# THE EFFECT OF APPLIED PHOSPHATE ON THE UPTAKE OF ZINC BY FLAX

By J. F. Loneragan*

[*Manuscript received November* 21, 1950]

*Summary*

An experiment is described in which flax plants were grown in pots in Kojonup gravelly sand containing varying amounts of added phosphate, both with and without added zinc. Dry weight, phosphorus content, and zinc content of the total tops were determined at four periods in the early stages of growth.

Symptoms of zinc deficiency appeared after four weeks in high phosphate treatments. The symptoms were soon apparent in all phosphate treatments and reached a maximum in six weeks, after which recovery of the plants commenced. Marked growth increases due to phosphate and to zinc treatments were apparent.

The degree of severity of the zinc deficiency symptoms and the extent of the response to zinc treatment increased with the level of phosphate application. Analyses showed that, while phosphorus content increased, relative and absolute zinc contents were significantly reduced at all harvests with increasing phosphate application.

In this experiment, the reduction of relative zinc content in the tops of flax plants was sufficient to account for the effect of phosphate application on zinc deficiency symptoms and on response to zinc treatment.

## I. Introduction

Several workers have reported that applied phosphate has induced or accentuated zinc deficiency symptoms in plants. West (1938) described phosphate-induced zinc deficiency in citrus at Griffith. Millikan (1946, 1947), in Victoria, found that excess phosphate, applied in the field as superphosphate and in pots as disodium hydrogen phosphate, induced zinc deficiency symptoms in flax. Cass Smith and Harvey (1948) reported that superphosphate application accentuated zinc dieback in flax in Western Australia. Reuther and Crawford (1948) found that a very heavy application of triple superphosphate to a Californian soil produced foliage symptoms typical of zinc deficiency in grapefruit.

There does not appear to be any evidence that zinc is fixed in the soil by the formation of an insoluble zinc phosphate. On the other hand, there is some evidence that zinc is not fixed by phosphate application. Peech (1941), investigating three light Florida soils, found that, though the phosphorus content varied considerably, they reacted similarly to zinc fixation. In a survey study, Jamison (1943*a*) produced strong evidence for several Florida soils that phosphate, both naturally occurring and applied as superphosphate, does not cause

* Institute of Agriculture, University of Western Australia, Nedlands, W.A.

fixation of zinc, except with extreme excesses of both applied phosphate and applied zinc (20,000 lb. per acre of superphosphate containing 6 per cent. water-soluble phosphoric anhydride in conjunction with 750 lb. per acre of zinc sulphate). Where treatments caused changes in pH, effects on the fixation of zinc were noticed, but they were shown to be due to pH changes. Further, he reported (Jamison 1943*b*) that the addition of superphosphate to the soil increased the mobility of zinc owing to the replacing action of calcium and the fact that zinc phosphate is somewhat more soluble than the humate.

It appears that phosphate affects the utilization of zinc by the plant in some other way. Millikan (1940), in a field experiment with wheat, obtained a linear relationship between zinc and phosphorus concentrations in the young plants. He postulated that application of superphosphate increased the "zinc requirement" of plants, and believed that this gave a partial explanation of phosphate-induced zinc deficiency. Over a wider range of zinc and phosphorus concentrations, Piper and Walkley (1943) confirmed the linear zinc-phosphorus relationship for mature oats, but they suggested that zinc impurities in the superphosphate used (Walkley 1940) might have been responsible for the relationship found. In an investigation on the effect of varying rates of application of phosphate (dicalcic phosphate) on the uptake of zinc by oat plants, Rogers and Wu (1948) found in young and mature plants that, as the phosphorus content of the plants increased, zinc content decreased to an almost constant value. Their results strongly support the view that zinc impurities in the superphosphate were responsible for the linear zinc-phosphorus relationship obtained by Millikan (1940) and Piper and Walkley (1943). Furthermore, although they did not discuss their results in the light of phosphate-induced zinc deficiency, the significant decrease in relative zinc content with high phosphate application found by Rogers and Wu (1948) gives a possible explanation of the phosphate-induced zinc deficiency discussed earlier.

The work reported in this paper was undertaken to obtain further information on the effect of phosphate on zinc uptake and its relationship to zinc deficiency in plants. Data for dry weight, phosphorus content, and zinc content of flax plants grown under varying levels of phosphate application are reported for different stages of development.

## II. Experimental

### (*a*) *Description of Experiment*

Flax plants (*Linum usitatissimum* var. WADA) were grown in glazed porcelain pots each containing 2 kg. of Kojonup gravelly sand (pH 6.2) obtained from an area that had shown severe zinc deficiency symptoms and marked yield increases with zinc treatment in flax and oat crops. The pots were kept in a glass-roofed bird-cage—the galvanized wire netting surrounding the bird-cage was painted with a bitumen-base enamel to prevent zinc contamination.

Seventy seeds were sown in each pot on May 18, 1950. Germination commenced on May 23 and plants were thinned to 65 per pot seven days later and finally to 55 per pot 12 days after germination.

All pots received the following nutrients (in g. per pot) two days after sowing:

$CaCl_2.6H_2O$, 0.28 g. ; $KNO_3$, 0.28 g. ; $K_2SO_4$, 0.14 g. ; $MgSO_4.7H_2O$, 0.07 g. ; $CuSO_4.5H_2O$, 0.009 g. ; $FeSO_4.7H_2O$, 0.009 g. ; $MnSO_4.4H_2O$, 0.018 g. ; $Na_2B_4O_7$, 0.003 g. ; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.001 g. On a surface area basis, 0.14 g. per pot = 112 lb. per acre.

All pots were maintained at 60 per cent. water-retaining capacity of the soil by frequent application of water distilled through pyrex glass apparatus.

Six treatments were imposed: phosphorus was applied at three levels with and without zinc. The scheme may be represented:

$$\left.\begin{matrix} P_0 \\ P_1 \\ P_2 \end{matrix}\right\} 3 \times \left.\begin{matrix} Zn_0 \\ \\ Zn_1 \end{matrix}\right\} 2$$

where $P_0$ = no treatment, $P_1$ = 0.32 g. per pot $NaH_2PO_4.2H_2O$, $P_2$ = 0.96 g. per pot $NaH_2PO_4.2H_2O$, $Zn_0$ = no treatment, $Zn_1$ = 0.018 g. per pot $ZnSO_4.7H_2O$. On a surface area basis, 0.32 g. per pot $NaH_2PO_4.2H_2O$ = 560 lb. per acre superphosphate (20 per cent. water-soluble $P_2O_5$). The salts (AR grade) were applied in solution three days after sowing. The pH of the soil was 6.2 and was not affected by treatment.

Harvests were made on the following days after germination:

Harvest I, 11 days, 9 pots; Harvest II, 21 days, 6 pots; Harvest III, 43 days, 3 pots; Harvest IV, 71 days, 3 pots. The number of pots refers to the number per treatment harvested. At each harvest, the plants were cut at the cotyledons and the tops dried overnight in an oven at 105°C., cooled in a desiccator, and weighed. At Harvests III and IV, the freshly harvested plants were sorted into groups based on the severity of zinc deficiency symptoms. The material was then divided into portions for zinc and phosphorus analyses (care being taken to see that plants from each severity group were equally represented) before drying in the oven. Dry weight data for the total tops are presented in Table 1A. Analysis for variance was used in the statistical analysis.

### (*b*) *Analytical Methods*

Phosphorus was determined in the tops of the flax plants by the molybdenum blue procedure. At Harvest I, two replicate determinations were made on each treatment. One pot was used for each determination. For all other harvests, three replicate determinations were made on each treatment. At Harvest II, the material from one pot was used for each determination, while at Harvests III and IV, an aliquot from each pot (separated as described above) was used for each determination. The means of the determinations are presented in Table 1*D*.

Zinc was determined polarographically in a Cambridge Voltamoscope using a nitrogen gas chain. The method used was that employed by Walkley (1942). Three replicate determinations were made for each treatment at each

TABLE 1

| Harvest / Treatment | | I $Zn_0$ | I $Zn_1$ | I Mean | II $Zn_0$ | II $Zn_1$ | II Mean | III $Zn_0$ | III $Zn_1$ | III Mean | IV $Zn_0$ | IV $Zn_1$ | IV Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *A.* Mean dry weight of total tops (g. per pot) | $P_0$ | 0.249 | 0.243 | 0.246 | 0.398 | 0.394 | 0.396 | 0.64 | 0.72 | 0.68 | 1.36 | 1.34 | 1.35 |
| | $P_1$ | 0.259 | 0.262 | 0.261 | 0.439 | 0.417 | 0.428 | 0.97 | 1.15 | 1.06 | 2.71 | 3.00 | 2.86 |
| | $P_2$ | 0.243 | 0.235 | 0.239 | 0.427 | 0.441 | 0.434 | 1.02 | 1.28 | 1.15 | 2.47 | 3.30 | 2.89 |
| | Mean | 0.250 | 0.247 | | 0.421 | 0.414 | | 0.88 | 1.05 | | 2.18 | 2.55 | |
| *B.* Relative amounts of zinc (p.p.m. dry wt.) | $P_0$ | 64 | 83 | 74 | 33 | 40 | 37 | 41 | 45 | 43 | 45 | 52 | 49 |
| | $P_1$ | 58 | 72 | 65 | 31 | 38 | 35 | 27 | 32 | 30 | 20 | 23 | 22 |
| | $P_2$ | 54 | 65 | 60 | 26 | 28 | 27 | 24 | 25 | 25 | 18 | 20 | 19 |
| | Mean | 59 | 73 | | 30 | 35 | | 31 | 34 | | 28 | 32 | |
| *C.* Absolute amounts of zinc (μg. per pot) | $P_0$ | 16 | 20 | 18 | 13 | 16 | 15 | 26 | 32 | 29 | 61 | 70 | 66 |
| | $P_1$ | 15 | 19 | 17 | 14 | 16 | 15 | 26 | 37 | 32 | 54 | 69 | 62 |
| | $P_2$ | 13 | 15 | 14 | 11 | 12 | 12 | 24 | 32 | 28 | 44 | 66 | 55 |
| | Mean | 15 | 18 | | 13 | 15 | | 25 | 34 | | 53 | 68 | |
| *D.* Relative amounts of phosphorus (% dry wt.) | $P_0$ | 0.37 | 0.34 | 0.36 | 0.22 | 0.23 | 0.23 | 0.14 | 0.13 | 0.14 | 0.12 | 0.11 | 0.12 |
| | $P_1$ | 0.50 | 0.46 | 0.48 | 0.25 | 0.33 | 0.29 | 0.41 | 0.32 | 0.37 | 0.21 | 0.19 | 0.20 |
| | $P_2$ | 0.90 | 0.82 | 0.86 | 0.53 | 0.51 | 0.52 | 0.84 | 0.64 | 0.74 | 0.55 | 0.40 | 0.48 |
| | Mean | 0.59 | 0.54 | | 0.33 | 0.36 | | 0.46 | 0.36 | | 0.29 | 0.23 | |

| | Level of Sig. | I 5% Level | I 1% Level | II 5% Level | II 1% Level | III 5% Level | III 1% Level | IV 5% Level | IV 1% Level |
|---|---|---|---|---|---|---|---|---|---|
| Analysis of variance—differences required for significance | *A* All values | — | — | — | — | 0.171 | 0.239 | 0.174 | 0.244 |
| | Mean Zn | — | — | — | — | 0.099 | 0.138 | 0.100 | 0.141 |
| | Mean P | 0.0170 | — | — | — | 0.121 | 0.169 | 0.123 | 0.172 |
| | *B* All values | 13.5 | 18.9 | 6.9 | 9.6 | 5.1 | 7.1 | 5.1 | 7.2 |
| | Mean Zn | 7.8 | 8.9 | 4.0 | — | 2.9 | 4.1 | 3.0 | — |
| | Mean P | 9.5 | — | 4.9 | 6.8 | 3.6 | 5.0 | 3.7 | 5.1 |
| | *D* All values | 0.171 | 0.240 | 0.143 | 0.200 | 0.112 | 0.157 | 0.044 | 0.062 |
| | Mean Zn | — | — | — | — | 0.065 | 0.091 | 0.026 | 0.036 |
| | Mean P | 0.121 | 0.170 | 0.101 | 0.142 | 0.079 | 0.111 | 0.031 | 0.044 |

harvest. At Harvest I, two pots were bulked for each determination. At Harvest II, one pot was used for each replicate. At Harvests III and IV, determinations were made on aliquots from each pot (see above). Mean values for relative zinc and statistical analyses are presented in Table 1*B*.

Absolute zinc was calculated from the means of the dry weight and the means of relative zinc, and the values are presented in Table 1*C*. In interpreting these data, it must be remembered that, to obtain sufficient material for analysis, 65 plants per pot were harvested at Harvest I and only 55 at succeeding harvests.

## III. Results and Discussion

### (*a*) *Effects of Zinc and Phosphorus on Growth*

At Harvest I, there were no visible effects of zinc on phosphorus treatments, but analysis of the yield data showed that $P_2$ gave a small significant depression of dry weight relative to $P_1$. Williams (1936) obtained a similar reduction of dry weight in oats during adolescence. By Harvest II, no significant differences were apparent from the dry weight data. However, the apices of plants of treatments $P_1$ and $P_2$, were visibly more vigorous than $P_0$ and, within another two days, growth increases due to phosphorus application were clearly visible ($P_2 > P_1 > P_0$).

On June 19, 28 days after germination, a necrotic spotting of leaves near the crown in some plants of $P_2Zn_0$ was noticed, and crowns of the plants of this treatment appeared less healthy than those of $P_2Zn_1$. The symptoms were suggestive of zinc deficiency. An examination on June 26 showed widespread zinc deficiency symptoms from mild (leaf bronzing, mild rosetting) to severe (severe rosetting, dieback) as described by Millikan (1942, 1946) and Cass Smith and Harvey (1948). The severity of the symptoms was dependent on phosphate treatment. $P_2Zn_0$ plants were the most severely affected; $P_2Zn_1$ were less severely affected, while $P_1Zn_0$ showed symptoms in relatively few plants. There were no symptoms of zinc deficiency in $P_1Zn_1$, $P_0Zn_0$, or $P_0Zn_1$.

The severity of the symptoms continued to increase until the time of Harvest III, when they reached a maximum. At this stage, the mean numbers of affected plants per pot were as follows:

| | $P_0$ | $P_1$ | $P_2$ |
|---|---|---|---|
| $Zn_0$ | 0 | 11 | 34 |
| $Zn_1$ | 0 | 1 | 17 |

The dry weight data for this harvest (Table 1*A*) show no significant effect of zinc at $P_0$, a significant increase at $P_1$, and a highly significant increase at the $P_2$ level. Both the relative and absolute effect of zinc on growth increased with each phosphate application. Also, it is obvious that phosphate treatments increased growth. The increase of $P_2$ over $P_1$ is not significant, but both treatments show highly significant increases over $P_0$. The possible influence of these growth differences on the zinc responses through dilution of zinc is discussed later.

Within a week after Harvest III, some diseased plants showed the typical symptoms of recovery from zinc deficiency by regrowth from rosetted apices or from the axils of the cotyledons. By Harvest IV all diseased plants showed signs of recovery but yield data revealed that responses to zinc and phosphorus were of the same order as in the previous harvest. With the significant depression in growth of $P_2Zn_0$ relative to $P_1Zn_0$ the effect of increasing phosphate application on response to zinc was even more obvious than at Harvest III.

From the evidence presented here it is clear that application of sodium dihydrogen phosphate to Kojonup gravelly sand induced zinc deficiency symptoms in flax plants and produced marked responses to zinc treatment. The severity of the symptoms and the relative response to zinc increased with increasing phosphate application.

### (*b*) *Zinc and Phosphorus Analyses*

The relative amount of phosphorus in the tops of the flax plants increased with increasing phosphate application at all harvests. In Harvests III and IV the differences in relative phosphorus content between zinc treatments may be explained by the promotion of growth by zinc application resulting in dilution of phosphorus.

The relative amount of zinc in the tops was depressed at every harvest by phosphate application. The depression of relative zinc content at Harvests III and IV may be partly accounted for by dilution of zinc through more rapid growth of phosphate treatments. But it is obvious that some factor other than dilution must have caused the significant depression of relative zinc content at Harvests I and II. Furthermore, the absolute amount of zinc in the tops was depressed at all harvests by phosphate application.

The data for relative zinc content confirm the results of Rogers and Wu (1948) discussed earlier. There is no evidence that high phosphorus content interferes with utilization of zinc in the tops of flax plants as suggested by Millikan (1940). However, the depression of relative zinc content in the tops of flax plants by the application of phosphate provides an adequate explanation for the effect of phosphate on growth responses to zinc treatment and on the incidence of zinc deficiency symptoms. This depression cannot be entirely accounted for by dilution effects resulting from growth responses; applied phosphate apparently exercises a direct effect on either the uptake of zinc by the roots, or the movement of zinc (Wood and Sibly 1950) from the roots to the tops of flax plants.

## IV. Acknowledgments

Grateful acknowledgment is made to the Commonwealth Research Grants to Universities for financial assistance and to Mr. R. C. Rossiter, Division of Plant Industry, C.S.I.R.O., and Dr. T. C. Dunne of the Western Australian Department of Agriculture for help and advice.

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