

THE DISTRIBUTION OF ZINC IN OAT PLANTS

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Summary

Three experiments are described in which oat plants (*var.* Mulga) were grown in Laffer sand or culture solutions containing varying amounts of added zinc. In each case, zinc contents of living and dead leaves, stems, roots, inflorescence, and grain were determined at intervals until final harvest.

Zinc was absorbed continuously throughout the life cycle of the plant. In the soil used, much of the zinc, whether native or added, was unavailable, and large increases in the amount added to the soil did not result in proportional increases in amount of zinc in the plant. It is shown that there is a threshold value for grain production in oats, but large increases in zinc contents of the plants did not cause proportionate increases in yield either of dry matter or of grain. Symptoms of zinc deficiency appeared on leaves when the concentration of zinc was less than 20 parts per million of dry matter at a time prior to exertion of the inflorescence.

Leaves contained 20-30 per cent. of total zinc in the plant. This zinc was not translocated from the leaves during senescence, but remained within the dead leaves; nor could zinc be removed by dialysis of macerated leaf material. Of the total zinc in the leaves, 15 to 20 per cent. was localized in the chloroplasts, though in spinach about 50 per cent. of the total leaf zinc occurs in the chloroplasts.

The amount of zinc in the roots increased in amount up to the time of grain development, but thereafter decreased and was translocated to other organs. The greater part of the zinc in grain and inflorescence was accounted for by uptake from the medium.

I. INTRODUCTION

Throughout large areas of South Australia, cereals and pasture plants cannot absorb from the soil sufficient zinc and copper for normal growth and development. Riceman and Anderson (1941) showed that addition of zinc in the presence of copper increased the yield of grain in oats grown on the calcareous Robe sand; and Riceman (1945) that on Laffer sand the yield of oats was increased by dressings of zinc in the presence of applied phosphate.

Reed (1939) and Reed and Beck (1939) grew maize plants in a zinc-deficient Californian soil and showed that production of cobs and kernels was depressed more than that of stalks and leaves. Reed (1942) showed, for peas and beans grown in water culture, that there was a threshold value for zinc below which plants produced only small, seedless pods; above this threshold value the weight of seeds bore a close relation to the amount of zinc supplied. Tops and roots of peas grown in solution containing 0.005-0.2 part per million

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Zn did not show significant differences in dry weight, but with beans grown in the same range of concentrations small increases in dry weight occurred with increasing concentrations of zinc. With *Andropogon sorghum*, however, there was a threshold value for development of tops and heads.

Systematic determinations of amount of zinc in plant tissues have been few and restricted chiefly to contents at time of flowering or at final harvest, with the object of determining the minimum zinc contents required for normal development. Piper and Walkley (1943) concluded from the percentage distribution of zinc in grain and straw of oat plants towards the end of the life cycle that zinc was translocated from straw to grain as maturity approached.

However, analyses at time of harvest can give little information about uptake and translocation during development. As pointed out by Gregory (1937), growth in cereals is an exponential process, determined by development of new meristems, during which the external supply of a nutrient in minimum is depleted and its rate of supply to the plant falls. When demand by the plant for the nutrient overtakes supply, internal starvation occurs and towards the end of the life cycle plants tend to possess the same concentration of the nutrient on a dry weight basis; this is low and may be independent of the magnitude of the original external supply. In this paper the authors report data for dry weights and zinc contents of the different plant organs of oat plants at different stages of development of the plant, the plants themselves being under a variety of conditions. From these data, movements of zinc within the plant are traced.

II. EXPERIMENTAL

(a) Description of Experiments

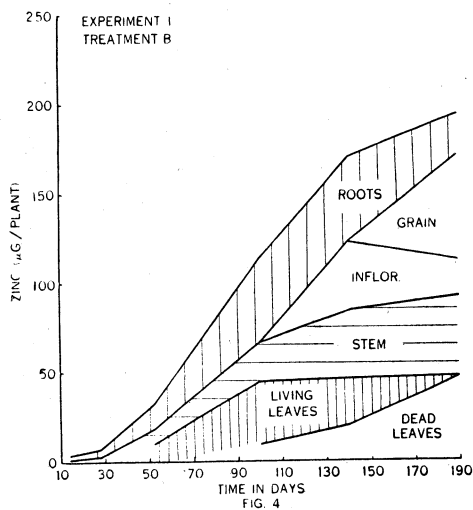
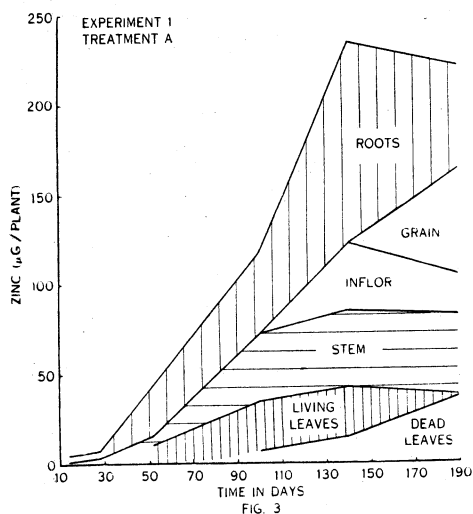
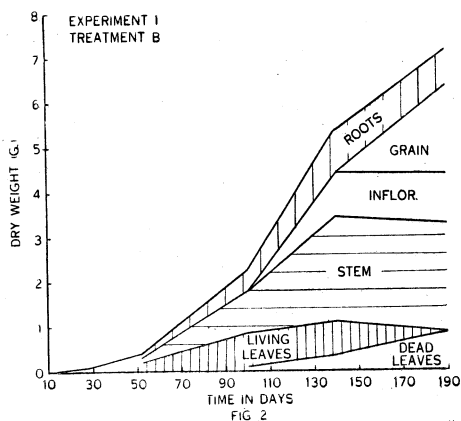
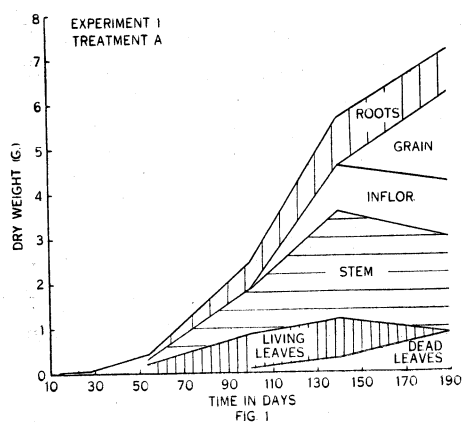
For each experiment, plants of a pure line of oats, *Avena sativa* var. *Mulga*, were grown in jars in a glass house, care being taken by use of paper shields that leaves did not come in contact with the soil.

Experiment 1.—Plants were grown in glazed earthenware pots, each containing 3 kg. Laffer sand obtained from an area which had been cropped once previously with a legume which showed symptoms of zinc deficiency. Six dehusked grains were sown in each pot on April 28, 1946 and thinned to three even plants per pot 9 days after sowing. Additional seedlings were grown for the first harvest but on subsequent occasions three pots from each treatment were harvested.

All pots received the following nutrients (in grams per pot) eleven days after sowing: $\text{Ca}(\text{H}_2\text{PO}_4)_2$, 0.5g.; NaNO_3 , 1.0g.; KCl , 0.4g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.14g.; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.015g.; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.01g.; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.04g.; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.003g. An additional 1.0g. NaNO_3 per pot was added 94 days after sowing.

Prior to addition to the sand, solutions of NaNO_3 , KCl , and MgSO_4 were freed from zinc by extraction with dithizone and removal of excess of the latter. $\text{Ca}(\text{H}_2\text{PO}_4)_2$ was prepared from CaCl_2 and KH_2PO_4 solutions after removal of zinc by dithizone extraction. Fe, Mn, Cu, and Mo salts were prepared by recrystallizing AR grade chemicals.

The pots were divided into two series according to the zinc treatment designed for them, viz. pots of Treatment A received 0.01g. $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (2300 μg . Zn) per pot; those of Treatment B received no additional zinc. The pots were maintained at 60 per cent. water-retaining capacity of the soil by frequent additions of water redistilled from a Pyrex glass still.



Figs. 1-4.—Dry weights (g. per plant) and absolute amounts of zinc in plant organs, Experiment 1.

Appropriate census data concerning numbers of leaves, tillers, etc., were made at frequent intervals. Plants showed signs of nitrogen deficiency about 90 days after sowing and additional nitrate (see above) was added. Inflorescences first appeared 114 days after sowing.

Harvests were made on the following days after sowing: Harvest I, 15 days; Harvest II, 27 days; Harvest III, 52 days; Harvest IV, 101 days; Harvest V, 140

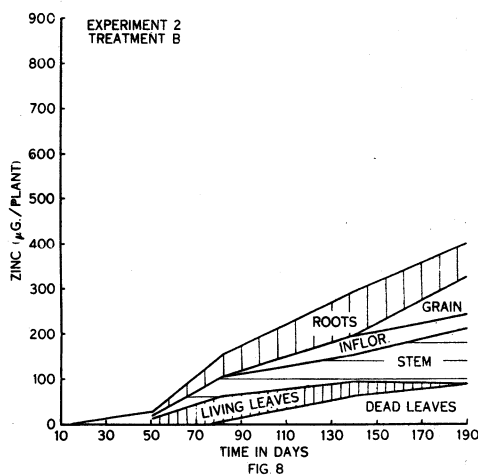
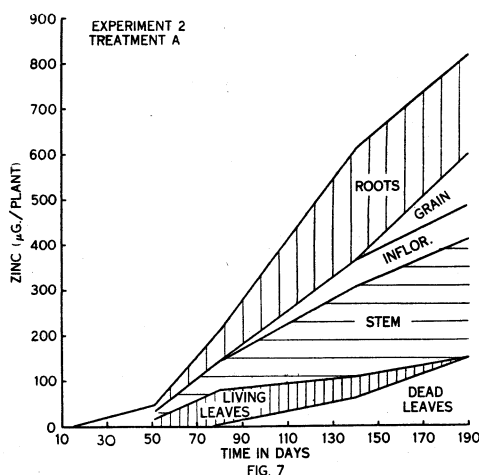
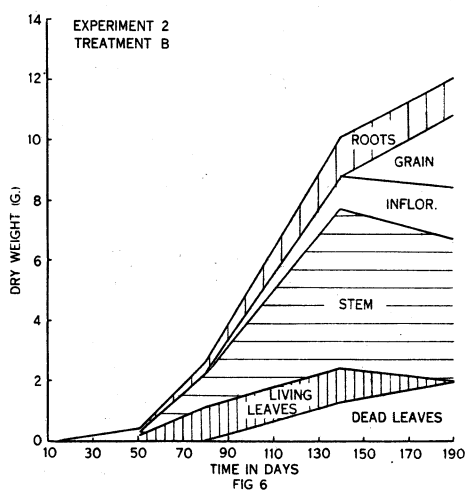
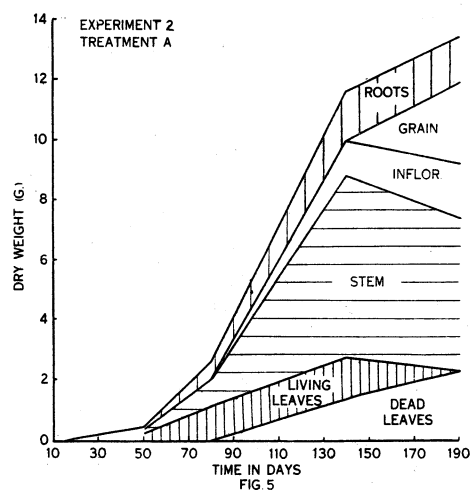
days; and Harvest VI, 190 days (maturity). At each harvest, plants were separated into appropriate parts, viz. living leaves, dead leaves, stem, root, inflorescence, and grain. After separation and before drying, roots were rinsed in dilute acetic acid and then in zinc-free distilled water in order to remove adsorbed zinc from the surface. All plant parts were rapidly dried under forced draught at 90°C. For each harvest, replicate material for analysis was drawn from the bulked samples of each treatment. Dry weights of plant organs per plant, plotted cumulatively, at different harvests are shown in Figures 1 and 2 and zinc contents in Figures 3 and 4. Percentage amounts of zinc are given in Table 1.

TABLE 1
RELATIVE AMOUNTS OF ZINC (MG. Zn PER KG. DRY MATTER)

| | Harvest | I | | II | | III | | IV | | V | | VI | |
|--------------|----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|----|----|
| | | A | B | A | B | A | B | A | B | A | B | A | B |
| Experiment 1 | Living leaves | 67 | 47 | 61 | 53 | 51 | 49 | 35 | 40 | 31 | 30 | — | — |
| | Dead leaves | — | — | — | — | — | — | 75 | 100 | 45 | 60 | 42 | 58 |
| | Stem | — | — | — | — | 51 | 66 | 39 | 25 | 20 | 17 | 21 | 19 |
| | Inflorescence | — | — | — | — | — | — | — | — | 35 | 37 | 18 | 15 |
| | Grain | — | — | — | — | — | — | — | — | — | — | 30 | 29 |
| | Roots | 550 | 420 | 237 | 269 | 308 | 169 | 71 | 97 | 106 | 53 | 34 | 32 |
| | Whole plant | 200 | 158 | 99 | 96 | 90 | 66 | 47 | 51 | 41 | 31 | 31 | 27 |
| Experiment 2 | Living leaves | 58 | 58 | 68 | 54 | 61 | 48 | 39 | 27 | — | — | — | — |
| | Dead leaves | — | — | — | — | 318 | 317 | 47 | 49 | 69 | 46 | — | — |
| | Stem | — | — | 97 | 57 | 74 | 44 | 32 | 14 | 50 | 28 | — | — |
| | Inflorescence | — | — | — | — | — | — | 51 | 38 | 43 | 20 | — | — |
| | Grain | — | — | — | — | — | — | — | — | 43 | 33 | — | — |
| | Roots | 222 | 222 | 180 | 88 | 128 | 124 | 133 | 79 | 145 | 59 | — | — |
| | Whole plant | 159 | 159 | 95 | 60 | 82 | 61 | 53 | 29 | 60 | 33 | — | — |
| Experiment 3 | Living leaves | 76 | 76 | 276 | 87 | 119 | 27 | 41 | 11 | 19 | 7 | — | — |
| | Dead leaves | — | — | — | — | 671 | 117 | 288 | 40 | 40 | 23 | 35 | 18 |
| | Symptom leaves | — | — | — | — | — | — | — | 14 | — | 5 | — | — |
| | Stem | — | — | — | — | 91 | 19 | 41 | 9 | 14 | 5 | 12 | 8 |
| | Inflorescence | — | — | — | — | — | — | — | — | 55 | — | 21 | 16 |
| | Grain | — | — | — | — | — | — | — | — | — | — | 33 | 16 |
| | Roots | 256 | 256 | 396 | 39 | 35 | 2 | 19 | 29 | 23 | 39 | 33 | 17 |
| | Whole plant | 164 | 164 | 305 | 73 | 105 | 20 | 50 | 14 | 19 | 11 | 21 | 12 |

Experiment 2.—Plants were grown in Laffer sand obtained from an area carrying only a sparse, natural grass covering. Cultural technique was similar to that described in Experiment 1, except that all pots received 0.004g. $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ initially plus 0.002g. H_3BO_3 ; $\text{Fe}_2(\text{SO}_4)_3$ was substituted for FeCl_3 , being prepared by oxidation of recrystallized FeSO_4 by Zn-free sulphuric and nitric acids. An additional 1.0g. NaNO_3 was added per pot 50 days after sowing. Pots of Treatment A received 0.08g. $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (18,200 μg . Zn) per pot; pots of Treatment B received no additional zinc.

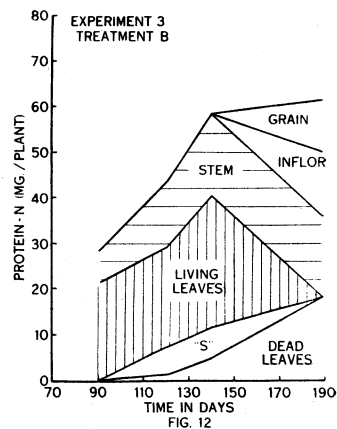
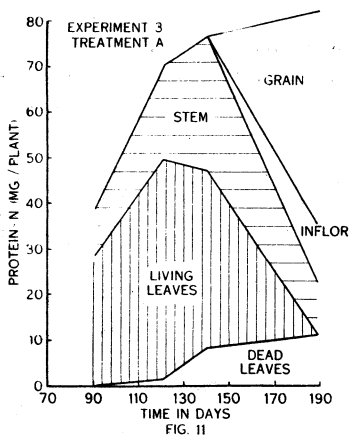
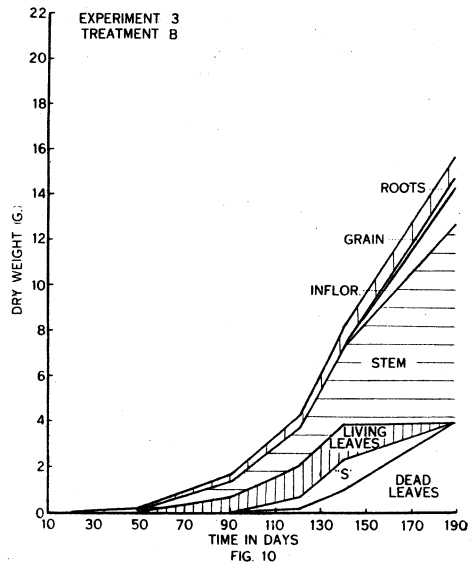
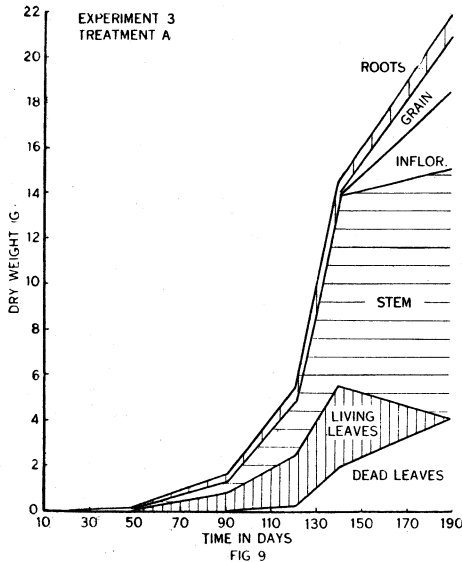
Seeds were sown on April 28, 1947 and inflorescences appeared 126 days after sowing. Harvests were made on the following days after sowing: Harvest I, 16 days; Harvest II, 51 days; Harvest III, 80 days; Harvest IV, 140 days; and Harvest V, 191 days (maturity). Separation of plant organs was made as described above. Dry weights of plant organs, plotted cumulatively, are shown in Figures 5 and 6, and their zinc contents in Figures 7 and 8. Percentage amounts of zinc are given in Table 1.



Figs. 5-8.—Dry weights (g. per plant) and absolute amounts of zinc in plant organs, Experiment 2.

Experiment 3.—Oat seedlings were germinated on waxed mosquito netting over glass-distilled water and when 16 days old were transferred to glass museum jars blackened externally, and each containing 2.5 litres of nutrient solution with the following composition (in g. per litre): KNO_3 , 1.0g.; KH_2PO_4 , 0.5g.; NaCl ,

0.1g.; $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 0.5g.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5g.; $\text{Fe}_2(\text{SO}_4)_3$, 0.017g.; H_3BO_3 , 0.5mg.; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ equivalent to 0.5 mg. Mn.; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ equivalent to 0.1mg. Mo; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ equivalent to 0.5mg. Cu. The pH of the solution was adjusted to pH 5.2. Salts were purified as in previous experiments. Nutrient

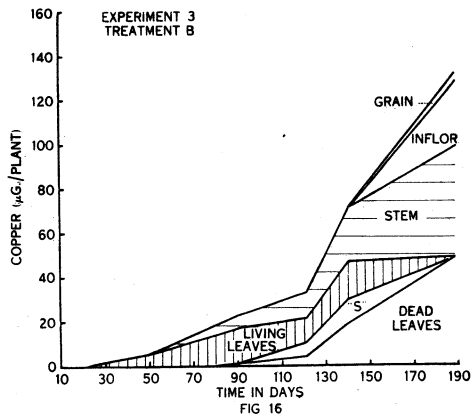
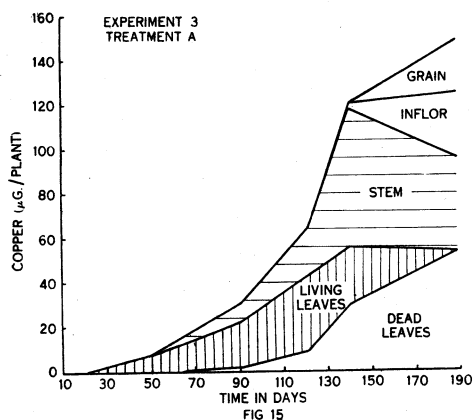
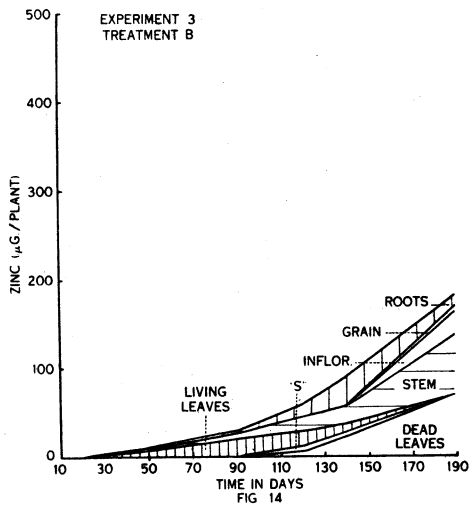
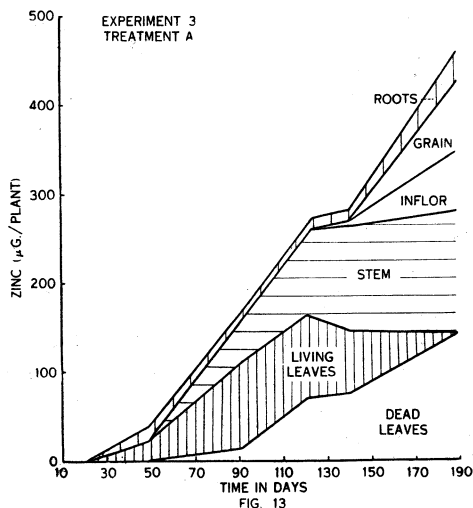


Figs. 9-12.—Dry weights (g. per plant) and absolute amounts of protein in plant organs, Experiment 3.

solutions were changed completely on days 58 and 88 and an additional 1.0g. KNO_3 per litre was added to all pots on day 73 and 0.5g. per litre on day 126. All nutrient solutions were aerated mechanically throughout the duration of the experiment. Six plants were grown in each jar.

Two treatments were applied: pots of Treatment A received 0.2mg. Zn per litre as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; pots of Treatment B received no additional zinc.

Seeds were germinated on May 17, 1948; lesions characteristic of zinc deficiency appeared on leaves of plants of Treatment B 95 days after sowing. These leaves were harvested and analysed separately. Inflorescences emerged in plants of Treatment A 146 days after sowing and in those of Treatment B 8 days later. Harvests were made on the following days after sowing: Harvest I, 21 days; Harvest II, 49 days; Harvest III, 91 days; Harvest IV, 121 days; Harvest V, 140 days; and Harvest VI, 189 days (maturity).



Figs. 13-16.—Absolute amounts of zinc and copper in plant organs, Experiment 3.

In this experiment, in addition to zinc contents, protein and copper contents of plant organs were determined. Dry weights of plant organs, plotted cumulatively, are shown in Figures 9 and 10; protein contents in Figures 11 and 12; zinc contents in Figures 13 and 14; and copper contents in Figures 15 and 16. Values for leaves exhibiting deficiency symptoms are labelled "S" in Figures 10, 12, 14, and 16. Percentage amounts of zinc are given in Table 1.

(b) Analytical Methods

Zinc and copper were determined polarographically in a Leeds and Northrup polarograph, using a nitrogen gas-chain. Wet digestion of one gram of dried plant material and subsequent procedures up to entry into the polarizing cell followed those employed by Walkley (1942). In Experiments 1 and 2, the basal solution used was similar to that used by Walkley, the quantity for each estimation being greater owing to the different size of cell used; in Experiment 3, the material prepared for polarization was dissolved in 2.5 ml. basal solution consisting of equal parts of 1N ammonia and 1N ammonium chloride solutions containing 0.02 per cent. gelatin; from this solution 2 ml. were placed in the electrolytic cell and both zinc and copper determined in successive steps on the polarogram. Standards for zinc and copper were run with each batch of five or six digestions. In the solution used, the zinc and cobalt steps coalesce, but the amount of cobalt present is negligible.

Protein-N was determined by the micro-Kjeldahl method after extraction of dried material and precipitation at pH 4.5 with trichloroacetic acid.

Chloroplasts were isolated and chlorophyll determined according to the methods described by Hanson, Barrien, and Wood (1941).

III. RESULTS AND DISCUSSION

(a) Total Zinc Content and its Relation to the Whole Plant

In all experiments the zinc content (in $\mu\text{g.}$ per plant) increased throughout the life cycle of the plant, indicating continuous uptake from the medium (Figs. 3, 4, 7, 8, 13, 14).

The absolute amount of zinc present in the whole plant at final harvest (approximately 190 days in each experiment) showed considerable variation between the different experiments.

In Experiment 3 the plants apparently absorbed the zinc completely from the culture solutions. In Treatment B each group of six plants at final harvest contained 1122 $\mu\text{g.}$ Zn absorbed from the culture solution and derived from contamination in distilled water, chemicals, and atmospheric dust; in Treatment A, 1500 $\mu\text{g.}$ Zn were added to the nutrient solution in each jar over the growing period and each group of six plants in this treatment contained 2700 $\mu\text{g.}$ Zn. This latter figure is approximately the sum of that in the untreated plants plus that supplied in solution. It would seem, therefore, that the efficient use by plants of relatively small quantities of zinc supplied in solution is analogous to that described for copper by Piper (1942).

On the other hand, in the experiments in the soil cultures it is clear that large amounts of zinc were not readily available to oat plants and were apparently immobilized in the soil. In Experiments 1 and 2, the Laffer sand contained 1 part per million of zinc, i.e. 3000 $\mu\text{g.}$ Zn were present in the soil in each pot.

In Experiment 1, 2300 $\mu\text{g.}$ Zn per pot were added to the sand in Treatment A, yet at final harvest the three plants in each pot of this treatment contained 678 $\mu\text{g.}$

Zn whilst that of the controls contained 585 $\mu\text{g.}$, i.e. the treated plants contained only 93 $\mu\text{g.}$ more than those untreated.

In Experiment 2, 18,200 $\mu\text{g.}$ Zn were added to the sand in Treatment A; the three plants in each pot at final harvest contained 2467 $\mu\text{g.}$ Zn whilst the three control plants contained 1206 $\mu\text{g.}$ Zn; i.e. the treated plants contained about twice as much zinc as the controls but this increase was small compared with the proportionate increase in amount of zinc added to the medium.

When treated plants contained much greater amounts of zinc at final harvest than the controls, as in Experiments 2 and 3, the increased zinc content was obvious at early harvests and was maintained throughout the growth cycle (see Figs. 7, 8, 13, 14).

It appears, therefore, that zinc uptake was increased when the amount of available zinc in the medium was increased, but in the Laffer sand much zinc was not readily available to the plant.

Differences in yield of dry matter between experiments in different years are probably due to the different seasons and difference in cultural conditions, and no comparison can be made on this basis.

Within any one experiment the total yield of dry matter of the whole plant and its parts at final harvest did not show significant differences (*t* test) between treated and untreated series, except in Experiment 3. In this case, characteristic zinc-deficiency symptoms appeared on the leaves and the yield of grain was greatly depressed.

In Experiment 1, treated plants contained only 31 $\mu\text{g.}$ Zn per plant more than the controls, the increase in zinc content occurring chiefly in the roots of the treated plants; there was no significant difference in dry weight at final harvest between treatments.

In Experiment 2 also there was no significant difference in dry weight at final harvest although the treated plants contained approximately twice as much zinc as the controls. This confirms the effects of "luxury" amounts of zinc which have been described by Reed (1942) for peas and beans, and by Lyon and Beeson (1947) for turnips and tomatoes.

Experiment 3 indicates clearly that there is a threshold value for zinc below which grain is not produced.

Zinc contents on a relative basis (mg. Zn per kg. dry matter) for the whole plant are shown for the three experiments in Table 1. In all cases this relative value for zinc decreased with increased age of the plant. This decrease was caused by exponential increase in dry matter associated with a decrease in rate of uptake owing to depletion of external supply. The small change in value of this ratio towards the end of the life cycle shown in all experiments suggests that, over this period, the amounts of dry matter produced may be determined by the amount of zinc absorbed.

The minimum amount of zinc required by an oat plant for full development depends upon its size; the oat plants in Experiments 2 and 3 (Treatment A) were average, well-developed plants and the data suggest that about 400 $\mu\text{g.}$ Zn per plant are necessary for full development. The data for Experiment 3 suggest

that deficiency symptoms and failure of grain formation occur when the concentration of zinc in the whole plant is less than 20 parts per million on a dry weight basis at a time when the inflorescence is developing rapidly and prior to its exsertion.

(b) *Distribution of Zinc in Plant Organs*

The absolute amounts of zinc and also the dry weights of the whole plant and its component organs in each experiment are shown in Figures 1-14. The distribution of zinc in each organ as a percentage of the total zinc content is given in Figures 17-19.

Up to the time of penultimate harvest the absolute zinc content of all plant organs increased whilst the relative content (parts per million of dry matter) decreased. This period was one of active growth and the reasons for decrease in the ratio have been discussed.

Between penultimate and final harvests in all experiments the inflorescence and grain developed; the root system had attained its maximum weight at penultimate harvest and thereafter decreased owing to translocation of material to the developing inflorescence; no new leaves were produced during this period but already-formed leaves became senescent and material was transported from them. Transport of protein from leaves and stems in Experiment 3 during this period is shown in Figures 11 and 12. Differences between Treatments A and B in Experiment 3 are noteworthy in this respect for deficiency of zinc in Treatment B resulted in little grain formation, consequently these plants remained vegetative for a longer time than in Treatment A and the effect of the small "sink" caused by the grain is reflected in the smaller percentage loss of protein from the leaves and stems; in this experiment copper also was transported from the stem in Treatment A but not in Treatment B.

During this period, the demand for zinc by the developing inflorescence and grain must be met either by translocation from other organs or by absorption from the medium. It has been pointed out already that the relative amounts of zinc in the whole plant in each treatment showed little change between penultimate and final harvests. Between these harvests the zinc uptake for the whole plant showed high correlation ($r = 0.843$) with increase in dry weight of stems *plus* inflorescence *plus* grain over that period. It would appear, therefore, that during development of the inflorescence and grain much of the zinc is absorbed from the medium, though it is apparent from Figures 1-14 that some may be transported, especially from the roots. The amounts of zinc in individual organs and its distribution are discussed in detail below.

Leaves.—Usually the leaves contained from 20 to 30 per cent. of the total zinc in the plant over the greater part of the life cycle (*vide* Figs. 17-19). On a relative basis, the zinc content of the living leaves varied considerably with the cultural treatment but within any one treatment the ratio decreased in a regular manner throughout the life cycle (Table 1) and for the reasons discussed previously.

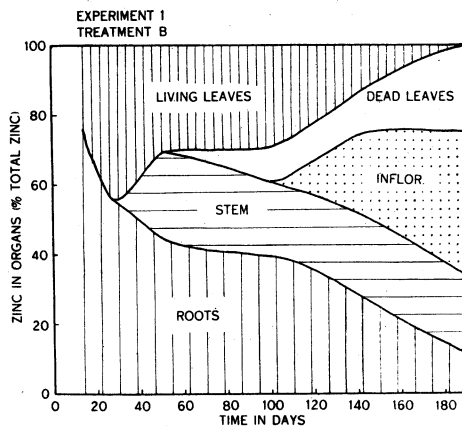
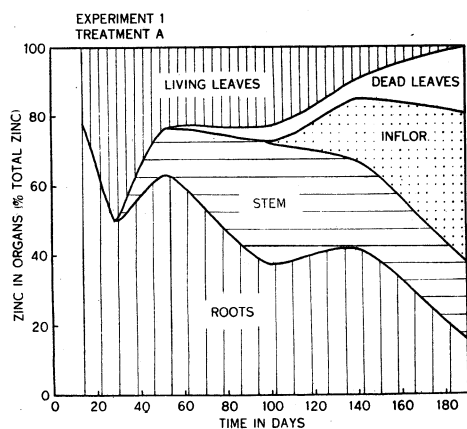


FIG 17

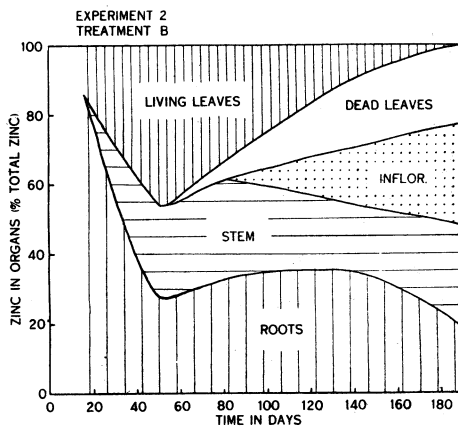
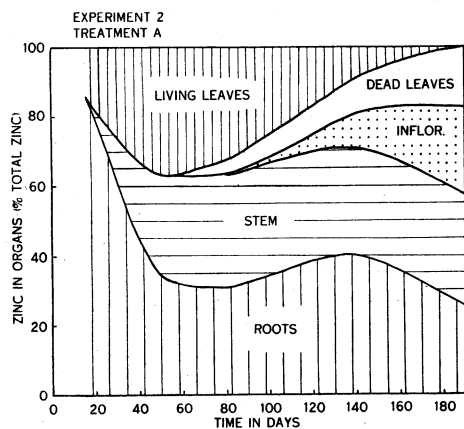


FIG 18

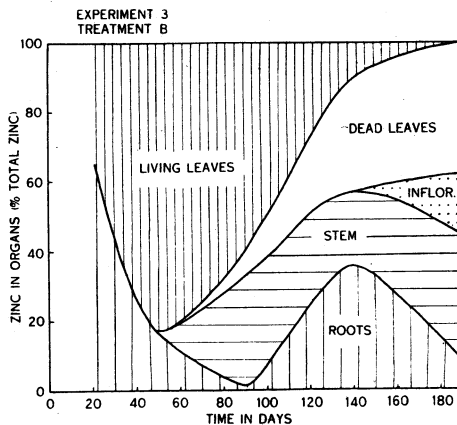
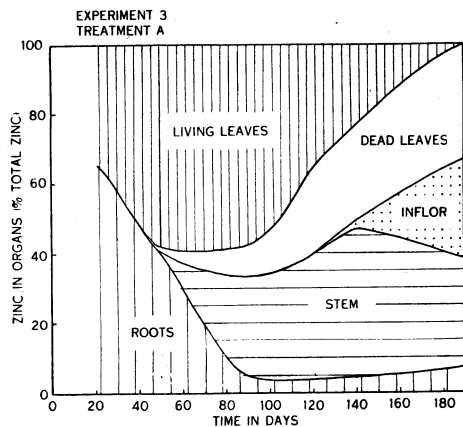


FIG 19

Figs. 17-19.—Amounts of zinc in plant organs as percentages of total zinc per plant, Experiments 1, 2, and 3.

The outstanding feature about the behaviour of zinc in leaves in these experiments is that, in every treatment, zinc was apparently immobilized in the leaves and was not transported to other organs as the leaves became senescent and died. This is shown in Figures 3, 4, 7, 8, 13, and 14 where it may be seen that the absolute zinc content of dead leaves at final harvest is equal to the sum of the living *plus* dead leaves at the penultimate harvest except in Treatment B, Experiment 3, where failure of grain formation permitted accumulation of zinc.

Up to the time of penultimate harvest it cannot be said with certainty that zinc was not transported from the leaves; the absolute amount of zinc increased up to this time but immobility is suggested by the high relative zinc contents of dead leaves. The relative zinc content of dead leaves was considerably higher than that of living leaves (Table 1) since the bases of expression were not comparable, dead leaves having lost protein as well as carbohydrates. Our experience in other experiments (Wood and Womersley 1946) has been that at the earlier harvests the dry weight of dead leaves was 50-70 per cent. of that of the dry weight of the same leaves when they were living.

In Experiment 3, leaves exhibiting symptoms of zinc deficiency were collected and analysed separately. The symptoms appeared on third and fourth leaves behind the growing point which were nearing maturity. The symptoms consisted of grey-fawn patches which appeared first on the upper parts of the leaves and the lesions then extended down the blade, purple colourings appearing as well. Chloroplast disintegration and lysis of cell contents occurred in these leaves. The final purple colour suggests connection of zinc deficiency with deranged phosphorus metabolism.

Whether translocation of zinc occurred from leaves under these circumstances is difficult to determine. Leaves exhibiting symptoms contained considerably less zinc at Harvest V in Experiment 3 than the whole sample of living leaves at that Harvest, but it must be remembered that the leaves exhibiting symptoms were late-formed and consequently would be expected to have lower zinc contents on a dry weight basis than those formed earlier which made up the bulk of the whole sample. Whilst, therefore, it cannot be said with certainty that no translocation of zinc occurred under these conditions, the data for absolute amounts of zinc indicate that if translocation occurred the amount must have been very small.

It appears, therefore, that in oat leaves zinc behaves in a similar manner to copper which Wood and Womersley (1946) have shown is not transported from leaves. The copper contents of plant organs in Experiment 3 confirm these findings (Figs. 15 and 16).

As might be expected from the different behaviour of zinc and protein in leaves (see Figs. 13, 14, 15, and 16) there was no simple relationship between these two variables. It is probable that zinc in leaves is bound with a colloidal constituent for zinc was not removed from leaves by dialysis. Fifty grams of oat leaves were macerated in a Waring Blendor for 3 minutes with

125 ml. iced water; the mass was centrifuged at a low speed sufficient to remove cell wall debris, etc. and of the supernatant suspension 15 ml. were dialysed in a Viskin membrane against distilled water for 24 hours. Zinc estimations showed that before dialysis the suspension contained 14.2 μ g. Zn and after dialysis 15.2 μ g. Zn, i.e. no zinc was removed by dialysis under these conditions.

The distribution of zinc within the leaf was further investigated by isolating pure whole chloroplasts according to the method described by Hanson, Barrien, and Wood (1941), determining the zinc, copper, and chlorophyll contents of the chloroplasts and comparing the amounts so obtained with those present in the original whole leaf material. The percentage of total leaf zinc present in the chloroplasts of oats in two separate determinations was 16 and 20 per cent. respectively; the amount of copper present in chloroplasts as a percentage of total copper was 35 and 40 per cent. respectively.

This work was extended by isolating chloroplasts from spinach (in which isolation is easier) and determining in a similar way zinc, copper, protein, and chlorophyll contents. In three separate estimations the percentage of total zinc occurring in the chloroplasts was 51, 50, and 53 per cent. respectively. In the last two estimations protein-N and copper contents were also determined; the chloroplasts contained 40 and 41 per cent. total leaf protein-N and 45 and 42 per cent. respectively of the total leaf copper.

It is apparent, therefore, that part of the leaf zinc was localized in the chloroplast although it would seem that variations in the proportion so localized occur between species—only about one-fifth of the total leaf zinc being present in the chloroplasts in oats and about one-half of the total leaf zinc in spinach. This distribution is in contrast with that of copper where there was little difference between the two species in percentage distribution between chloroplasts and whole leaf.

Grain.—The zinc contents of grain on a dry weight basis are given in Table 1; they reflect the zinc status of the whole plant at maturity, being most marked in the small amount of grain produced by plants in Treatment B in Experiment 3 which exhibited zinc-deficiency symptoms in leaves. The mean value for oat grains from a number of experiments, some not recorded here, in which zinc-deficiency lesions were not evident was found to be 35 μ g. Zn per g. of dry weight of grain. The developing grain represents a "sink" for zinc; since zinc was not transported from leaves it is apparent that the source of the zinc must be stems, roots, or absorption from the medium.

Stems and Inflorescence.—The absolute zinc content of stem *plus* inflorescence increased throughout the life cycle except in Experiment 1, where little change occurred between penultimate and final harvests. In these experiments, therefore, there was no evidence of translocation from the stem—from the data of Experiment 3 (Figs. 11-14) it is clear that the stem was losing protein whilst accumulating zinc.

Roots.—The absolute amount of zinc in the roots increased up to the penultimate harvest and thereafter decreased up to the time of final harvest. Decrease in amount of zinc between these two harvests was correlated with

decrease in dry weight of the roots over the same period ($r = 0.826$). The zinc apparently moved from the root along with other metabolites.

The absolute amounts of zinc in the roots varied considerably in the different experiments, being noticeably low in the poorly developed root systems of plants in the water culture (Experiment 3). In other experiments, with a different nutrient regime (to be described elsewhere) the authors have found relatively large absorption of zinc by the roots with considerable loss between penultimate and ultimate harvests, the general picture conforming to that shown in Figure 3.

The greater proportion of total zinc found in the roots of Treatment B compared with that found in Treatment A in Experiment 3 is possibly the result of renewal of the culture solutions on day 88 associated with partial failure of flower formation at that time.

Summing up these studies, it is apparent that zinc is absorbed continuously throughout the life cycle of oat plants and redistribution of zinc occurs during the development of grain and inflorescence. No zinc is translocated from leaves to other organs but zinc is supplied to inflorescence and grain from the root and from the medium. Piper and Walkley (1947) have stated that zinc is translocated to the grain from straw as maturity approaches. However, this conclusion was reached from consideration of the percentage distribution of zinc between grain and straw at final harvest. Such a deduction in fact cannot be made from relative figures at final harvest, but only from absolute contents per plant determined at intervals throughout the life cycle.

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