each plant was subsampled for radioassay purposes, thus enabling the total dry weight and $^{65}Zn$ concentration of each plant to be calculated.

Each leaf of the plants obtained on days 35, 44, and 51 was subsampled into leaf edge (outer third of each leaflet), leaf centre (remainder of lamina), and distal and proximal halves of the petiole. Because of the size of the 63-day plants, each leaf was subsampled into lamina and petiole only.

The total number of subterranean clover subsamples radioassayed was 3570. The $^{65}Zn$ radioassays were made with a scintillation counter fitted with a deep-welled sodium iodide crystal. All the day-63 subsamples of the tops of the plants were retained after radioassay, and a composite sample for each treatment was obtained. The total zinc and phosphorus concentrations in each composite sample were then determined by the Victorian State Government Laboratories, Melbourne.

(ii) *Flax*

Two plants per treatment (one from each duplicate pot) were harvested on each of days 38, 45, and 52, and four or six plants on day 60. Subsamples for radioassay of $^{65}Zn$ consisted of the top rosette including the growing point, and leaves and stem sections from the upper, middle, and basal regions of the main stems. The total number of flax subsamples radioassayed was 450.

### (*f*) *Statistical Analyses*

In the tables which follow, the values presented are mean values for all factors, including those not explicitly mentioned, which implies that there are no interactions between any of these factors whatsoever.

## III. Results

### (a) *Subterranean Clover*

#### (i) *Development of Zinc-deficiency Symptoms*

Up to the time of the first harvesting on day 35 no zinc-deficiency symptoms were apparent in plants of any of the Zn1 or Zn2 cultures. However, by day 40, the bronze coloration at the bases of the laminae of the middle leaves was apparent in the Zn1 P3 plants. Marginal necrosis of the oldest leaves (as described by Millikan 1963 for the zinc-deficient cultivar Clare) was also apparent in those plants. Only bronzing occurred in the Zn1 P2 plants.

On day 50, at both the Zn1 and Zn2 levels, the P1 plants were darker green in color than the corresponding P2 or P3 plants, and their old leaves remained free of marginal necrosis which had become severe at the P2 and P3 levels. The typical "goblet" shape symptoms described by Riceman and Jones (1956) and Millikan (1963) occurred in the middle leaves, and first signs of chlorosis and "little" leaf (i.e. leaves showing typical zinc-deficiency symptoms) formation in the youngest leaves were apparent at the two higher phosphorus levels of the Zn1 and, to a lesser extent, Zn2 series.

By the time of the final sampling on day 63, little leaf formation was apparent in all Zn1 and, to a lesser degree, Zn2 cultures, but at each zinc level was least marked at the P1 level. Also, the oldest leaves of the Zn1 P1 and Zn2 P1 plants showed no signs of necrosis, whereas in the plants grown at the P2 or P3 levels in either the Zn1 or Zn2 series (the former in particular) death of many of these leaves had occurred. These dead leaves are shown in the autoradiographs of the Zn1 P3 and Zn2 P3 plants in Figures 4 and 6. They are identifiable by their withered petioles.

Death of the unifoliate leaf and the oldest trifoliate leaf of the Zn3 P3 plants also occurred on day 63 (Fig. 8). However, no plants in the Zn3 series showed any of the characteristic zinc-deficiency symptoms described above.

#### (ii) *Radioassays and Statistical Analyses*

The mean concentrations (expressed as $\log_{10}$ counts/min/mg dry matter) of $^{65}Zn$ in the lamina plus petiole of the oldest leaves up to leaf 5 produced by the plants to day 63 are presented in Table 1, and the retransformed values of the concentrations and of absolute amounts of $^{65}Zn$ (as counts/min) in individual leaves in Figures 1 and 2. Leaves younger than leaf 5 were not included in this analysis as this was the maximum number of leaves common to harvests 2–4. At harvest 1 there was a maximum of only three leaves per plant. A separate statistical analysis was made on the results of the four harvests for leaves to number three, but this is not presented in detail in this paper. However, the results of the two analyses were combined to follow changes between harvests in the absolute amounts of $^{65}Zn$ in each old leaf to leaf 5 (Table 2). It was found that the required differences for significance of the two analyses were almost identical. In each instance the higher value has been used in Table 2. Autoradiographs of plants from harvest 4 are presented in Figures 3–8.

At each zinc level, decreases in the $^{65}Zn$ concentration occurred between harvests 2 and 4 (Table 1). With few exceptions they were general for each leaf and

TABLE 1

CONCENTRATION OF $^{65}Zn$ IN INDIVIDUAL LEAVES OF SUBTERRANEAN CLOVER PLANTS

Plants were grown at different zinc (Zn1, Zn2, Zn3) and phosphorus (P1, P2, P3) levels and harvested on days 44 (H2), 51 (H3), and 63 (H4). Concentrations are expressed as $\log_{10}$ counts/min/mg dry matter. $D_H$, $D_P$, and $D_L$ refer to a difference of means of any two harvests, phosphorus levels, and leaf types respectively. $D_D$ refers to a difference of any two such means

| | Zn1 | | | | Zn2 | | | | Zn3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H2 | H3 | H4 | (H4−H2) | H2 | H3 | H4 | (H4−H2) | H2 | H3 | H4 | (H4−H2) |
| P1 | 2·49 | 2·33 | 2·03 | −0·46 | 2·63 | 2·36 | 1·92 | −0·71 | 2·36 | 2·30 | 2·19 | −0·17 |
| P2 | 2·36 | 2·30 | 1·99 | −0·37 | 2·63 | 2·26 | 2·13 | −0·50 | 2·31 | 2·27 | 2·13 | −0·18 |
| P3 | 2·47 | 2·35 | 2·31 | −0·16 | 2·67 | 2·40 | 2·13 | −0·54 | 2·33 | 2·19 | 2·14 | −0·19 |
| L.S.D. ($P = 0·05$) | $D_H = 0·07$; $D_P = 0·19$; $D_D = 0·10$ | | | | $D_H = 0·10$; $D_P = 0·41$; $D_D = 0·14$ | | | | $D_H = 0·07$; $D_P = 0·14$; $D_D = 0·09$ | | | |
| L.S.D. ($P = 0·01$) | $D_H = 0·09$; $D_P = 0·40$; $D_D = 0·13$ | | | | $D_H = 0·14$; $D_P = 0·87$; $D_D = 0·19$ | | | | $D_H = 0·09$; $D_P = 0·28$; $D_D = 0·12$ | | | |
| Cotyledon | 1·88 | 1·88 | 1·54 | −0·34 | 2·37 | 2·04 | 1·67 | −0·70 | 2·04 | 1·97 | 1·92 | −0·12 |
| Unifoliate leaf | 2·39 | 2·37 | 2·24 | −0·15 | 2·65 | 2·35 | 2·28 | −0·37 | 2·45 | 2·37 | 2·42 | −0·03 |
| Leaf 1 | 2·50 | 2·43 | 2·25 | −0·25 | 2·69 | 2·48 | 2·23 | −0·46 | 2·46 | 2·38 | 2·35 | −0·11 |
| Leaf 2 | 2·55 | 2·45 | 2·28 | −0·27 | 2·65 | 2·39 | 2·15 | −0·50 | 2·42 | 2·34 | 2·23 | −0·19 |
| Leaf 3 | 2·49 | 2·36 | 2·21 | −0·28 | 2·64 | 2·36 | 2·09 | −0·55 | 2·37 | 2·29 | 2·14 | −0·23 |
| Leaf 4 | 2·58 | 2·36 | 2·13 | −0·45 | 2·71 | 2·36 | 2·03 | −0·68 | 2·35 | 2·24 | 2·05 | −0·30 |
| Leaf 5 | 2·68 | 2·45 | 2·11 | −0·57 | 2·81 | 2·38 | 1·95 | −0·86 | 2·25 | 2·20 | 1·97 | −0·28 |
| L.S.D. ($P = 0·05$) | $D_H$, $D_L = 0·11$; | | $D_D = 0·15$ | | $D_H$, $D_L = 0·16$; | | $D_D = 0·22$ | | $D_H$, $D_L = 0·10$; | | $D_D = 0·14$ | |
| L.S.D. ($P = 0·01$) | $D_H$, $D_L = 0·14$; | | $D_D = 0·20$ | | $D_H$, $D_L = 0·21$; | | $D_D = 0·29$ | | $D_H$, $D_L = 0·13$; | | $D_D = 0·19$ | |

TABLE 2

ABSOLUTE AMOUNTS OF $^{65}Zn$ IN INDIVIDUAL LEAVES OF SUBTERRANEAN CLOVER PLANTS

Conditions of experiment and explanation of symbols as in Table 1. Amounts of $^{65}Zn$ in leaves expressed as $\log_{10}$ counts/min

| | Zn1 | | | | | Zn2 | | | | | Zn3 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H1* | H2 | H3 | H4 | (H4−H2) | H1* | H2 | H3 | H4 | (H4−H2) | H1* | H2 | H3 | H4 | (H4−H2) |
| P1 | — | 3·45 | 3·32 | 3·23 | −0·22 | — | 3·54 | 3·49 | 3·15 | −0·39 | — | 3·18 | 3·36 | 3·37 | +0·19 |
| P2 | — | 3·27 | 3·25 | 3·08 | −0·19 | — | 3·57 | 3·27 | 3·26 | −0·31 | — | 3·25 | 3·36 | 3·26 | +0·01 |
| P3 | — | 3·36 | 3·22 | 3·37 | +0·01 | — | 3·59 | 3·47 | 3·18 | −0·41 | — | 3·12 | 3·09 | 3·25 | +0·13 |
| L.S.D. ($P = 0·05$) | $D_H = 0·15$; $D_P = 0·31$; $D_D = 0·21$ | | | | | $D_H = 0·14$; $D_P = 0·47$; $D_D = 0·19$ | | | | | $D_H = 0·09$; $D_P = 0·25$; $D_D = 0·13$ | | | | |
| L.S.D. ($P = 0·01$) | $D_H = 0·20$; $D_P = 0·61$; $D_D = 0·28$ | | | | | $D_H = 0·18$; $D_P = 0·98$; $D_D = 0·26$ | | | | | $D_H = 0·12$; $D_P = 0·52$; $D_D = 0·17$ | | | | |
| Cotyledon | 2·84 | 2·77 | 2·64 | 2·33 | −0·44 | 3·31 | 3·18 | 2·87 | 2·33 | −0·85 | 2·88 | 2·77 | 2·68 | 2·58 | −0·19 |
| Unifoliate leaf | 3·11 | 3·10 | 3·01 | 2·97 | −0·13 | 3·57 | 3·36 | 2·96 | 2·93 | −0·43 | 2·95 | 3·16 | 2·98 | 3·07 | −0·09 |
| Leaf 1 | 3·64 | 3·69 | 3·52 | 3·46 | −0·23 | 3·97 | 3·80 | 3·63 | 3·33 | −0·47 | 3·53 | 3·55 | 3·47 | 3·47 | −0·08 |
| Leaf 2 | 3·74 | 3·83 | 3·67 | 3·64 | −0·19 | 3·92 | 3·85 | 3·66 | 3·44 | −0·41 | 3·49 | 3·59 | 3·57 | 3·50 | −0·09 |
| Leaf 3 | — | 3·70 | 3·62 | 3·60 | −0·10 | — | 3·82 | 3·69 | 3·50 | −0·32 | 3·15 | 3·51 | 3·56 | 3·57 | +0·06 |
| Leaf 4 | — | 3·39 | 3·40 | 3·34 | −0·05 | — | 3·70 | 3·61 | 3·46 | −0·24 | — | 3·19 | 3·44 | 3·47 | +0·28 |
| Leaf 5 | — | 3·06 | 3·01 | 3·26 | +0·20 | — | 3·26 | 3·46 | 3·41 | +0·15 | — | 2·53 | 3·14 | 3·37 | +0·84 |
| L.S.D. ($P = 0·05$) | $D_H$, $D_L = 0·23$; | | | $D_D = 0·32$ | | $D_H$, $D_L = 0·21$; | | | $D_D = 0·30$ | | $D_H$, $D_L = 0·14$; | | | $D_D = 0·20$ | |
| L.S.D. ($P = 0·01$) | $D_H$, $D_L = 0·30$; | | | $D_D = 0·43$ | | $D_H$, $D_L = 0·28$; | | | $D_D = 0·39$ | | $D_H$, $D_L = 0·19$; | | | $D_D = 0·26$ | |

* H1 excluded from the statistical analysis: however, the appropriate differences for significance are initially the same as those presented.

phosphate level of the Zn1 and Zn2 series, and for leaves 2–5 of the Zn3 plants. The effect of increase in phosphate level from P1 to P3 on the $^{65}Zn$ concentration depended on the level of zinc supply and time of harvest. Thus at the Zn1 level increase in phosphate level had no effect at harvests 2 and 3, but at harvest 4 resulted in significant increases in $^{65}Zn$ concentration in all tissues. At the Zn2 and Zn3 levels, increase in phosphate level had no effect on $^{65}Zn$ concentration at any harvest.

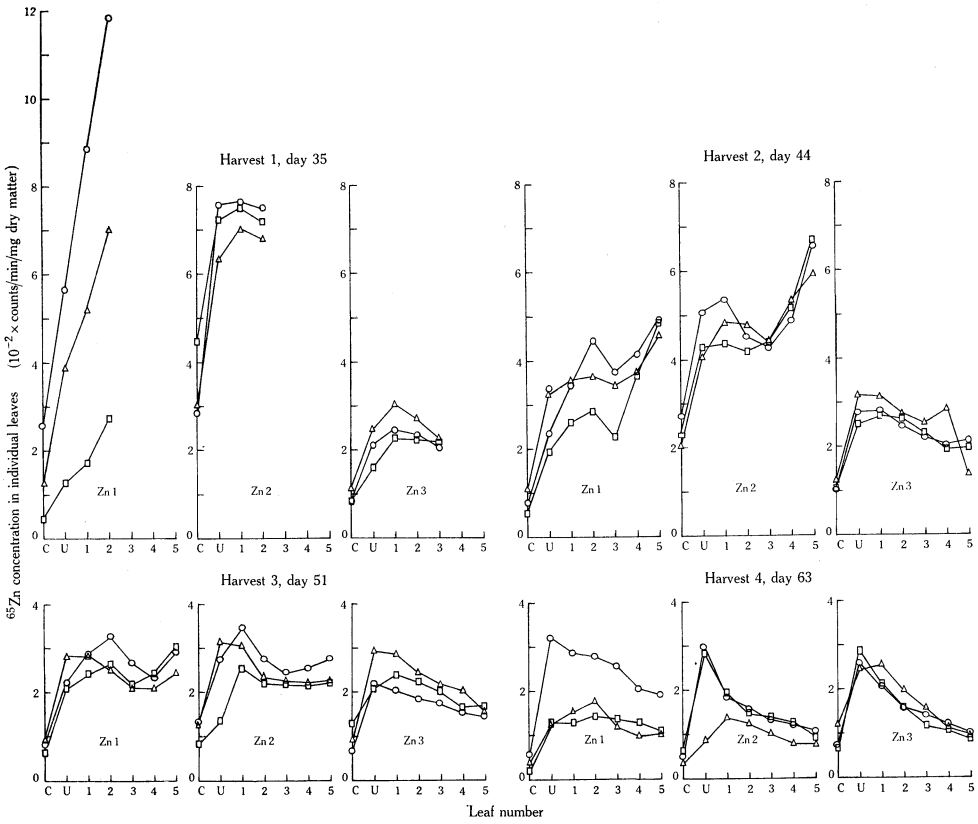



Fig. 1.—$^{65}Zn$ concentration in individual leaves (lamina plus petiole) of subterranean clover grown at different levels of zinc and phosphorus supply. Values are expressed as counts/min/mg dry matter, retransformed from logarithmic means. C, cotyledons; U, unifoliate leaves.

The effect of time of harvest on the total amount of $^{65}Zn$ in the cotyledons and oldest leaves was dependent on the level of zinc supplied to the plants (Table 2). Apart from sampling fluctuations the table shows a steady trend with time at all zinc levels, from an efflux of $^{65}Zn$ from the cotyledons, to an influx into leaf 5. The effect of zinc level was to change the net loss at the Zn1 and Zn2 levels to a net gain at the Zn3 level. The relatively lower efflux of $^{65}Zn$ from the oldest leaves of the Zn1 than the Zn2 plants may be attributable to the earlier death of the oldest leaves of the former.

Where a non-significant change with time in the amount of $^{65}Zn$ is recorded in Table 2 it is not possible to tell whether this is the result of no movement of $^{65}Zn$ or of a

balance of influx and efflux of the isotope. In view of the trend described above, the latter appears feasible.

At each level of zinc supply and time of harvest, increase in phosphate level from P1 to P3 had no effect on the $^{65}Zn$ content of any tissue (Table 2). Thus the significant increase at harvest 4 in $^{65}Zn$ concentration at the Zn1 level resulting from the increase in phosphate level (Table 1) was attributable to a reduction in growth induced by the high phosphate rather than to any movement of $^{65}Zn$ into the leaves.

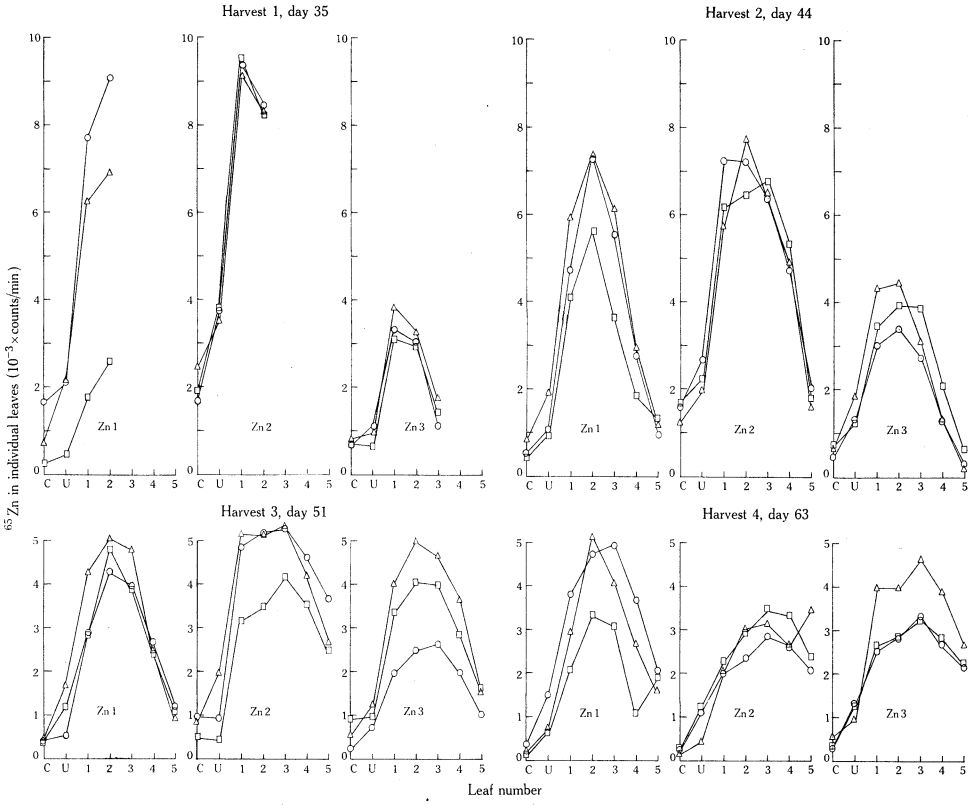



Fig. 2.—Absolute amounts of $^{65}Zn$ in individual leaves (lamina plus petiole) of subterranean clover grown at different levels of zinc and phosphorus supply. Values are expressed as counts/min, retransformed from logarithmic means. C, cotyledons; U, unifoliate leaves.

An analysis was also made of the effects of treatments on the relative concentrations of $^{65}Zn$ in the distal and proximal halves of the petioles (PD/PP ratio), and between the leaf edge and leaf centre (LE/LC ratio) of trifoliate leaves 1–5 for harvests 1–3. The results are presented in Table 3.

It was evident that there were appreciable differences in the relative distribution of $^{65}Zn$ within petiole and lamina between zinc-deficient (Zn1) and normal (Zn3) plants. Between harvests 1 and either 2 or 3 there were falls in the PD/PP ratio of $^{65}Zn$ concentrations at the Zn1 level (except at the highest phosphorus level) but

TABLE 3

RATIOS OF $^{65}Zn$ CONCENTRATION BETWEEN THE DISTAL AND PROXIMAL HALVES OF PETIOLES (PD/PP) AND LEAF EDGE AND LEAF CENTRE (LE/LC) OF TRIFOLIATE LEAVES 1–5 OF SUBTERRANEAN CLOVER

Plants were harvested on days 35 (H1), 44 (H2), and 51 (H3). Other experimental conditions and explanation of symbols as in Table 1, except that $D_{Zn}$ refers to a difference of means of any two zinc levels

| | Zn1 | | | | Zn2 | | | | Zn3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H1 | H2 | H3 | (H3−H1) | H1 | H2 | H3 | (H3−H1) | H1 | H2 | H3 | (H3−H1) |
| PD/PP ratio* | | | | | | | | | | | | |
| P1 | 1·15 | 1·02 | 1·12 | −0·03 | 0·91 | 0·90 | 1·06 | +0·15 | 0·83 | 0·88 | 0·90 | +0·07 |
| P2 | 1·32 | 0·94 | 1·02 | −0·30 | 0·92 | 0·98 | 1·04 | +0·12 | 0·70 | 0·92 | 1·02 | +0·32 |
| P3 | 1·00 | 0·90 | 1·06 | +0·06 | 0·87 | 0·90 | 1·13 | +0·26 | 0·86 | 0·85 | 0·94 | +0·08 |
| LE/LC ratio† | | | | | | | | | | | | |
| P1 | 0·92 | 1·04 | 1·06 | +0·14 | 1·00 | 1·02 | 1·18 | +0·18 | 0·94 | 1·00 | 1·38 | +0·44 |
| P2 | 0·94 | 1·18 | 1·03 | +0·09 | 1·00 | 1·06 | 1·04 | +0·04 | 0·93 | 0·95 | 1·16 | +0·23 |
| P3 | 0·88 | 0·94 | 1·05 | +0·17 | 0·91 | 1·10 | 1·10 | +0·19 | 0·89 | 0·77 | 1·16 | +0·27 |

* Least significant differences at the 5% level for $D_H$, $D_P$, and $D_{Zn} = 0·17$, and for $D_D = 0·24$. Corresponding values at the 1% level are 0·23 and 0·32 respectively.

† Least significant differences at the 5% level for $D_H$, $D_P$, and $D_{Zn} = 0·22$ and for $D_D = 0·32$. Corresponding values at the 1% level are 0·30 and 0·43 respectively.

increases at the Zn2 and Zn3 levels. The difference between these two effects was highly significant. In the Zn1 plants the PD/PP ratio was greater than that of the Zn3 plants. This difference occurred irrespective of phosphate level and was apparent at harvests 1 and 3 with a similar trend at harvest 2. However, within the Zn1 level only, increase in phosphate level from P1 to P3 reduced the PD/PP ratio.

Between harvests 1 and 3 there were increases in the LE/LC ratio irrespective of zinc level. There was a significant harvest × zinc level interaction. Thus, at harvest 2 there was a higher LE/LC ratio in the Zn1 than in the Zn3 plants, but by harvest 3 the reverse was true. Increase in phosphate level had no effect on the LE/LC ratio of the Zn1 and Zn2 plants, but reduced the ratio in the Zn3 plants. This latter effect was obviously further strengthened by harvest 4, as is shown by the autoradiographs of the plants presented in Figures 3–8. A comparison of these autoradiographs shows that at the Zn3 but not at the Zn1 or Zn2 levels, a marked marginal accumulation of $^{65}Zn$ occurred in the older leaves of the P1 but not of the P3 plants.

TABLE 4

RELATION OF $^{65}Zn$ CONCENTRATION TO LEAF SIZE IN SUBTERRANEAN CLOVER

Plants grown at three levels of zinc supply. Concentrations expressed as $\log_{10}$ counts/min/mg dry matter, and re-transformed values (counts/min/mg dry matter) shown in parenthesis

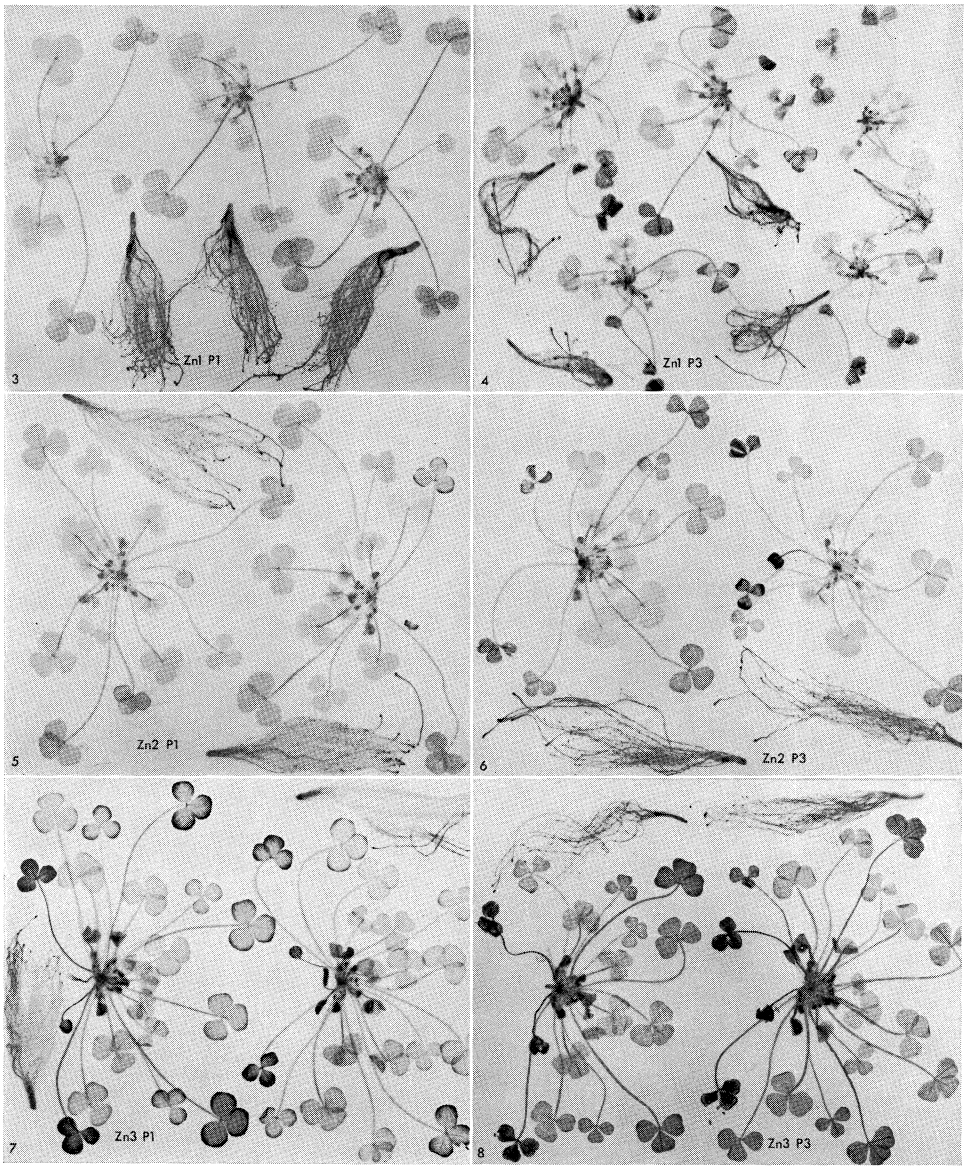
| Leaf Size* (mm) | Zn1 | Zn2 | Zn3 |
|---|---|---|---|
| < 15 | 2·40 (250) | 2·08 (120) | 2·05 (112) |
| 15–30 | 2·21 (161) | 2·00 (98) | 2·00 (99) |
| 31–45 | 2·12 (133) | 1·91 (81) | 2·01 (103) |
| > 45 | 2·22 (165) | 2·06 (114) | 2·08 (119) |
| Least significant differences | | | |
| $P = 0·05$ | 0·12 | 0·03 | 0·05 |
| $P = 0·01$ | 0·17 | 0·05 | 0·07 |

* Length of lamina plus petiole.

The relationship between zinc concentration and the occurrence of "little" leaves was examined. For this purpose each leaf of each plant in harvest 4 was grouped according to the length of its lamina plus petiole into one of the following classes: < 15 mm, 15–30 mm, 31–45 mm, and > 45 mm. The mean $^{65}Zn$ concentration of the leaves in each class was then calculated, the results being presented in Table 4.

The leaves in the < 15-mm category from the Zn1 and Zn2 plants were little leaves typical of zinc deficiency (Millikan 1953), while those from the Zn3 plants were merely immature normal leaves. It is characteristic of these little leaves that they do not enlarge, and are the last to die under conditions of acute zinc deficiency. The $^{65}Zn$ concentration of the little leaves of the Zn1 plants was very significantly higher than that of the leaves in each other class. In the Zn2 plants the $^{65}Zn$ concentration of the little leaves was higher than that of all other leaves except those in the > 45-mm

class where the trend was similar but did not attain significance. With the normal (Zn3) plants, however, the $^{65}Zn$ concentration of the small immature leaves was higher than that of the next larger class (15–30 mm) only. The significance of this




Figs. 3–8.—Autoradiographs of $^{65}Zn$ in subterranean clover plants from harvest 4 on day 63. Zinc and phosphorus treatments are indicated. Times of exposure were 3 days (Figs. 3 and 4), 6 days (Figs. 5 and 6), and 11 days (Figs. 7 and 8).

difference was in no way comparable to that between the little and larger leaves of the Zn1 and Zn2 plants respectively. At each zinc level the lowest $^{65}Zn$ concentrations occurred in the leaves of intermediate size.

The high $^{65}Zn$ concentration in "little" leaves as compared with that in other leaves on the same plant is shown in the autoradiograph in Figure 9. This autoradiograph also shows relatively high concentrations of $^{65}Zn$ in the meristematic regions of the root tips.

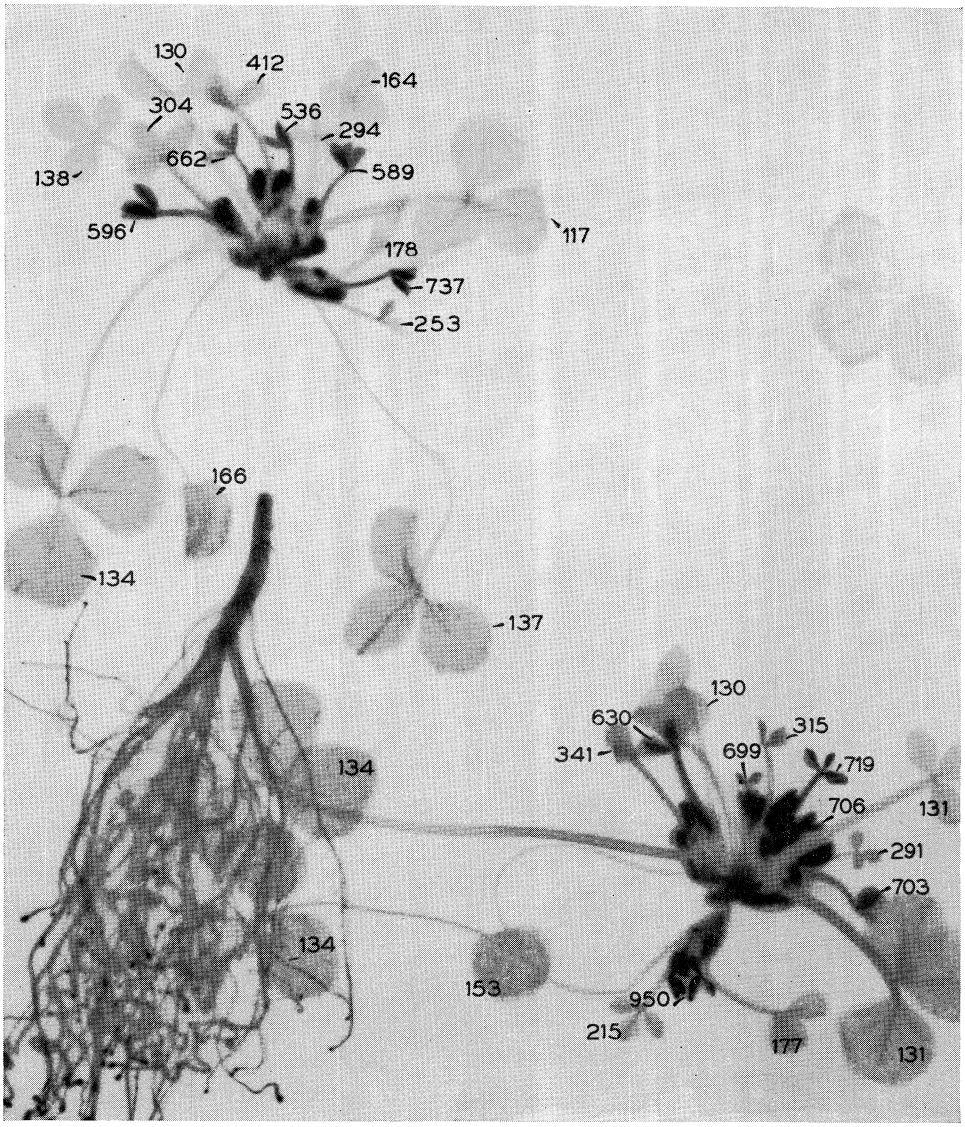
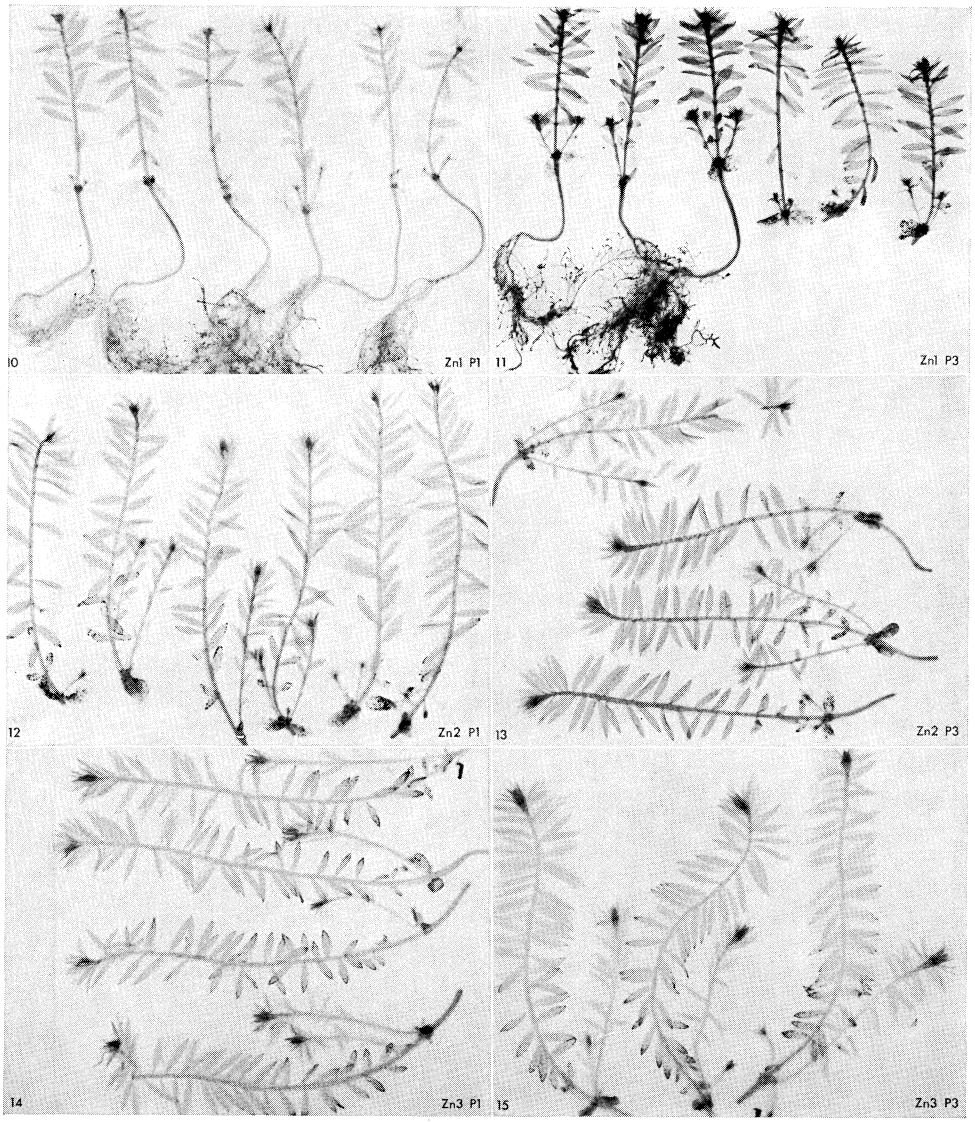



Fig. 9.—Autoradiograph of $^{65}Zn$ in zinc-deficient subterranean clover plants with "little" leaves, harvested on day 63. Numbers represent $^{65}Zn$ concentrations, expressed as counts/min/mg dry matter, in lamina of individual leaves. Time of exposure, 3 days.

It is concluded from the results of the radioassays (Table 4) and the autoradiographs that a deficient supply of zinc promotes a marked difference from normal in the relative distribution of zinc between old and young leaves of subterranean clover.

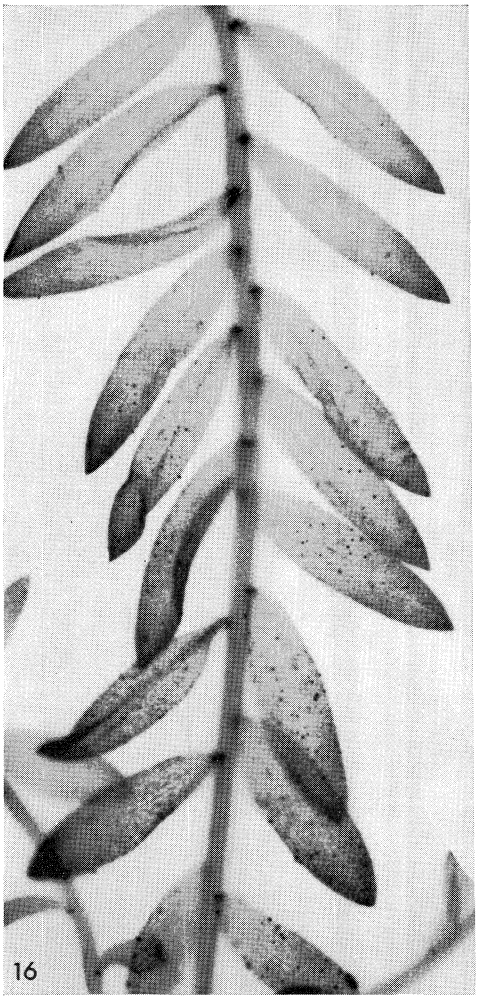
The total dry weight of tops plus roots and percentage weight of the top of the plants from harvests 3 and 4 are presented in Table 5. The retransformed values for tops and roots separately for harvest 4 are presented in Figure 18. Increases in total

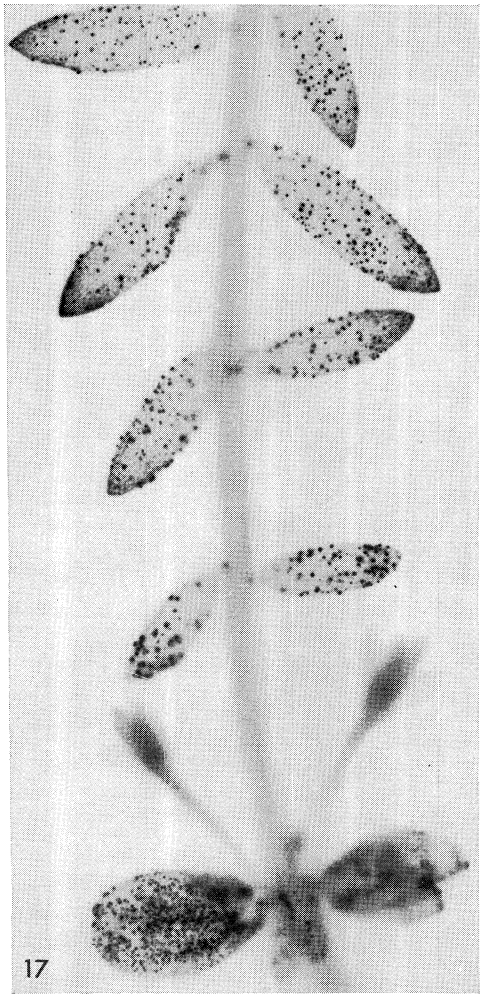



Figs. 10–15.—Autoradiographs of $^{65}Zn$ in flax plants from harvest 4 on day 60. Zinc and phosphorus treatments are indicated. Times of exposure were 4 days (Figs. 10 and 11), 6 days (Figs. 12 and 13), and 7 days (Figs. 14 and 15). All the Zn1 and Zn2 plants were showing dieback symptoms typical of zinc deficiency.

dry weight occurred between harvests, but they were greatest at the P1 level of phosphate and the Zn3 level of zinc. An increase in either zinc or phosphate level had no effect on total dry weight at harvest 3, but by harvest 4 contrary effects had

developed, i.e. increase in zinc level resulted in significant and progressive increases in growth, whereas increase in phosphate level from P1 to P2 or P3 depressed growth at this harvest. This depressive effect of phosphate was associated with high phosphorus concentrations in the tops of all P2 and P3 plants (Table 8). In this regard Rossiter (1952) found that subterranean clover showing incipient phosphorus toxicity symptoms contained a phosphorus concentration of 1·43%.




Figs. 16 and 17.—Autoradiographs of $^{65}Zn$ in flax plants grown at the Zn3 level showing nodal accumulation of the isotope in plants sampled on day 45 (Fig. 16) and accumulation of islands of $^{65}Zn$ in the cotyledons and lower leaves of plants sampled on day 52 (Fig. 17). Times of exposure were 11 and 7 days, respectively.

There was no consistent effect on the percentage weight of the top due to difference in time of harvest, between any zinc or phosphate level. However, at both harvests there was a marked main effect of increase in percentage weight of the top between Zn1 and Zn3, i.e. zinc deficiency reduced top growth more than root

growth. This result is supported by previous work of Millikan (1953). This effect was apparent at harvest 3 even though there was no significant difference in total dry weight between Zn1 and Zn3 at that time, although a trend was apparent. At harvest 4 increase in phosphate level from P1 to P3 significantly increased the percentage weight of the top. In contrast with such an increase due to increase in zinc level, this phosphate effect was associated with a reduction in total dry weight, i.e. the toxic effect of phosphate was manifested in a relatively greater reduction in root growth than in top growth.

TABLE 5

DRY WEIGHT OF TOPS AND ROOTS AND PERCENTAGE WEIGHT OF TOPS OF SUBTERRANEAN CLOVER

Plants grown at different zinc and phosphorus levels, and harvested on days 51 (H3) and 63 (H4). Other symbols as defined in previous tables

| | Dry Weight of Tops and Roots (mg)* | | | Percentage Weight of Tops† | | |
|---|---|---|---|---|---|---|
| | H3 | H4 | (H4−H3) | H3 | H4 | (H4−H3) |
| Zn1 | 128 | 252 | +124 | 72·4 | 70·9 | −1·5 |
| Zn2 | 173 | 364 | +191 | 76·6 | 72·6 | −4·0 |
| Zn3 | 153 | 466 | +313 | 78·6 | 78·1 | −0·5 |
| P1 | 161 | 451 | +290 | 74·4 | 69·1 | −5·3 |
| P2 | 154 | 336 | +182 | 76·4 | 75·9 | −0·5 |
| P3 | 138 | 294 | +156 | 76·8 | 76·6 | −0·2 |

* Least significant differences at the 5% level for $D_H$, $D_{Zn}$, and $D_P = 82$, and for $D_D = 115$; corresponding values at the 1% level are 112 and 158 respectively.

† Least significant differences at the 5% level for $D_H$, $D_{Zn}$, and $D_P = 3·1$, and for $D_D = 4·4$; corresponding values at the 1% level are 4·3 and 6·1 respectively.

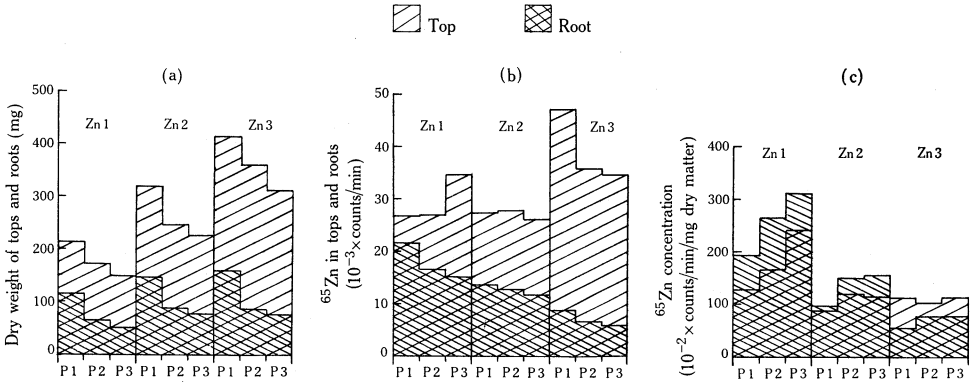



Fig. 18.—Mean dry weights, absolute amounts of $^{65}Zn$, and concentrations of $^{65}Zn$ in tops and roots of subterranean clover plants grown at different levels of phosphorus and zinc supply, and harvested on day 63.

The absolute amounts and concentrations of $^{65}Zn$ in tops plus roots of subterranean clover harvested on days 51 and 63 are shown in Table 6. No comparisons are possible between the levels of applied zinc owing to the difference in their specific

activities. There were no differences in the absolute amounts of $^{65}Zn$ between harvests or phosphate levels at either the Zn1 or Zn2 levels of zinc, but at the Zn3 level increases occurred between harvests, and a decrease between the P1 and P3 levels. This latter effect was merely a reflection of the decreased growth induced by increase in phosphate level (Table 5) as no significant difference in $^{65}Zn$ concentration occurred (Table 6). However, the increased uptake of $^{65}Zn$ by the Zn3 plants between harvests was not proportional to the concomitant increase in growth (Table 5), since there was a significant decrease in $^{65}Zn$ concentration.

TABLE 6

ABSOLUTE AMOUNTS AND CONCENTRATIONS OF $^{65}Zn$ IN TOPS AND ROOTS OF SUBTERRANEAN CLOVER

Absolute amounts expressed as $10^{-3} \times$ (counts/min) and concentrations as $10^{-2} \times$ (counts/min/mg dry matter). Experimental conditions and explanation of symbols as for Table 5

| | Absolute Amount of $^{65}Zn$ | | | $^{65}Zn$ Concentration | | |
|---|---|---|---|---|---|---|
| | Zn1 | Zn2 | Zn3 | Zn1 | Zn2 | Zn3 |
| H3 | 36·4 | 44·9 | 26·0 | 2·86 | 2·67 | 1·70 |
| H4 | 47·9 | 40·7 | 46·4 | 2·03 | 1·15 | 1·01 |
| (H4−H3) | +11·5 | −4·2 | +20·4 | −0·83 | −1·52 | −0·69 |
| L.S.D. ($P = 0·05$) | 16·6 | 15·7 | 8·9 | 0·78 | 0·88 | 0·19 |
| L.S.D. ($P = 0·01$) | 26·1 | 24·6 | 14·0 | 1·22 | 1·37 | 0·29 |
| P1 | 43·8 | 44·6 | 43·4 | 2·11 | 1·87 | 1·40 |
| P2 | 40·8 | 39·5 | 35·8 | 2·49 | 1·87 | 1·34 |
| P3 | 41·9 | 44·4 | 29·3 | 2·74 | 2·00 | 1·32 |
| L.S.D. ($P = 0·05$) | 20·4 | 19·2 | 10·9 | 0·95 | 1·07 | 0·23 |
| L.S.D. ($P = 0·01$) | 32·0 | 30·2 | 17·1 | 1·49 | 1·68 | 0·36 |

An analysis of the effects of treatments on the percentage of the absolute amount of $^{65}Zn$ in the tops of subterranean clover is presented in Table 7. There was a nearly significant reduction in percentage $^{65}Zn$ between harvests 3 and 4, and this matched a similar reduction in percentage weight of top (Table 5).

At both harvests and each phosphate level (since there were no two- or three-factor interactions) there were increases in the percentage $^{65}Zn$ in the tops between the Zn1 and Zn3 levels. Increase in phosphate level did not significantly affect the magnitude of these increases. These increases in percentage $^{65}Zn$ in the tops due to increase in zinc supply parallel but were relatively greater than the increases in the percentage weight of the tops resulting from the same treatments (Table 5). Thus it is apparent that under conditions of zinc deficiency relatively more than normal of the total zinc in the plants is located in the roots.

The results in Table 7 also show that an increase in phosphate level from P1 to P3 tended to increase the percentage of $^{65}Zn$ in the tops, i.e. promoted the movement of $^{65}Zn$ from roots to tops. Thus the more acute zinc-deficiency symptoms which occurred in the tops of the Zn1 P3 when compared with the Zn1 P1 plants could not be attributed to a lack of translocation of zinc from roots to tops due to the high phosphate supply.

There is general agreement between the relative differences in concentrations of $^{65}Zn$ (Fig. 18) and total zinc (Table 8) in the plant tops due to differences in phosphate level at each zinc level. It is, of course, not valid to compare $^{65}Zn$ concentration between zinc levels because of differences in labelling. In the case of the Zn1 P2 treatment the concentrations of total zinc showed a decrease, and that of $^{65}Zn$ an increase, over the corresponding Zn1 P1 value. It was not possible to check the result of the total zinc analysis as no sample remained.

TABLE 7

PERCENTAGE OF $^{65}Zn$ IN TOPS OF SUBTERRANEAN CLOVER

Experimental conditions and explanation of symbols given in Table 5

| Zinc Level | $^{65}Zn$ (%) | Phosphorus Level | $^{65}Zn$ (%) | Harvest | $^{65}Zn$ (%) |
|---|---|---|---|---|---|
| Zn1 | 63·8 | P1 | 72·6 | H3 | 75·4 |
| Zn2 | 72·6 | P2 | 72·4 | H4 | 71·8 |
| Zn3 | 84·5 | P3 | 75·7 | | |
| Least significant differences | | | | | |
| $P = 0·05$ | 4·6 | | 4·6 | | 3·7 |
| $P = 0·01$ | 6·3 | | 6·3 | | 5·1 |

TABLE 8

TOTAL PHOSPHORUS AND ZINC (BOTH LABELLED AND UNLABELLED) CONCENTRATIONS (ON DRY WEIGHT BASIS) IN TOPS OF SUBTERRANEAN CLOVER

Plants harvested on day 63

| | P1 | | | P2 | | | P3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | P (%) | Zn (p.p.m.) | P/Zn Ratio | P (%) | Zn (p.p.m.) | P/Zn Ratio | P (%) | Zn (p.p.m.) | P/Zn Ratio |
| Zn1 | 0·37 | 38 | 97 | 2·95 | 30 | 957 | 3·53 | 41 | 860 |
| Zn2 | 0·23 | 37 | 62 | 2·29 | 55 | 417 | 2·61 | 48 | 544 |
| Zn3 | 0·22 | 85 | 26 | 1·59 | 67 | 238 | 1·81 | 76 | 236 |

From the results described above it is apparent that the increased severity of zinc deficiency symptoms in subterranean clover grown at high phosphate level was not related to a lower zinc concentration in the tops of the plants. However, plants with severe zinc-deficiency symptoms had P/Zn ratios of over 400 (Table 8).

### (*b*) *Flax*

(i) *Development of Zinc-deficiency Symptoms*

At the time of the first harvest on day 38, no differences in growth were apparent between treatments. The first symptom of zinc deficiency in the form of a tan discoloration of the main stem immediately below the growing point (Millikan 1951*a*), was observed in the Zn1 P3 plants on day 40, but by day 45 when the second harvest

TABLE 9

$^{65}$Zn CONCENTRATION IN SELECTED TISSUES OF FLAX PLANTS

Plants grown at different zinc (Zn1, Zn2, Zn3) and phosphorus (P1, P3) levels and harvested on days 38 (H1), 45 (H2), 52 (H3), and 60 (H4). $^{65}$Zn concentrations expressed as counts/min/mg dry matter. The level of phosphorus supply had no significant effect on $^{65}$Zn concentration in any of the tissues studied. $D_{PT}$ refers to a difference of mean values for two plant tissues. Other symbols as in earlier tables

| Plant Tissue | Zn1 | | | | | Zn2 | | | | | Zn3 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H1 | H2 | H3 | H4 | (H4−H1) | H1 | H2 | H3 | H4 | (H4−H1) | H1 | H2 | H3 | H4 | (H4−H1) |
| Top rosette | 332 | 182 | 121 | 104 | −228 | 343 | 210 | 98 | 50 | −293 | 79 | 70 | 61 | 49 | −30 |
| Top leaves | 262 | 149 | 105 | 99 | −163 | 221 | 129 | 72 | 42 | −179 | 108 | 58 | 50 | 31 | −57 |
| Mid leaves | 190 | 128 | 109 | 85 | −105 | 175 | 91 | 71 | 46 | −129 | 88 | 69 | 60 | 28 | −60 |
| Lower leaves | 165 | 137 | 96 | 88 | −77 | 198 | 114 | 111 | 79 | −119 | 94 | 89 | 86 | 65 | −39 |
| Top stem | 231 | 163 | 102 | 88 | −143 | 236 | 146 | 90 | 43 | −193 | 74 | 55 | 43 | 28 | −42 |
| Mid stem | 148 | 83 | 69 | 61 | −87 | 146 | 63 | 38 | 23 | −123 | 69 | 40 | 31 | 16 | −53 |
| Lower stem | 109 | 73 | 50 | 50 | −59 | 122 | 47 | 30 | 18 | −104 | 59 | 31 | 24 | 13 | −46 |
| L.S.D. ($P = 0{\cdot}05$) | $D_H = 45$; | | $D_{PT} = 36$; | | $D_D = 50$ | $D_H = 51$; | | $D_{PT} = 38$; | | $D_D = 54$ | $D_H = 19$; | | $D_{PT} = 19$; | | $D_D = 26$ |
| L.S.D. ($P = 0{\cdot}01$) | $D_H = 64$; | | $D_{PT} = 47$; | | $D_D = 67$ | $D_H = 72$; | | $D_{PT} = 51$; | | $D_D = 72$ | $D_H = 26$; | | $D_{PT} = 25$; | | $D_D = 35$ |

was made this symptom was also apparent in the Zn1 P1 plants. At harvest 3 on day 52, dieback and bronze spotting of the upper leaves was occurring on the Zn1 P1 and Zn1 P3 plants, and the tan discoloration was apparent in the Zn2 P1 and Zn2 P3 plants.

When the final samples were obtained on day 60, dieback was occurring in all plants of both the Zn1 and Zn2 series. At no time were any zinc-deficiency symptoms observed in plants grown at the Zn3 level of zinc supply.

(ii) *Radioassays and Statistical Analyses*

The results of radioassays made on selected tissues of plants from each harvest are presented in Table 9 and autoradiographs of the day 60 samples in Figures 10–15. No reference to phosphate level is included in Table 9 as it had no significant effect on $^{65}Zn$ concentration at any harvest or zinc level.

Between harvests there was a fall in $^{65}Zn$ concentration in the top rosette and leaves and stem sections of each plant, which became progressively slower with time, irrespective of phosphorus and zinc levels. However, the relative differences in $^{65}Zn$ concentrations between the various tissues differed between zinc levels and harvests. At the Zn1 and Zn2 levels the top rosettes had the highest $^{65}Zn$ concentrations of any tissue at harvests 1 and 2, even though those of the Zn1 plants were showing zinc-deficiency symptoms by harvest 2. However, by harvests 3 and 4 the $^{65}Zn$ concentration in the top rosette of the Zn1 plants was significantly higher than that of the mid and lower stem sections only, while in the tops of the Zn2 plants the value recorded was actually less, although not significantly so, than that of the lower leaves. By contrast, at no harvest did the top rosette of the Zn3 plants have the highest $^{65}Zn$ concentration — at all harvests it was lower than that of the lowest leaves.

With respect to the relative $^{65}Zn$ concentrations in leaves and stems, the Zn1 plants differed markedly from the Zn2 and Zn3 plants. In the Zn1 plants there was, in general, a gradient in $^{65}Zn$ concentrations from upper to lower tissues, at each harvest or between harvests, which had the same sense for both leaves and stem. The top leaves had a significantly higher $^{65}Zn$ concentration than the lower leaves at the first harvest and at subsequent harvests the recorded value did not fall below that of the lower leaves. Also, at each harvest the top leaves had a comparable $^{65}Zn$ concentration to the top stem, but the mid and lower leaves were higher in $^{65}Zn$ than the corresponding stem tissue. In the Zn2 and Zn3 plants the $^{65}Zn$ concentration varied independently between upper and lower leaves and stem tissues respectively. In the Zn2 plants, the concentration recorded for the top leaves was higher at harvests 1 and 2, and lower at harvests 3 and 4 than that of lower leaves. The difference between these two effects was significant. By contrast there was a progressive fall in $^{65}Zn$ concentration from upper to lower stem tissue, and in the latter it was less than in the associated lower leaves at each harvest. In the Zn3 plants the concentration of $^{65}Zn$ in the upper leaves was not significantly higher than that of the lower leaves at harvest 1 and from harvest 2 onwards there was an accumulation of $^{65}Zn$ in the lower leaves where the level of the isotope became higher than that in any other aerial part of the plants. In the stems, however, there was a progressive fall in $^{65}Zn$ concentration from upper to lower stem sections.

The autoradiographs of the plants showed that by day 52 the lower leaves, particularly of the Zn2 and Zn3 plants, contained relatively high $^{65}Zn$ concentrations in small isolated areas or islands scattered throughout the lamina, and along the distal leaf edges (Fig. 17). Relatively high concentrations of $^{65}Zn$ also occurred in the nodes (Fig. 16), and in the root tips (Figs. 10 and 11).

## IV. Discussion

We have shown that $^{65}Zn$ concentration within zinc-deficient plants may vary considerably, and that this variation is relatively different from that in normal plants. The $^{65}Zn$ concentration in tissues actually involved in phosphate-induced zinc-deficiency symptoms, e.g. little leaves of subterranean clover, or the apical tissues of flax plants affected with dieback, has been found to be relatively much higher than that of other parts of the same plant. Such differences obviously cannot be detected by analyses of whole plants.

Zinc deficiency may stimulate considerable retranslocation of zinc within the plant, particularly from tissues which become necrotic. Because of this, the concentration or absolute amount of $^{65}Zn$ in affected tissues, when compared with that of comparable tissues of normal plants, may show very different relative changes with time. For example, the fall in $^{65}Zn$ concentration in the top rosette of zinc-deficient flax amounted to a minimum of approximately 300% between days 38 and 60, whereas that in similar tissue of plants with a normal zinc supply was approximately 40% over the same period. Symptoms were first recognized in the zinc-deficient plants on day 40. Again, relatively large and progressive decreases in the absolute amount of $^{65}Zn$ in the oldest leaves of zinc-deficient subterranean clover plants occurred with time, as they became senescent, whereas similar leaves of normal plants remained active and continued to import $^{65}Zn$, at least to days 44 or 51. This result supports the conclusion of Riceman and Jones (1960), based on the appearance of autoradiographs only, that some zinc appeared to be transported out of old leaves of subterranean clover plants as they became senescent due to zinc deficiency. Riceman and Jones (1956) also observed that increase in dry weight in various plant parts was accompanied by a rapid decline in zinc concentration. Millikan (1963) also observed that the zinc concentration in subterranean clover fell rapidly with time.

It is clear that the $^{65}Zn$ translocated from the old leaves of zinc-deficient subterranean clover moved into the little leaves as they were produced. Also the movement of $^{65}Zn$ into secondary shoots produced from the crown node would account for the general fall in $^{65}Zn$ concentration in the zinc-deficient flax plants. However, $^{65}Zn$ may have also moved into the meristematic regions of the root tips as these had relatively high concentrations of the isotope at the final harvest (Figs. 9–15).

In the present experiments, important differences occurred between zinc treatment levels with respect to the effects of increase in phosphate level on the zinc concentration in the plants. Thus, high, when compared with low, phosphate level caused increases in zinc concentrations in the tissues of zinc-deficient but not of normal plants. With zinc-deficient subterranean clover, the higher $^{65}Zn$ concentration induced by high phosphate level was only partly due to the depression in growth caused

by this treatment, as the total uptake of the isotope into the tops was also increased. In this species the plants with severe zinc-deficiency symptoms had P/Zn ratios of over 400 in the tops on day 63. However, Millikan (1963) showed that the critical value of this ratio in relation to the occurrence of zinc-deficiency symptoms in subterranean clover altered appreciably with time. In a study of zinc deficiency in potatoes Boawn and Leggett (1964) found a critical P/Zn ratio in the region of 400.

It is concluded that, in a study of zinc–phosphorus relationships in plants, more precise information on zinc concentration in relation to symptom severity and phosphate treatment may be obtained by comparative analyses of selected tissues rather than whole plants, and by sampling such tissues immediately after the appearance of symptoms.

Previous non-recognition of the fact that a given increase in phosphate level may have different effects on zinc concentration within plants depending on whether a deficient or normal supply of zinc is available to them, and also of the extent of the variability of zinc concentration within zinc-deficient plants, both between tissues, and with time, may be responsible for much of the conflict in the literature, as reviewed in the Introduction, with respect to zinc–phosphorus relationships in plants.

It has been shown by different workers, including Biddulph *et al.* (1958) and Norton and Wittwer (1963), that phosphorus shows preferential movement into areas of high metabolic activity including the young leaves, stem apex, and root tips. These are the same general areas in which the highest concentrations of $^{65}Zn$ were found in flax and subterranean clover, particularly when zinc was in deficient supply, and during the early development of zinc-deficiency symptoms.

The occurrence of islands of relatively high $^{65}Zn$ concentration in the old leaves of flax plants, particularly in those receiving the normal zinc treatment, is comparable to the development of $^{54}Mn$ islands in the old leaves of this species, described by Millikan (1951*b*). The function of these islands is unknown.

The nodal accumulation of $^{65}Zn$ found in flax (Fig. 16) extends the known occurrence of this phenomenon to an additional species to those listed by Millikan and Hanger (1967).

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