THE EFFECT OF NITROGEN ON ZINC DEFICIENCY IN SUBTERRANEAN CLOVER

By P. G. Ozanne*

[*Manuscript received September* 9, 1954]

Summary

Data are presented from four sand culture and one water culture trial.

Subterranean clover plants growing on a soil low in zinc showed increased severity of zinc deficiency symptoms when the nitrogen supply was increased. This effect was produced by applications of $NaNO_3$, L-asparagine, NH_4NO_3, and $(NH_4)_2SO_4$. It was not due to changes in soil pH, nor to increased growth demand arising from applied nitrogen.

Under conditions of low zinc supply, the zinc concentration in roots was found to be correlated with the percentage protein N present. The proportion of total zinc absorbed that was translocated to the plant tops was also found to be related to the percentage protein N in the roots.

It seems likely that increased nitrogen supply caused more of the zinc to be retained by the roots in zinc protein complexes. Under conditions of low zinc supply, this root retention led to severe symptoms of zinc deficiency in the plant tops.

I. Introduction

Responses to zinc-containing fertilizers are obtained over wide areas in the south-west of Western Australia, and have been reported by Teakle (1942), Dunne and Throssell (1948), Rossiter (1951*a*), and others. A feature of this zinc deficiency is that, in the same locality, the symptoms vary in severity from season to season and from year to year. During a study of the influence of light intensity on this varying response (Ozanne, unpublished data), it became apparent that the zinc requirement of subterranean clover is markedly affected by the supply of nitrogen available to the plant. As subterranean clover is an important component of some pastures in Western Australia, this zinc-nitrogen relationship was examined further.

Haas (1936), Chapman, Vanselow, and Liebig (1937), and Camp and Fudge (1945) mentioned that liberal nitrogen applications may produce zinc deficiency symptoms on citrus plants growing with a low zinc supply. These authors suggested that this zinc deficiency was partly induced by the increased growth with high nitrogen, and also partly by the rise in pH of the culture medium when $NaNO_3$ was used as a nitrogen source.

Reuther and Smith (1950), also working with citrus, reported a trial in which increasing levels of applied nitrogen led to increasingly severe symptoms of zinc deficiency. They suggested a nitrogen-zinc ratio effect on symptom

* Division of Plant Industry, C.S.I.R.O., Institute of Agriculture, University of Western Australia, Nedlands, W.A.

expression; but, as fruit yields increased considerably with each increase in added nitrogen, the crop demand must also be taken into account.

In this paper, nitrogen supply and nitrogen content are examined with regard to their effects on the growth and zinc content of subterranean clover.

II. Experimental Methods

(a) Soil Culture

Subterranean clover (*Trifolium subterraneum* L.) (Dwalganup strain) was grown from commercial seed inoculated with an effective strain of *Rhizobium*. The plants were grown in white glazed porcelain pots containing 2 kg of Muchea Sand which field trials by Rossiter (1951*a*) have shown to be deficient in zinc. This grey siliceous sand is too acid (pH 5·2) for good nodulation by subterranean clover and lime was mixed through the soil to raise the pH to about 6·7.

The following basal dressing (mg per pot) was applied at sowing: K_2SO_4, 210; $Ca(H_2PO_4)_2.H_2O$, 51; $MgSO_4.7H_2O$, 70; $MnSO_4.4H_2O$, 15; $FeSO_4.5H_2O$, 15; $CuSO_4.5H_2O$, 15; $(NH_4)_6 Mo_7O_{24}.4H_2O$, 1·5; H_3BO_3, 6. To keep some iron in solution, 15 mg of tartaric acid were added. In Experiments 1-4, 140 mg/pot is equivalent to 1 cwt/ac on an area basis.

All nutrients used were prepared from "analytical reagent" grade chemicals. "Pyrex" glass-distilled water only was used for watering the plants.

In each trial a factorial experimental design was used. To half the pots $ZnSO_4.7H_2O$ was added at 35 mg per pot to give a set of treatments with ample zinc.

In the absence of applied zinc, the plants showed zinc deficiency symptoms similar to those described by Rossiter (1951*b*) and Millikan (1953). In Experiments 1 and 3 the severity of the symptoms was recorded for each pot by counting the leaves present and expressing the number showing definite symptoms as a percentage of the total.

The tops and roots of all plants were harvested separately about 42 days after germination and while the plants were still in the vegetative stage of growth. At harvest the plant roots were washed from the soil, rinsed in diluted acetic acid, and then rinsed thoroughly in distilled water before being oven-dried at 85°C. The data presented in Tables 1-3 are mean values obtained from six pots.

Experiment 1.—Commencing at germination, 280 mg per pot of $NaNO_3$ were added at fortnightly intervals to the "high-nitrogen" treatments.

Experiment 2.—L-Asparagine monohydrate at 70 mg per pot was applied weekly to the high-nitrogen treatments as a source of nitrogen.

Experiment 3.—NH_4NO_3 (280 mg per pot) was applied at sowing to the high-nitrogen pots.

Experiment 4.—Four nitrogen treatments were used: no applied nitrogen; $NaNO_3$ at 70 mg per pot; $(NH_4)_2SO_4$ at 55 mg per pot; and L-asparagine at 62 mg per pot.

The latter three treatments were applied weekly and contained equivalent amounts of nitrogen.

(*b*) *Water Culture*

In Experiment 5, the subterranean clover plants were grown in 3-l. "Pyrex" beakers filled with a basal solution containing the following ions (mM per beaker): K^+, 8·5; Ca^{++}, 2·5; Mg^{++}, 2·0; NO_3^-, 5·0; $H_2PO_4^-$, 3·0; $SO_4^=$, 4·0; also the following elements (p.p.m.) Cu, 0·02; Mn, 0·50; B, 0·50; Mo, 0·01; Fe, 0·50 p.p.m. in sequestered form with "Versene."

Three levels of N supply were obtained by adding NH_4NO_3 at nil, 5mM and 15mM per beaker. The two levels of zinc added were nil, and 30 μg per beaker.

Stock solutions of the major elements were saturated with H_2S to remove zinc impurities. All culture solutions were kept at approximately pH 6·4.

TABLE 1

EFFECT OF NITROGEN AND ZINC SUPPLY ON YIELD AND COMPOSITION OF PLANT TOPS, EXPERIMENT 1. VALUES GIVEN REPRESENT MEANS FROM 6 POTS

Treatments	Dry Weight (g)	Increase in Weight with Zn (g)	No. of Leaves with Symptoms (%)	Protein N in Tops (% dry wt.)	Zn in Tops (p.p.m.)
Low Zn, low N	1·91	—	34	2·05	25·0
Low Zn, high N	1·08	—	84	2·91	17·0
High Zn, low N	2·70	0·79	—	2·20	71·3
High Zn, high N	2·97	1·89	—	2·22	64·6
L.S.D. of two means at $P = 0·01$	0·77	1·08	—	—	—

(*c*) *Chemical Analysis*

Zinc determinations were made using the wet digestion method of Piper (1944) and a modification of the photometric technique described by Cowling and Miller (1941).

Protein N was determined by the micro-Kjeldahl method. In Experiments 1, 3, and 4, the fresh plant material was dried at 85°C, and then the protein N precipitated from a trichloracetic acid solution at pH 4·5. In Experiment 5, the freshly harvested plant roots were rinsed, then immersed in boiling 80 per cent. alcohol for 1 min. After standing overnight in the 80 per cent. alcohol, the insoluble material was filtered off and protein N determined in it. Soluble zinc was determined in the filtrate.

III. RESULTS AND DISCUSSION

(*a*) *Effect of Nitrogen Supply on Growth and Zinc Content*

Experiment 1.—Previous experience has shown that clover readily uses $NaNO_3$ as a source of nitrogen, and in Experiment 1 the increase in protein

N shows that the nitrate was assimilated (Table 1). Early in this trial the plants nodulated well and, where zinc was added, the growth response to nitrogen was small.

In the absence of applied zinc, plant growth was markedly reduced by the addition of nitrogen. Decreased growth was associated with increased severity of zinc deficiency symptoms, and reduced zinc concentration in the plant tops.

In this experiment the addition of $NaNO_3$ caused a rise in soil pH from 6·65 to 7·15.

Experiment 2.—The treatments here were similar to those in Experiment 1 but an attempt was made to prevent the possible effects of change in soil pH on zinc absorption by using L-asparagine as a source of nitrogen. Ghosh and Burris (1950) and Vantsis and Bond (1951) reported that clover is able to use nitrogen from L-asparagine. This compound was applied to the "high nitrogen" pots and gave a large growth response in the presence of zinc (Table 2). No differences in soil pH were caused by the addition of asparagine. At the low zinc level, on the other hand, additional nitrogen gave an insignificant change in plant size. However, the amount of zinc present in the plant leaves was greatly reduced.

Table 2

INTERACTION OF NITROGEN WITH ZINC RESPONSE, EXPERIMENT 2. VALUES GIVEN REPRESENT MEANS FROM 6 POTS

Treatments	Dry Weight (g)	Increase in Weight with Zn (g)	Zn in Leaves (p.p.m.)
Low Zn, low N	1·06	—	34·1
Low Zn, high N	1·15	—	22·7
High Zn, low N	2·24	1·18	120·1
High Zn, high N	5·13	3·98	132·6
L.S.D. of two means at $P = 0·01$	0·22	0·31	—

Experiment 3.—NH_4NO_3, used as nitrogen source, gave the same marked nitrogen by zinc interaction as did asparagine in Experiment 2 (Table 3). The soil pH was unchanged by the added NH_4NO_3.

In this trial the plant roots were analysed for zinc as well as the tops. Surprisingly, the "low Zn, high N" plants, showing severe symptoms, were found to contain more zinc in the roots than the "low Zn, low N" plants. Even under ample zinc conditions, where added nitrogen gave considerably larger plants, it also caused greater root concentration of zinc.

In Experiments 1, 2, and 3, when the nitrogen status of low zinc plants was increased, more severe symptoms developed and zinc content of the aerial parts was reduced. High nitrogen supply must then have (1) reduced zinc absorption by the roots, or (2) reduced translocation of absorbed zinc to the plant tops. The increased zinc content of "high N" roots in Experiment 3 indicates that the latter cause was the most likely.

(*b*) *Zinc Distribution in Relation to Protein Nitrogen*

Jacobson and Overstreet (1947) suggested that absorbed ions are fixed in the plant cells in the form of chemical compounds which are stable in living tissue but quite labile in dead. They mentioned proteins, amino acids, and organic acids as forming such chelated compounds, especially with polyvalent cations.

TABLE 3

EFFECT OF NITROGEN ON YIELD, ZINC CONTENT, AND ZINC DEFICIENCY SYMPTOMS, EXPERIMENT 3. VALUES REPRESENT MEANS FROM 9 POTS

Treatments	Total Tops Dry Weight (g)	Increased Weight with Zn (g)	No. of Leaves with Symptoms (%)	Protein N* (% dry wt.)	Zn Content	
					Leaves (p.p.m.)	Roots (p.p.m.)
Low Zn, low N	0·550	—	33	1·69	22·9	132
Low Zn, high N	0·485	—	63	2·20	21·5	162
High Zn, low N	0·928	0·378	—	1·51	60·3	210
High Zn, high N	1·350	0·865	—	1·72	70·6	359
L.S.D. of two means at $P = 0{\cdot}01$	0·225	0·318	—	0·15	2·9†	25†
					11·1‡	36‡

* Protein nitrogen in stems and petioles.
† Low Zn.
‡ High Zn.

In Experiment 3, increased nitrogen supply gave rise to increased protein N, and also increased retention of zinc within the roots. It was considered likely that protein compounds in the roots may have formed poorly dissociated chelate complexes with some of the absorbed zinc, and thus prevented its translocation to the plant tops. Two trials were carried out to investigate this possibility.

Experiment 4.—Here plants using the various nitrogen sources NH_4NO_3, $(NH_4)_2SO_4$, and asparagine were compared with plants relying solely on atmospheric N made available after symbiotic fixation in the root nodules. As in the previous trials, increased nitrogen supply from all sources gave increased zinc response.

After harvest the plant material was dried and the protein compounds precipitated from solution by trichloracetic acid. Zinc determinations were made on both the precipitated material and on the soluble filtrate. Only some 12 per cent. of the total zinc in the plant roots was found to be precipitated with the protein material using this method.

To see if a relationship existed between the protein content and zinc content of the plant roots, the percentage protein N found was graphed against the p.p.m. total zinc present. As may be seen from Figure 1, the concentration of zinc present in the plant roots was positively correlated with the percentage protein N.

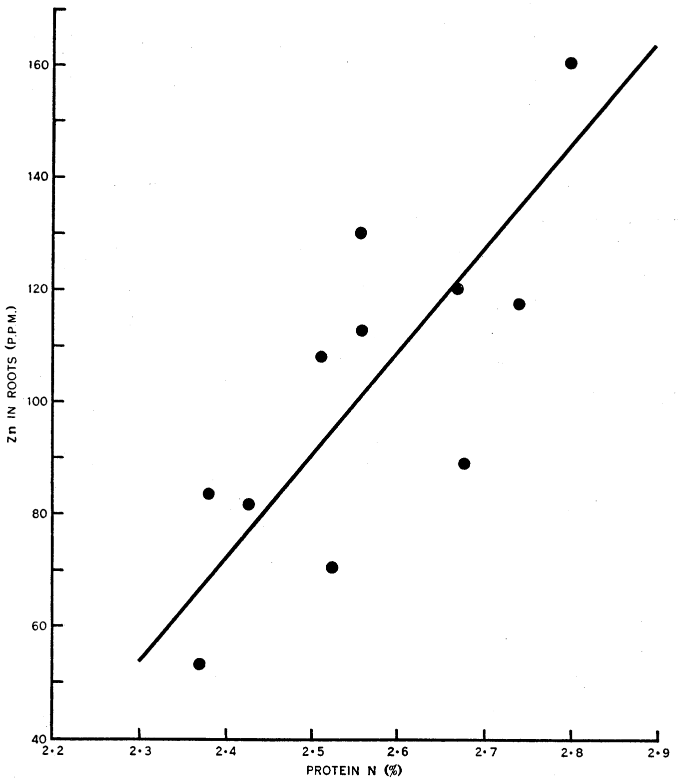

Fig. 1.—Relationship of protein nitrogen to concentration of zinc present in plant roots. The line drawn from the regression equation $y = -370 + 184x$; and $r = 0{\cdot}81$ significant at $P = 0{\cdot}01$.

Only values from "low Zn" roots were included for it was thought that the relationship might be different under the conditions of luxury absorption with "high Zn." Although all plants had the same low zinc supply, those roots which formed more protein also contained more zinc. The work of Bean (1940), Wood and Sibly (1952), and others has shown that zinc is necessary for protein synthesis. However, Glasstone (1947) found a very low zinc requirement for tomato roots. Also experiments by the author have shown that clover roots may continue to grow in an almost zinc-free medium without decrease in protein N. These observations suggest that the requirement of zinc by subterranean clover roots is at least no higher than the requirement of the tops, and that the relatively high concentrations of zinc found in the roots serve no useful purpose. It was shown in Figure 1 that increased protein N is associated with increased zinc in the roots. Figure 2 shows that protein N is closely related to the power roots have to retain zinc at the expense of the plant tops.

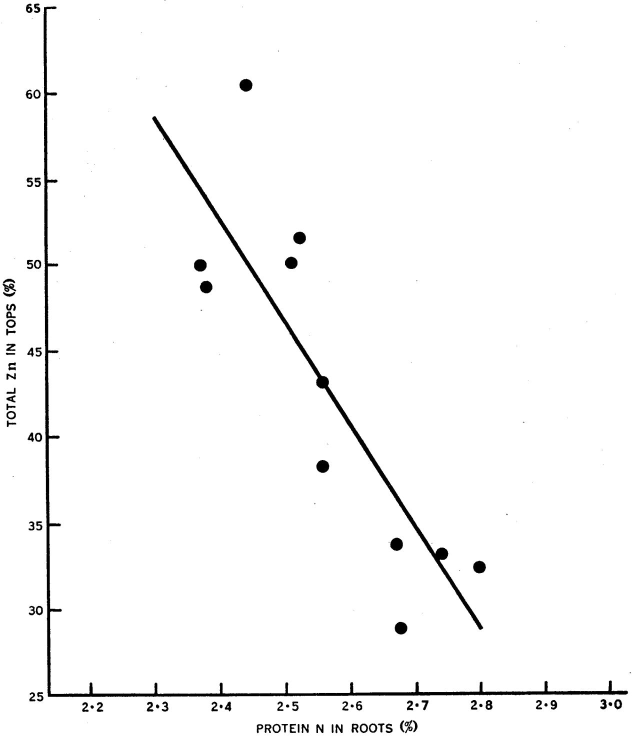

Fig. 2.—Relationship between concentration of protein nitrogen in the roots and the proportion of the total absorbed zinc translocated to the plant tops. The line is drawn from the regression equation $y = 196 - 59{\cdot}7x$; and $r = -0{\cdot}85$ significant at $P = 0{\cdot}001$.

As so little of the zinc in the plant roots was precipitated from solution with the protein material the possibility remained that the zinc had not formed complexes with the root proteins, but perhaps with some other compounds which varied in concentration with protein N.

Experiment 5.—In this trial the plants were grown in culture solutions containing three levels of nitrogen supply and two of zinc. At harvest, fresh weights were taken and aliquots of the plant roots were killed immediately in boiling 80 per cent. ethanol. After standing, protein N and insoluble zinc were determined on the precipitate from this solution and soluble zinc from the filtrate plus washings.

Using this less severe technique, 75 per cent. of the root zinc was recovered from the protein precipitate, compared with the 12 per cent. in Experiment 4. This suggested that the root zinc is held in a labile metallo-organic complex which was largely decomposed by the analytical technique used in Experiment 4. The small amounts of soluble zinc obtained from the roots of Experiment 5 were expressed as p.p.m. of the oven-dry root weights and graphed against percentage protein N as shown in Figure 3. The close positive correlation between soluble zinc and protein N suggests that this fraction of the zinc was also linked to the protein compounds of the living plant.

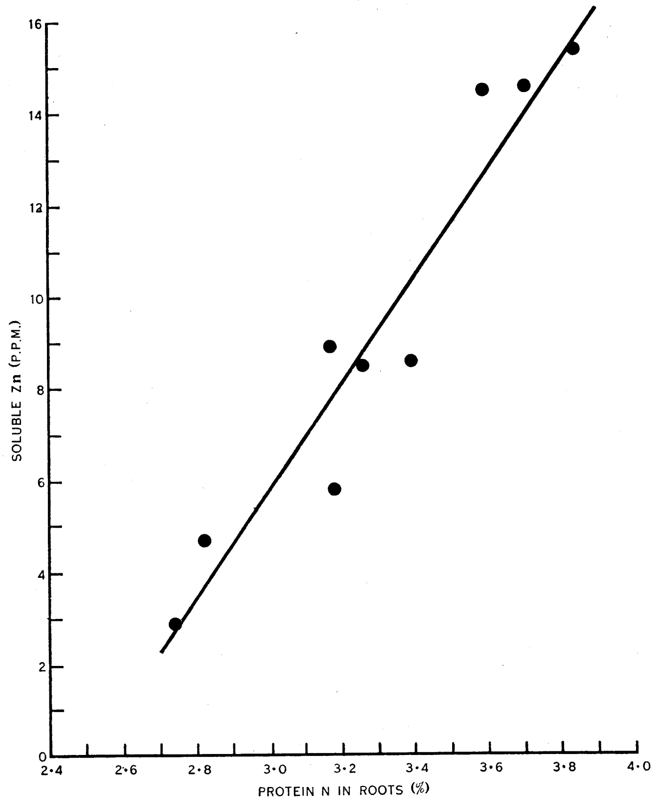

Fig. 3.—Relationship of soluble zinc to protein nitrogen precipitated from solution by boiling 80 per cent. ethanol. The line is drawn from the regression equation $y = -29 \cdot 3 + 11 \cdot 7x$; and $r = 0 \cdot 96$ significant at $P = 0 \cdot 001$.

Thus it seems probable that all the zinc present in the plant roots was in combination with the root protein in the living plant. As roots can retain relatively high concentrations of zinc while the tops are suffering severely from zinc deficiency it might be expected that very little soluble or uncomplexed zinc would be present in roots when the zinc supply is low.

From the experiments described here it was concluded that increasing the nitrogen supply available to subterranean clover causes more of the absorbed zinc to be retained by the roots in a poorly dissociated zinc-protein complex.

Under conditions of low zinc supply, this increased root retention is able to cause severe symptoms of zinc deficiency in the plant tops.

It is of interest to compare the results described above with recent work on copper accumulation by plants. Seymour (1951) found that tung plants given a high nitrogen supply did not translocate copper to the leaves. From work on citrus trees, Smith (1953) reported that copper accumulation by citrus roots appears to be directly related to the non-replaceable protein nitrogen of the roots.

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