THE EFFECT OF LIGHT ON ZINC DEFICIENCY IN SUBTERRANEAN CLOVER (*TRIFOLIUM SUBTERRANEUM* L.)

By P. G. OZANNE*

[*Manuscript received March* 3, 1955]

Summary

The effects of light on zinc response were investigated by growing subterranean clover under daylight of various intensities. Plants were grown in limed Muchea sand and the zinc response was measured as the difference in dry weight between plants with and without added zinc. The light intensities are given as the mean daily maximum intensities.

Zinc responses increased as light intensities increased from 200 f.c. up to 3000-4000 f.c. Further increases up to 11,000 f.c. caused a decrease in zinc response. Maximum zinc responses occurred at light intensities which were near saturation levels for photosynthesis.

Increased light intensities gave decreased concentration of zinc in the green leaves of the plants.

Plants grown under an 11¾-hr day showed a much greater zinc response than plants under a 7½-hr day. The long day plants took up more zinc than the short day plants but retained a relatively larger proportion of zinc in the roots.

The relation between light intensity and zinc response is discussed.

I. INTRODUCTION

In the south-west of Western Australia, plant responses to zinc-containing fertilizers have been frequently reported (e.g. Teakle 1942; Dunne and Elliot 1950). Although these responses to zinc are widespread, they are by no means consistent, and in the same field, may vary from month to month and year to year. Deficiency symptoms are usually most severe in the winter months under low light intensity and short day conditions, the affected plants recovering towards summer (Dunne, Smith, and Cariss 1949; Rossiter 1952).

Experiments reported by Hoagland (1944) and Trumble and Ferris (1946) show that light intensity and daily light duration can influence zinc deficiency. However, in contrast to the field observations mentioned above, Skoog (1940) and Millikan (1953) reported that zinc deficiency symptoms were more severe under brighter light. These workers grew most of their experimental plants in water culture. Under such conditions the effects which light may have on the ability of plants to absorb zinc from the relatively unavailable sources in the soil cannot be shown.

The experiments described in this paper were designed to examine the effects of a wide range of light intensities on plants growing in a zinc deficient soil.

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

II. Experimental Methods

Subterranean clover plants (*Trifolium subterraneum* L.) were grown from commercial seed inoculated with an effective strain of *Rhizobium*. The plants were grown in white, glazed porcelain pots each containing 2 kg of Muchea sand which field trials by Rossiter (1951*a*) have shown to be zinc deficient. The topsoil used is a grey sand consisting mainly of silica with about 3 per cent. organic matter; it contains approximately 0·7 p.p.m. of zinc. The sand is too acid (pH 5·2) for good nodulation by subterranean clover and in all experiments 2·8 g $CaCO_3$ were mixed in dry to each pot. This raised the soil pH to 6·5 and led to good nodulation.

In addition to the 2·8 g of $CaCO_3$, 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; $(NH_4)_6Mo_7O_{24}.4H_2O$, 1·5; H_3BO_3, 6. To prevent iron deficiency symptoms, 15 mg of tartaric acid were added to the iron solution. This basal dressing was prepared from A.R. chemicals.

Pots were maintained at 40 per cent. of the water holding capacity of the soil by additions of water distilled from a "Pyrex" all-glass apparatus.

In each trial half the plants were grown as low zinc (Zn_0) treatments with no added zinc, and to the remainder, 35 mg of $ZnSO_4.7H_2O$ per pot were added to give a set of high zinc (Zn_1) or complete nutrient control treatments.

The various light intensity treatments used over the range of 5000-12,000 f.c. represent full daylight in the glasshouse at different seasons between midwinter and midsummer. Light intensities between 200 and 4000 f.c. were obtained by shading the plants with white cloth screens of the required thickness. These screens were open at the sides to allow free air circulation (see Plate 1, Fig. 1). The light intensities were read at plant level on an E.E.L. light-meter.* They are expressed as the mean of the maximum daily readings taken during each experiment.

The mean day lengths reported were measured from sunrise to sunset as the light intensities at other times were considered to be too low to affect the vegetative growth of subterranean clover.

In every case, a factorial experimental design was used.

The tops and roots of all plants were harvested separately 42 days after germination and while still in the vegetative stage. At harvest, the roots were washed from the soil with tap water, then washed in dilute acetic acid, washed in distilled water, and finally dried in an oven at 85°C. The tops were hand separated into green leaves, dead leaves, and stems plus petioles, then oven dried at 85°C.

(*a*) *Experiments* 1-3

Experiment 1 germinated on January 1, 1952. Mean day length during the trial was 13 hr 50 min. At sowing, 140 mg of $NaNO_3$ plus an equivalent amount of HNO_3 were applied to each pot. Two light intensities were used—340 and

* Made by Evans Electroselenium Limited, Harlow, Essex.

750 f.c. Two strains of clover were grown—Dwalganup and Bacchus Marsh. Mean daily maximum and minimum temperatures were 30·0 and 18·9°C.

Experiment 2 germinated on February 26, 1952. Mean day length was 13 hr 7 min. At sowing, 35 mg of $NaNO_3$ plus an equivalent amount of HNO_3 were applied to each pot. Two light intensities were used—220 and 600 f.c. Dwalganup and Bacchus Marsh strains of subterranean clover were sown. Each treatment was replicated four times. Mean daily maximum and minimum temperatures were 30·0 and 18·9°C.

Experiment 3 germinated on April 7, 1952. Mean day length was 10 hr 57 min. No nitrogen was applied. Dwalganup strain only was sown. Light intensities used were 1050 and 7600 f.c. Three replications were sown. Mean daily maximum and minimum temperatures were 31·2 and 14·5°C.

(*b*) *Experiment* 4

Seed germinated on March 3, 1953. At sowing, NH_4NO_3 (350 mg per pot) was applied to half the pots. Three light intensities were used–"low" light at 750 f.c., "medium" light at 1400 f.c., and "high" light at 8000 f.c. Half the plants were grown under "long" day conditions of 11¾ hr mean day length. The "short" day plants were covered daily at 2.0 p.m. with large light-tight black cardboard boxes and only received a mean of 7½ hr daylight.

The quantity of light received daily under the different treatments was found by recording the light intensity at short intervals throughout the day and plotting these values against time. By measuring the area under the curve so obtained, the mean quantity of light received daily under the various light treatments was found.

Dwalganup strain only was sown. Three replications were used. To ensure an even air temperature under all light intensities, the treatments were laid out in one glasshouse through which air was passed at a rate of 1 ft/sec. The air temperature, as read on a shaded thermometer, or the soil temperature did not vary to any extent from treatment to treatment. The mean daily maximum and minimum air temperatures were 30·7 and 17·2°C. The mean daily maximum and minimum relative humidity values were 89 and 50 per cent.

Zinc content of tops and roots was estimated using the wet digestion method described by Piper (1944) and a modification of the photometric method described by Cowling and Miller (1941).

Eight other trials, details of which will be reported elsewhere, were carried out using the same technique as described above. The mean daily maximum light intensities during these trials were recorded, and also the zinc responses for those fertilizer treatments used in experiments 1-4.

III. Results

(*a*) *Experiments* 1-3

In these trials, zinc deficiency symptoms as described by Rossiter (1951*b*) and Millikan (1953) appeared first within 16 days of germination on the low zinc treatments. Symptoms were about 3 days later in appearing on plants under the low light treatments of experiments 1 and 2. The mean yields from the treatments in experiments 1-3 are shown in Table 1. As no strain by zinc interaction was shown in experiments 1 and 2 the yields of the two strains are combined. The high plant weights of experiment 1 are probably due to the long day conditions and the relatively high level of applied nitrogen.

TABLE 1

EFFECT OF LIGHT INTENSITY ON THE ZINC RESPONSE OF SUBTERRANEAN CLOVER

Expt.	Light Intensity (f.c.)	Mean Dry Wt. Yield of Tops (g)		Zinc Response (Zn_1-Zn_0)	L.S.D. of Two Means		Zinc × Light Interaction
		High Zinc (Zn_1)	Low Zinc (Zn_0)		$P = 0\cdot05$	$P = 0\cdot01$	
1	340	0·765	0·566	0·199	—	—	Positive
	750	1·981	1·351	0·630			
2	220	0·259	0·222	0·037	0·059	0·081	Positive**
	600	0·808	0·647	0·161			
3	1050	0·875	0·648	0·227	0·121	0·183	Negative
	7600	0·662	0·554	0·108			

** Significant at $P = 0\cdot01$ level.

Increasing the light intensity from 340 to 750 f.c. in experiment 1 gave markedly increased growth and also greatly increased zinc response as measured by the difference in yield between the low and high zinc treatments. In experiment 2, these light effects were confirmed as both plant growth and zinc response were much greater under a light intensity of 600 than 220 f.c. However, these two trials were carried out at relatively low light intensities.

During experiment 3 the higher light value used was the full autumn sunshine received through the roof of the glasshouse. In contrast to the effects in experiments 1 and 2, increasing the light intensity in experiment 3 from 1050 to 7600 f.c. gave no increase in plant weight and the zinc response was decreased.

A marked effect of light intensity on plant form was observed in these trials. The plants grown under a mean daily maximum of only 220 or 340 f.c. were tall and spindly in appearance; plants under 1050 f.c. were large with broad leaves; while the plants under sunlight at 7600 f.c. were dense and compact with short petioles and small leaves.

TABLE 2

EFFECT OF LIGHT INTENSITY AND DAY LENGTH ON THE ZINC RESPONSE OF SUBTERRANEAN CLOVER (EXPT. 4)

Day Length	Light Treatment	Light Intensity (f.c.)	Light Quantity (f.c.h.)	Mean Dry Wt. Yield of Tops (g)		Zinc Response (Zn_1-Zn_0)	L.S.D. of Two Means		Zinc × Light Interactions
				High Zinc (Zn_1)	Low Zinc (Zn_0)		$P = 0 \cdot 05$	$P = 0 \cdot 01$	
Short day ($7\frac{1}{2}$ hr)	Low Medium High	750 1400 8000	3,700 7,100 43,900	0·354 0·740 0·500	0·252 0·429 0·341	0·102 0·311 0·159	0·093	0·126	Low *v.* Medium —Positive** Medium *v.* High —Negative*
Long day ($11\frac{3}{4}$ hr)	Low Medium High	750 1400 8000	5,000 9,600 61,300	0·875 1·803 1·691	0·460 0·664 0·979	0·415 1·139 0·712	0·230	0·314	Low *v.* Medium —Positive*** Medium *v.* High —Negative* Short day *v.* Long day—Positive***

* Significant at $P = 0 \cdot 05$ level.
** Significant at $P = 0 \cdot 01$ level.
*** Significant at $P = 0 \cdot 001$ level.

(b) *Experiment* 4

To clarify the apparently contradictory light effects of experiments 1 and 2 as compared with 3, experiment 4 was carried out using one relatively low light intensity, one intensity between those of experiment 3, and one bright sunshine intensity. The yields of this trial and the zinc responses obtained are shown in Table 2. As there was no significant interaction between applied nitrogen and zinc responses at different light intensities or at different day lengths, the yields of the two nitrogen treatments were combined.

Zinc deficiency symptoms occurred in experiment 4 as in the earlier trials, but did not appear on the low light short day treatments until day 24. However, by day 35, symptoms were showing on at least 40 per cent. of the leaves of all low zinc plants.

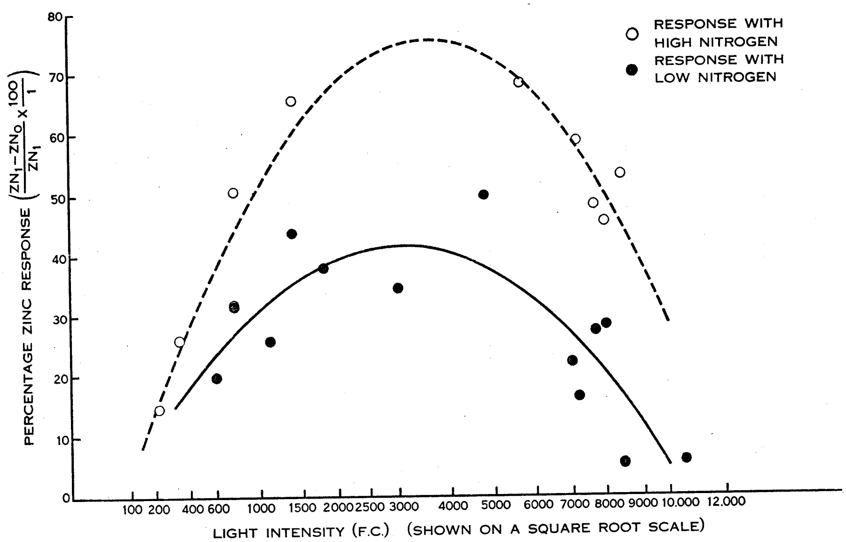


Fig. 1.—Zinc responses of subterranean clover plotted against light intensity. ○ Responses of plants supplied with ample combined nitrogen. The broken curve has been fitted to these points, and the multiple correlation coefficient (r) for these values is 0·94. ● Responses of plants relying on nitrogen from symbiotic nitrogen fixation in the root nodules. The solid curve has been fitted to these points, and $r = 0{\cdot}82$ for these values.

(i) *Light Intensity.*—In experiment 4 the increase in light intensity from 750 to 1400 f.c. gave rise to a large increase in plant weight and also in response to applied zinc. However, the increase in light from 1400 to 8000 f.c. intensity failed to increase the dry weight production of the high zinc plants, and gave a decreased zinc response.

The trend of dry weight of tops between medium and high light in long day-low zinc plants differed from that of the other three treatments. However, as in all other trials, under high light intensity the yield of the low zinc plants was closer to that of the high zinc plants, and the severity of zinc deficiency symptoms was greatly reduced.

The yields from experiment 4, taken in conjunction with those from experiments 1-3, show an increase with increased light intensity up to 1400 f.c. of daylight. Near this intensity light seems to reach the saturating level for photosynthesis, and increasing the light intensity above 3500 f.c. did not give increased plant weight.

As in experiments 1-3, the zinc responses obtained in experiment 4 suggest that, up to a certain level, increasing light intensity leads to increasing plant responses to zinc. In an attempt to find the level of light giving the maximum zinc response with subterranean clover, the experimental data from experiments 1-4 and the other eight trials referred to above were used. The zinc responses obtained in these trials are plotted against the mean daily light intensity in Figure 1.

TABLE 3

EFFECT OF LIGHT ON ZINC CONTENTS AND ROOT WEIGHT RATIOS (EXPT. 4)

Day Length	Light Intensity	Low Zinc Plants				Root Wt. Ratio* (%)
		Zinc in Green Leaves (p.p.m.)	Zinc in Roots (p.p.m.)	Total Zinc in All Leaves (μg)	Total Zinc in Roots (μg)	
Short day ($7\frac{1}{2}$ hr)	Low	30·7	187	3·5	8·8	15·9
	Medium	25·7	151	6·8	15·6	19·4
	High	24·2	174	5·3	20·4	26·0
Long day ($11\frac{3}{4}$ hr)	Low	26·7	145	5·6	18·0	19·2
	Medium	21·4	147	8·1	26·0	21·2
	High	20·9	127	11·6	40·0	27·1
		High Zinc Plants				
Short day ($7\frac{1}{2}$ hr)	Low	130	450	26	25	13·5
	Medium	74	278	32	44	17·8
	High	82	297	25	46	23·4
Long day ($11\frac{3}{4}$ hr)	Low	73	312	32	55	16·8
	Medium	54	246	51	116	20·9
	High	56	218	50	143	28·1

* Root wt./total wt. × 100.

For ease of comparison the zinc responses are graphed on a relative basis as the "percentage zinc response," or the difference in yields between high and low zinc treatments expressed as a percentage of the high zinc yield. As the responses to zinc are greater in plants with a high nitrogen supply (Ozanne 1955), these treatments are shown separately.

From Figure 1 it may be seen that the maximum response to zinc was given by the plants at about 3500 f.c. light intensity, and this peak was not moved to any extent by increasing the nitrogen status of the plants.

In experiment 4, as in the earlier trials, the increase in intensity of light had a progressive effect on the appearance of the plants which became more dense and compact with increasing light intensity as shown in Plate 1, Figure 2.

(ii) *Day Length and Quantity of Light*.—The plant yields under the various day length and light energy treatments are shown in Table 2. A large decrease in plant weight was obtained by reducing the daily duration of photosynthesis from 11¾ to 7½ hr. No increase in light intensity was able to compensate for the shorter day length. Although the medium light and high light treatments under short day received 7000 and 43,000 f.c.h. of light respectively, the plants were still smaller and their dry weight less than those under low light, long day conditions receiving only 5000 f.c.h. of light.

In spite of their smaller size, the short day plants responded in growth form to the different levels of light intensity in a similar way to the long day plants. But the zinc responses obtained at the shorter day length were smaller on both an absolute and relative basis than the response under the longer day conditions.

(iii) *Zinc Contents*.—The living green leaves are the principal light receptors of the plant and most of the photosynthetic processes are carried out within them. For this reason, the zinc content of the green leaves was determined separately from the other plant parts. In the case of the low zinc treatments, this separate analysis was carried out to find the minimum zinc concentration required by the leaves under the various light treatments.

In general, increased light led to greater total zinc uptake. However, growth increases in response to more light tended to produce a dilution effect on the absorbed zinc, i.e. greater production of dry matter than uptake of zinc. The zinc analyses of plant material from experiment 4 are given in Table 3.

The zinc concentration in green leaves of both high and low zinc plants decreased significantly between low and medium light treatments, though differences between medium and high light are probably not significant. The decreases in zinc content with increased light intensity are the more striking since the level of zinc concentration in roots is still high at higher light intensities. At both levels of zinc, the short day plants give a similar pattern of analyses to the long day plants, but zinc concentrations are higher, and total uptakes lower, under the short day.

IV. Discussion

The experimental evidence presented here indicates that the zinc requirement of subterranean clover is markedly affected by light intensity. Increasing zinc responses were obtained as light intensities were increased up to a daily maximum of about 3000-4000 f.c. Further increases in light intensity between 4000 and 11,000 f.c. gave corresponding decreases in zinc response. The latter range of light values was obtained by using light intensities received between midwinter and midsummer. Because of this, plants grown under light values of 5000-7000 f.c. received an appreciably shorter day length than plants grown under light of 8000-11,000 f.c. intensity. However, in experiment 4, it was found that the longer day length gave the greater zinc response. Hence the decrease in zinc response found over the range of 4000-11,000 f.c. is unlikely to have been caused by the increasing day length.

Strong solar radiation falling on the growing plant has many complex effects, e.g. increase in leaf blade temperature, increase in respiration rate, and increase in transpiration rate with a possible consequent decrease in leaf turgor and partial closure of the stomates. Hence the decreased zinc response at high light intensity probably has no single explanation. It is worth noting in experiment 4, however, that the concentration of zinc present in green leaves under full sunlight was substantially the same as that found under thin shade, even though the plants under thin shade suffered more severely from zinc deficiency. It appears then that the leaves growing under high light intensity were able to utilize the zinc present in them more efficiently than leaves growing under the medium light intensity.

Light showed some effect on the absorption and distribution of zinc. The long day plants took up more zinc than did the plants under short day, but they also retained a relatively larger amount of the absorbed zinc in the roots. A similar effect of long day on zinc distribution within the plant was reported by Ferres (1951), and is partly due to the increased root weight ratio under long day conditions.

The high concentration and high total zinc content of the roots relative to the leaves is very striking, especially in the treatments which gave rise to acute zinc deficiency. Work reported elsewhere (Ozanne 1955) indicates that much of the zinc found in roots is held in complexes and is not readily mobile. For this reason the total zinc content of the roots relative to the leaves is affected to a considerable extent by the root weight to total plant weight ratio. This ratio was increased by every increase in total light energy received, irrespective of day length or light intensity.

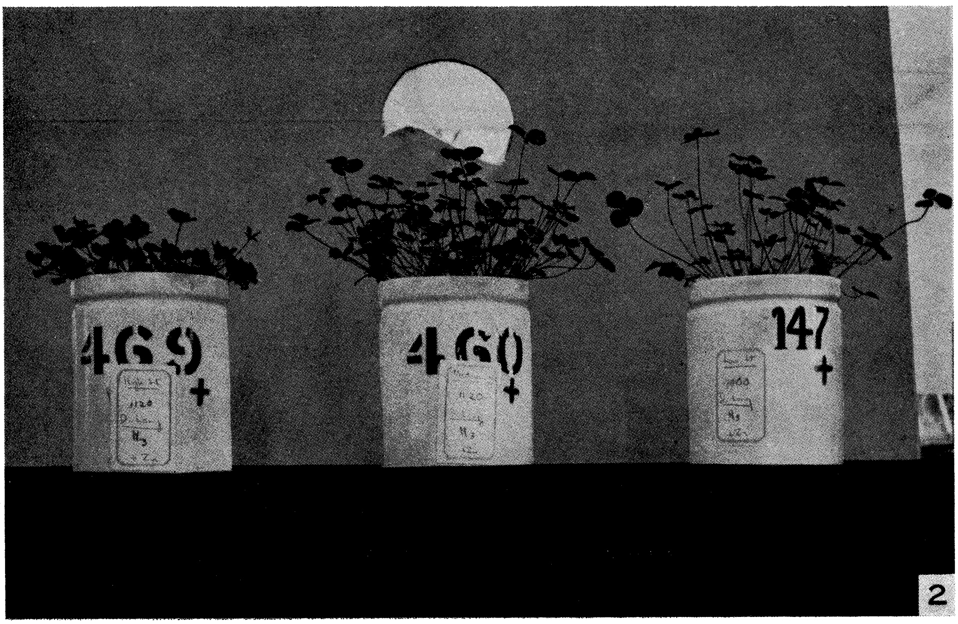
Reports from California (e.g. Hoagland 1944) state that corn, tomato plants, and citrus trees show zinc deficiency most frequently under summer conditions of high light intensity. By contrast, observations made in southern Australia indicate that subterranean clover, flax, and oats suffer most severely from zinc deficiency during the cloudy winter weather of lower light intensities. In so far as subterranean clover is concerned, these latter observations are supported by the experiments described here, in which the greatest responses to zinc were obtained under light values of the same order as those received in midwinter.

V. Acknowledgment

The author is indebted to Mr. N. Stenhouse, Division of Mathematical Statistics, C.S.I.R.O., for help with the statistical analyses presented in this paper.

EFFECT OF LIGHT ON ZINC DEFICIENCY IN CLOVER

1



2

VI. References

Cowling, H., and Miller, E. J. (1941).—Determination of small amounts of zinc in plant material. A photometric dithizone method. *Industr. Engng. Chem.* (*Anal.*) **13**: 145-9.

Dunne, T. C., and Elliot, H. G. (1950).—Zinc fertilizers for subterranean clover at Kendenup. *J. Agric. W. Aust.* **27**: 115-17.

Dunne, T. C., Smith, S. T., and Cariss, H. G. (1949).—Responses of oats to zinc, phosphate and copper on newly cleared light land in Western Australia. *J. Agric. W. Aust.* **26**: 75-82.

Ferres, H. M. (1951).—Influence of light and temperature on nutrient uptake and use, with particular reference to zinc. Proc. Spec. Conf. Plant Anim. Nutr. Aust. pp. 240-3.

Hoagland, D. R. (1944).—"Lectures on the Inorganic Nutrition of Plants." (Chronica Botanica Co.: Waltham, Mass.)

Millikan, C. R. (1953).—Relative effects of zinc and copper deficiencies on lucerne and subterranean clover. *Aust. J. Biol. Sci.* **6**: 148-78.

Ozanne, P. G. (1955).—The effect of nitrogen on zinc deficiency in subterranean clover. *Aust. J. Biol. Sci.* **8**: 47-55.

Piper, C. S. (1944).—"Soil and Plant Analysis." (University of Adelaide: Adelaide.)

Rossiter, R. C. (1951*a*).—Studies on the nutrition of pasture plants in the south-west of Western Australia. I. The effect of copper, zinc, and potassium on the growth of the Dwalganup strain of *Trifolium subterraneum* L. on sandy soils. *Aust. J. Agric. Res.* **2**: 1-13.

Rossiter, R. C. (1951*b*).—Studies on the nutrition of pasture plants in the south-west of Western Australia. II. Visual symptoms of mineral deficiencies on the Dwalganup strain of *Trifolium subterraneum* L. *Aust. J. Agric. Res.* **2**: 14-23.

Rossiter, R. C. (1952).—D.Sc. Thesis, University of Western Australia.

Skoog, F. (1940).—Relationships between zinc and auxin in the growth of higher plants. *Amer. J. Bot.* **24**: 939-51.

Teakle, L. J. H. (1942).—Experiments with micro-elements for the growth of crops in Western Australia. *J. Agric. W. Aust.* **19**: 242-53.

Trumble, H. C., and Ferres, H. M. (1946).—Responses of herbage legumes to applied nutrients on some southern Australian soils and their dependence on external factors. *J. Aust. Inst. Agric. Sci.* **12**: 32-43.

Explanation of Plate 1

Fig. 1.—General layout of light screens and pots in experiment 4. The medium light-short day plants are in the foreground, and in the background from left to right are: low light-short day, low light-long day, high light-short day, and high light-long day plants. Two of the light-tight boxes used to cover the short day plants may be seen.

Fig. 2.—From left to right—high light, medium light, and low light plants from experiment 4. The spindly growth of the low light plants and the short petioles of the high light plants contrast with the medium light growth.