RELATIVE EFFECTS OF ZINC AND COPPER DEFICIENCIES ON LUCERNE AND SUBTERRANEAN CLOVER

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Summary

The symptoms of zinc deficiency in subterranean clover (var. Dwalganup) and lucerne (var. Hunter River) are described. Bronze spotting was caused by the localized collapse of cells of the upper epidermis. Spherical coacervates which occurred in the underlying cells were different in appearance in the two species. Microchemical tests indicated the presence of phosphorus compounds in the membrane enveloping the coacervates of subterranean clover. The "little leaves" were variable in thickness owing to hypertrophy of the palisade cells. Reduced light delayed the date of appearance of these symptoms.

Copper deficiency produced a marked epinasty of the petioles of the younger leaves of lucerne. Grey to whitish necrotic spots later occurred on the lamina, and death of the top of the plant followed. In subterranean clover, this disorder induced an erect habit of growth and a marginal necrosis of the leaves.

Zinc deficiency caused a significantly greater reduction (1 per cent. level) in the top growth (dry weight) of subterranean clover when compared with lucerne, but there was no significant interaction in the effect of this treatment on roots or the whole plant (tops + roots) between the two species. For both species this treatment significantly reduced top growth more than root growth.

Copper deficiency had a significantly greater effect (1 per cent. level) on lucerne than on subterranean clover for dry weight of tops, roots, and whole plant.

The effect of zinc deficiency was significantly less (1 per cent. level) under reduced light than under full light conditions for both species. The amount of light had no effect on the severity of copper deficiency.

Zinc deficiency decreased the zinc concentration and increased the copper concentration in both lucerne and subterranean clover. However, with lucerne, the zinc concentration was lower, and the coefficient of utilization of zinc was higher, than for subterranean clover, at both normal and deficient levels of zinc in the substrate. For each species, the coefficient of utilization of zinc was higher under conditions of zinc deficiency.

In the control plants the coefficient of utilization of copper was highest in lucerne. Copper deficiency increased the value of this coefficient with subterranean clover, but reduced it in lucerne.

I. Introduction

It has been reported in the literature that lucerne will make satisfactory growth on certain soils on which other plants show severe symptoms of zinc deficiency. Thus Chandler, Hoagland, and Hibbard (1932); Ballard and Lindler (1934); Hoagland, Chandler, and Hibbard (1936); and Hoagland, Chandler,

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and Stout (1936) found that lucerne would grow without symptoms on soil in which fruit trees and other plants developed severe symptoms of "little leaf" or zinc deficiency. On soils of the Ninety-mile Plain in South Australia it has similarly been found that, whereas subterranean clover requires a combined dressing of copper and zinc, lucerne will respond to copper, but not to zinc (Riceman 1945, 1948).

These results suggest either that lucerne had a lower requirement for zinc than certain other types of plants, or that the lucerne roots were able to utilize soil zinc which was unavailable to the roots of the other plants.

Lucerne roots apparently have the property of making the soil zinc more available to other plants. Thus Ballard and Lindler (1934) reported that a cover crop of lucerne materially reduced the amount of "little leaf" in a vineyard so treated. Similarly Hoagland, Chandler, and Stout (1936) found that apricot and corn seedlings grown in pots of "little leaf" soil between lucerne plants established about a year remained almost free of "little leaf" and "white bud" symptoms whereas without the lucerne these symptoms of zinc deficiency were very acute.

In the water culture experiments of Hoagland, Chandler, and Hibbard (1936), the absence of zinc caused "little leaf" symptoms in a range of plants, but no symptoms occurred in the lucerne, although the yield of the control was "better than the minus zinc at one cutting." No yield data were given.

Zinc deficiency symptoms in lucerne grown in sand cultures have recently been described by Hewitt and Bolle-Jones (1951). Their data suggest that lucerne may make relatively better growth under conditions of zinc deficiency than certain other legumes. To date there has been no critical experiment reported in which the relative growth of lucerne and other plants has been studied under known identical conditions of zinc deficiency.

The purpose of the present work was to study the growth of lucerne and subterranean clover in zinc- and copper-deficient water cultures respectively, and thereby obtain data on the relative zinc and copper requirements of the two species. The interaction between light intensity and the effects of zinc or copper deficiency on plant growth was also examined.

II. Method

The solution used was that of Arnon (1938). Where possible, solutions of the nutrient salts were eluted several times with a solution of dithizone in carbon tetrachloride to remove heavy metal contaminants. The salts were then recrystallized. The other nutrients to which the dithizone purification technique could not be applied were recrystallized before use.

Double-distilled water produced by a Pyrex glass still was used, and the plants were grown in Pyrex glass beakers blackened on the outside and covered with plaster of paris tops soaked in paraffin. The subterranean clover (var. Dwalganup) and lucerne (var. Hunter River) seed was germinated in acid-washed sand and the plants were transferred to the cultures as soon as they could be conveniently handled. The experiment was commenced on July

3, 1951. In one series of cultures, two clover and two lucerne plants were grown in the same culture pot and in the other series four plants of either clover or lucerne were grown in separate pots. Each treatment in these two series was replicated four times. Half the replicates of each series were placed under full light conditions in the glass-house for the duration of the experiment. The remaining pots were shaded from the direct sunlight and received only approximately 50 per cent. of the illumination of the "full light" series for the first 8 weeks, after which they were transferred to the full light conditions for the remaining 4 weeks of the experiment. The solutions were renewed approximately every 3 weeks.

At the end of the experiment the tops and roots of the plants were harvested separately, oven dried, and weighed.

Histological examinations of certain tissues were made. The material selected was fixed in formalin-acetic-alcohol, dehydrated, embedded in paraffin, and sectioned with a microtome in the usual way. The sections were stained with Connant's Quadruple Combination (Johansen 1940).

III. Results

(*a*) *Symptoms*

The type of symptoms produced in each species by either zinc or copper deficiency was unaffected by growing the two species together or in separate pots, or under full or reduced light conditions. However, the difference in light intensity had a significant effect on the severity of zinc deficiency as described below.

Differences occurred between species with respect to the type of symptom produced by either copper or zinc deficiency.

(i) *Zinc Deficiency*

In both species, the first symptoms appeared approximately 5 weeks after setting up the cultures in full light conditions, but were a fortnight later in the "reduced light" cultures.

Subterranean clover.—The first leaves formed by the plants were normal both as regards size of the leaflets and length of the petioles (Plate 1, Fig. 1). Many of these leaves developed a reddish purple coloration around the midrib at the base of each leaflet. Light brown necrotic spots appeared later, particularly on the distal halves of these leaflets (Plate 1, Fig. 2). These necrotic spots coalesced, and the necrosis extended inwards from the margins of the leaflets. Necrosis of the petioles soon followed. Meanwhile, subsequently formed leaves were slightly chlorotic, and showed a typical "little leaf" condition (Plate 1, Figs. 1 and 3). The size of the leaflets and the length of the petioles were very reduced and the length of the internodes between leaves was shortened, giving the plants a rosetted appearance.

The "little" leaves were usually narrower in proportion to their length than normal leaflets, and many were deeply notched at the apex (Plate 1, Fig. 3).

They did not develop any necrotic spots as described above for the first-formed leaves. However, many of them showed a reddish purple coloration, together with localized chlorotic areas towards the margins, and visible in the upper surface.

A histological examination of extremely dwarfed little leaves was made. It was found that the thickness of the leaves was very variable — marked thickening occurred in places, particularly towards the edges (Plate 1, Fig. 4). Here the thickness of the little leaves was actually greater than that of normal leaves, whereas the thin portions of the former were of normal thickness. The little leaves were usually folded upwards to form a deep crease at the midrib (Plate 1, Fig. 4).

Severe disorganization of the tissues themselves was evident. The upper epidermis was uneven in appearance, and there were marked projections on many of the cells (Plate 2, Fig. 2). However, the palisade cells were the most seriously affected. In many cases they were divided to form rhomboidal rather than columnar-shaped cells. In the thin portions of the leaf, this cell division led to the virtual disappearance of the palisade layer, as its cells were little different in size and shape to the underlying spongy-mesophyll cells (Plate 2, Fig. 2). The thickening of portions of the little leaves was caused by a more extensive hypertrophy of the palisade cells. Several layers of these cells occurred, and many retained the normal columnar shape (Plate 2, Figs. 3 and 4).

Some of the plastids showed elongation. However, in localized areas, lytic factors had destroyed many of the plastids. In some cells the contents had been converted into materials which could not be detected microscopically (Plate 2, Fig. 4). This lysis of the plastids was correlated with the development of the reddish purple coloration or localized chlorosis referred to above.

Cell necrosis first occurred in the mesophyll cells. Identical manifestations of cellular disorganization in "little" leaves of various plants have been described by Reed (1938, 1939) and Reed and Dufrenoy (1935).

The cytology of the first-formed normal-sized leaves of the zinc-deficient Dwalganup plants was very different from that of the "little" leaves of the same plants as described above. In contrast with the latter, these first-formed leaves were normal with respect to the development of the epidermal, palisade, and spongy mesophyll layers. However, the tissues were abnormal in that many of the cells were devoid of plastids, and contained large, usually spherical bodies evidently identical in nature with the coacervates described as occurring in the cells of zinc-deficient plants by Reed and Dufrenoy (1942). Although they were most common in the palisade cells, the coacervates occurred also in the epidermal and mesophyll cells of the zinc-deficient subterranean clover leaflets (Plate 3, Figs. 1 and 2). The tissue towards the edge of the leaflet contained more coacervates than that near the midrib. The coacervation of the cell contents evidently preceded the extension of the marginal necrosis of the affected leaves as described above.

The phosphoric acid test, using the molybdenic reagent described by Reed and Dufrenoy (1942), was applied to sections of the clover leaflets. The result of the test was similar to that obtained by these workers, as the membrane enveloping the coacervate was stained blue, indicating the presence of phospholipoids.

The first signs of the necrotic spotting of the oldest, normal-sized leaves of the zinc-deficient subterranean clover plants (Plate 1, Fig. 2) consisted of a localized collapse of the upper epidermis (Plate 3, Figs. 1 and 2). Later the underlying tissues were also involved. This phenomenon of upper epidermal collapse is identical with that associated with the occurrence of bronze spotting of zinc-deficient flax leaves (Millikan 1951).

Lucerne.—The first symptoms consisted of bronze-coloured spots around the distal margins of the upper (but not the youngest) leaves of the plant (Plate 4, Fig. 1). These spots first occurred on the upper surface of the leaf, but later they could also be seen from the under surface. Subsequently the bronzed areas became white and necrotic. At a later stage some leaves showed bronze spots which were scattered in a random fashion over the surface of the leaflets rather than concentrated near the distal margins (Plate 4, Fig. 2).

Meanwhile, the rate of growth was retarded, tillering was reduced, and the whole plant assumed a slight chlorotic appearance. The younger leaves were smaller and much stiffer than normal and their margins were inrolled (Plate 4, Fig. 4). Reduction in leaf size was particularly severe in the tillers produced at the base of the plant (Plate 4, Fig. 4). Many had a deep notch at the apex (Plate 4, Fig. 3), and some showed a chlorotic mottling. They also had a leathery feel. The middle leaves of the plants finally developed a grey necrosis which started at the tips and extended down the lamina until the whole leaf and the top of the petiole was involved. There was a marked tendency for these affected leaves to fall from the plant.

A histological examination of various tissues from the zinc-deficient lucerne plants revealed similar cellular changes to those described above for subterranean clover. The normal lucerne leaf is characterized by a well-developed palisade player with long, columnar-shaped cells and large intercellular spaces (Plate 5, Fig. 5).

The first signs of the bronze spotting referred to above consisted of a collapse of the cells of the upper epidermis (Plate 5, Fig. 1). The underlying tissues appeared normal at first, but the cells soon showed signs of disorganization. The cell contents disintegrated and became necrotic. This sequence of cellular changes was identical with that associated with the bronze spotting of zinc-deficient subterranean clover leaves.

Small, spherical bodies with a marked affinity for safranin sometimes occurred in the zinc-deficient lucerne tissues directly associated with the development of a bronze necrotic spot (Plate 5, Fig. 3). These bodies did not occur in the cells of normal appearance in other parts of the leaf, nor did they occur in every lesion examined. They were apparently similar in nature to the coacervates in zinc-deficient subterranean clover tissue, but were smaller and stained differently to the latter. However, the upper epidermal cells of the lucerne leaves occasionally contained larger coacervates (Plate 5, Fig. 4).

In contrast with those of subterranean clover, the little leaves of lucerne were not variable in thickness, although they did show similar cellular changes.

Subdivision of the palisade cells, and the reduction in the intercellular spaces occurred (Plate 5, Fig. 6). This latter change probably accounted for the stiffer and leathery feel of the little leaves when compared with normal leaves.

(ii) *Copper Deficiency*

Symptoms first occurred on the same day both in the full light and reduced light cultures. This was approximately 5 weeks after the commencement of the experiment.

Subterranean clover.—Growth was retarded and the plants became slightly chlorotic and developed a habit of growth more erect than normal (Plate 3, Fig. 3). This erect habit of growth was apparent before any necrotic leaf symptoms developed. Leaf size was unaffected, but the edges of the younger leaflets commenced to roll upwards and inwards. Later the inrolled margin became necrotic, while the remainder of the lamina was distorted and puckered (Plate 3, Fig. 4). This marginal necrosis commenced in any place on the edge from the centre to the tip of the leaflet, and varied in colour from white or light brown to bluish green. In the latter case, the margin between necrotic and unaffected tissue was usually a light brown colour. The necrosis later involved the whole of the leaf and petiole. Many of the older leaves developed pinkish cinnamon-coloured necrotic spots approximately 1-2 mm. in diameter. No flowers were produced in the absence of copper, whereas the controls flowered freely.

Piper (1942) reports a fourfold reduction in dry weight of subterranean clover grown in copper-deficient solutions when compared with the yield of plants grown in solutions containing an adequate supply of copper. In the absence of copper his plants were at first slightly chlorotic, but he states that many of them later withered and died without developing any other symptoms. However, copper deficiency in alsike clover has been described by Hewitt and Bolle-Jones (1950). The symptoms of marginal cupping and necrosis of the leaflets were similar to the subterranean clover symptoms described in this paper. Rossiter (1951) observed less severe symptoms in Dwalganup subterranean clover plants grown in copper-deficient soil, which were similar in many respects to those outlined above.

Lucerne.—The first symptom consisted of a marked epinasty of the petioles of the younger leaves (Plate 6, Fig. 1), the terminal leaflets being bent right back against the petioles. Light grey to white necrotic spots soon appeared on these leaves, which were then slightly chlorotic. The spots sometimes appeared on the leaf margins, but most characteristically they occurred towards the base of the leaflets as shown in Plate 6, Figure 2. However, the necrosis soon involved the whole of the leaflets and petiole. The size of the youngest leaflets was very much reduced and death of the top of the plant followed (Plate 6, Fig. 3). Piper (1942) has also described symptoms of epinasty in the petioles of lucerne plants grown in copper-deficient water cultures.

(*b*) *Relative Effects on Growth*

The dry weights of the plants grown in these cultures are presented in Tables 1 and 2. A general view of portion of the experiment is shown in Plate 7. As the species grown in different pots had a larger error variance than those grown together in the same pot, it was necessary to use weights proportional to the inverse of the variances for statistical purposes. The transformed values given in Tables 1 and 2 are derived from the weighted means of the two sets of data.

TABLE 1

MEAN DRY WEIGHT (G. PER PLANT) OF SUBTERRANEAN CLOVER (VAR. DWALGANUP) AND LUCERNE (VAR. HUNTER RIVER) GROWN IN NUTRIENT SOLUTIONS

Transformed values* in parentheses

Treatment	Subterranean Clover			Lucerne		
	Tops	Roots	Tops+Roots	Tops	Roots	Tops+Roots
Complete solution	3·631 (3·491)	0·434 (2·630)	4·065 (6·121)	3·587 (3·541)	1·575 (3·192)	5·162 (6·733)
Zinc deficiency	0·356 (2·530)	0·160 (2·195)	0·516 (4·725)	0·891 (2·892)	0·491 (2·663)	1·382 (5·555)
Copper deficiency	0·534 (2·713)	0·111 (2·035)	0·645 (4·748)	0·333 (2·446)	0·118 (1·947)	0·451 (4·393)

Significant differences: interaction effect (transformed values)	5 per cent. Level	1 per cent. Level
Within species	0·109	0·148
Between species (tops or roots)	0·198	0·269
Between species (tops + roots)	0·350	0·490

* Log_{10} $(X \times 10^3)$ of weighted means.

A comparison of the results obtained from the series where the two species were grown in separate pots and that where they were grown together in the same pot revealed no significant difference in dry weight, nor did these two methods of culture have any significant interaction effects with zinc or copper deficiencies, or with the amount of light. Because of this negative result these figures are not included in this paper.

Zinc deficiency caused a significantly greater reduction in dry weight of the tops of subterranean clover than of lucerne, but the zinc species interaction was not significant either with respect to roots or roots + tops.

By contrast, copper deficiency had a significantly greater effect on lucerne than on subterranean clover for dry weight of tops, roots, and tops + roots.

As regards the effect on plant parts the results show that zinc deficiency caused a significantly greater reduction in top growth than root growth for both subterranean clover and lucerne.

The effect of copper deficiency on plant parts was not consistent for the two species. In subterranean clover the top growth was reduced by this treatment to a greater degree than root growth, whereas with lucerne root growth was affected more than top growth.

The interaction between light intensity and zinc deficiency was highly significant (Table 2), i.e. the reduction in growth due to the absence of zinc was less under reduced light than under full light conditions. The amount of light had no significant effect on the severity of copper deficiency symptoms.

TABLE 2

EFFECT OF LIGHT INTENSITY ON ZINC AND COPPER DEFICIENCY. COMBINED RESULTS FOR SUBTERRANEAN CLOVER AND LUCERNE (G. DRY WT. PER PLANT)

Transformed values* in parentheses

Treatment	Full Light Dry Wt. (g.)	Reduced Light Dry Wt. (g.)
Complete solution	6·094 (3·325)	3·130 (3·052)
Zinc deficiency	0·828 (2·547)	1·051 (2·593)
Copper deficiency	0·691 (2·382)	0·405 (2·188)

	5 per cent. Level	1 per cent. Level
Significant difference: interaction effect (transformed values)	0·175	0·245

* Log_{10} $(X \times 10^3)$ of weighted means. The results of the two species are combined in this table as the statistical analysis showed that there was no significant interaction between amount of light and either species or plant part.

(c) *Utilization of Zinc and Copper*

The results of copper and zinc analyses of plants from the series where the two species were grown together in the same pot are presented in Table 3.

There was a decrease in zinc concentration and the absolute amount of zinc in the tops, a marked increase in copper concentration, and a decrease in the Zn/Cu ratio under conditions of zinc deficiency in both subterranean clover and lucerne. On the other hand, the copper concentration of the copper-deficient plants was only slightly less than normal for subterranean clover and was actually higher for lucerne. However, the Zn/Cu ratio was comparable to that of the control treatment, while the total copper content in the tops was much reduced for both species. Piper (1942) has reported that the copper concentration in the tops of Algerian oats grown in a nutrient solution without the addition of copper was higher than that of plants grown in other cultures to which small amounts of copper had been added. The possible significance of this result has been discussed by Ulrich (1952).

TABLE 3

COPPER AND ZINC CONTENTS (ON DRY BASIS) OF TOPS OF SUBTERRANEAN CLOVER (VAR. DWALGANUP) AND LUCERNE (VAR. HUNTER RIVER)* SHOWING THE UTILIZATION OF THESE TWO ELEMENTS

Nutrient Solution	Light Conditions	Subterranean Clover								Lucerne							
		Top Dry Wt. per Plant (g.)	Copper (Cu) (p.p.m.)	Zinc (Zn) (p.p.m.)	Ratio Zn/Cu	Cu per Plant (μg.)	Zn per Plant (μg.)	Coefficient of Utilization†		Top Dry Wt. per Plant (g.)	Copper (Cu) (p.p.m.)	Zinc (Zn) (p.p.m.)	Ratio Zn/Cu	Cu per Plant (μg.)	Zn per Plant (μg.)	Coefficient of Utilization*	
								Cu	Zn							Cu	Zn
Complete solution	Full	4·814	6·2	76	12·3	29·8	366	0·162	0·013	5·415	3·9	48	12·3	21·1	260	0·257	0·021
	Reduced	1·895	6·5	90	13·8	12·3	171	0·154	0·011	2·365	4·4	39	8·9	10·4	92	0·228	0·026
Zinc deficiency	Full	0·320	25	25	1·0	8·0	8·0	—	0·040	0·762	10·8	13	1·2	8·2	9·9	—	0·077
	Reduced	0·336	27	24	0·9	9·1	8·1	—	0·042	0·869	10·4	13	1·3	9·4	11·3	—	0·077
Copper deficiency	Full	0·602	4·8	62	12·9	2·9	37·3	0·208	—	0·338	5·2	69	13·3	1·8	23·3	0·188	—
	Reduced	0·465	5·1	87	17·1	2·4	40·5	0·194	—	0·211	‡	74	—	—	15·6	—	—

* The plants are from the series where the two species were grown together in the same pots (see Plate 7).

† Dry matter produced (g.)/zinc or copper absorbed (μg.).

‡ Sample lost.

The zinc concentration of lucerne was less than that of subterranean clover except under copper deficiency where the figures were of comparable magnitude. Conversely, the coefficient of utilization of zinc (i.e. dry matter produced (g.)/zinc absorbed (μg.)) of both the normal and zinc-deficient lucerne was higher than for subterranean clover. These data indicate that the zinc requirement of lucerne was less than that of the subterranean clover and support the conclusions drawn from the data in Table 1.

IV. Discussion

The experiment described above has demonstrated the existence of highly significant differences in the requirements of zinc and copper between the two species studied. Lucerne grew better than subterranean clover under conditions of zinc deficiency, whereas subterranean clover grew better than lucerne when copper was deficient in the substrate.

The author (unpublished data) has shown that the Dwalganup variety of subterranean clover (which was used in the present experiment) is less affected by zinc deficiency than the later varieties Mt. Barker and Bacchus Marsh. Thus, if either of these two varieties had been used in the present experiment, the difference in the effect of zinc deficiency between subterranean clover and lucerne would doubtless have been greater.

There was no significant difference between the results obtained from cultures where the two species were grown together in the same pot and those where they were grown in separate pots. Thus there is no evidence that either of the two species, when grown together, utilized any limited supply of zinc or copper available to them in the deficient cultures at the expense of the other species.

Zinc deficiency caused a relatively greater reduction in top growth than in root growth of both species. A similar interaction between plant part and zinc deficiency in subterranean clover was obtained in previous experiments by Millikan (unpublished data). Wood and Sibly (1950) found that a greater percentage of the total zinc in the plant was contained in the roots of oats than in the other organs of this plant. The relatively greater effect of zinc deficiency on top as compared with root growth of lucerne and subterranean clover could, therefore, be attributed to the accumulation in the roots at the expense of the tops of the very limited amount of zinc available to the plants.

Although lucerne made better growth than subterranean clover in the zinc-deficient water cultures, the former species still showed very severe symptoms of zinc deficiency, indicating that an adequate supply of zinc in the substrate was essential for its normal development. However, the literature citations given in the introduction to this paper show that lucerne fails to develop zinc deficiency symptoms, or respond to zinc, when grown on soils on which other plants show severe symptoms, and also that the proximity of lucerne roots will cure zinc deficiency symptoms in more susceptible plants. The better utilization by lucerne of the zinc supply, as demonstrated in the present experiment, would only partly account for these phenomena. It is suggested that excretions from lucerne roots have the property of increasing the availability of soil zinc not only for their own use, but for the roots of other plants. The deep penetration of lucerne roots, which may perhaps enable them to exploit soil zinc outside the range of shallower-rooted species, does not affect this conclusion. This is indicated by the work of Hoagland, Chandler, and Stout (1936) cited earlier, where the roots of the lucerne and other species were confined to the same pot.

In the present experiment the growth of subterranean clover was significantly better than that of lucerne under identical conditions of copper deficiency, irrespective of whether the two species were grown together in the same pot or in different pots. This accords with the reports of Trumble and Ferres (1946) and Hewitt and Bolle-Jones (1951) that lucerne was the most responsive to copper of the legumes (including subterranean clover) which they grew in copper-deficient substrates. The results given in Table 3 indicate a difference in the capacities of lucerne and subterranean clover to utilize absorbed copper under conditions of copper deficiency rather than to differences in their absorptive capacity for copper.

However, differences in the absorptive capacity of plant species for copper have been reported. Rye is much more resistant than oats to copper deficiency (Piper 1938, 1942; Riceman 1943), while resistant species and varieties of oats occur (Rademacher 1937; Riceman 1943). In contrast with the present experiment, these differences have not been explained by differences in copper requirement as the resistant plants were able to extract more copper than susceptible plants from the copper-deficient soils (Rademacher 1937; Piper 1942). On normal soils there was no difference in copper values between resistant and susceptible varieties.

The increase in the severity of zinc deficiency symptoms with increasing light intensity demonstrated in this paper accords with the results of previous investigators (Haas 1936; Chapman, Vanselow, and Liebig 1937; Reed 1938; Hoagland 1944). Skoog (1940) further showed that zinc-deficient plants maintained a high auxin content and continued to elongate in red or weak light, but in blue light an early cessation of growth and reduction in auxin content occurred.

Differences in light duration, as distinct from light intensity, may also affect the plant's capacity to utilize zinc from a relatively unavailable source. In this regard Trumble and Ferres (1946) showed that on a zinc-deficient soil, the growth of subterranean clover (variety Mt. Barker) was significantly increased by increased light duration.

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EXPLANATION OF PLATES 1-7

PLATE 1

Fig. 1.—Zinc-deficient subterranean clover plants. The first leaves are normal in size, but leaves produced later show a marked reduction in size of lamina and length of petioles.

Fig. 2.—Necrotic spotting on the upper surfaces of oldest leaves of zinc-deficient subterranean clover.

Fig. 3.—Enlarged views of very dwarfed "little" leaves showing their abnormal shape.

Fig. 4.—Transverse section through a "little" leaf. The thickness of the leaf is very variable.

PLATE 2

Sections through subterranean clover leaves.

Fig. 1.—Leaf of normal plant grown in full nutrient solution.

Figs. 2-4.—"Little" leaves from zinc-deficient plants similar to those shown in Plate 1, Figures 3 and 4. Cellular disorganization in the little leaves is apparent. Projections from the epidermal cells occur. In Figure 2 the cells of the palisade layer are little different from those of the mesophyll layer as the former are rhomboidal rather than columnar in shape. The increased thickness in places of the little leaves (see Plate 1, Fig. 4) is due to the marked hypertrophy of the palisade layer (Fig. 3), which consists of several layers of more or less columnar-shaped cells. Lytic factors have destroyed the contents of the cells of the hypertrophied palisade layer in Figure 4. This lysis was associated with the occurrence of a chlorotic area in the leaf.
E, epidermis; *P*, palisade layer; *M*, mesophyll.

PLATE 3

Figs. 1 and 2.—Sections through normal-sized leaves of zinc-deficient subterranean clover similar to those shown in Plate 1, Figures 1 and 2. Necrotic spotting (Plate 1, Fig. 2) commences as a localized collapse of the cells of the upper epidermis (*E*). Large coacervates (*C*) occur in the cells of both the palisade and mesophyll layers.

Fig. 3.—Subterranean clover plants growing in full nutrient solution (left) and copper-deficient solution (right). The latter show abnormally erect habit of growth.

Fig. 4.—Younger leaves of copper-deficient subterranean clover showing marginal necrosis and cupping.

PLATE 4

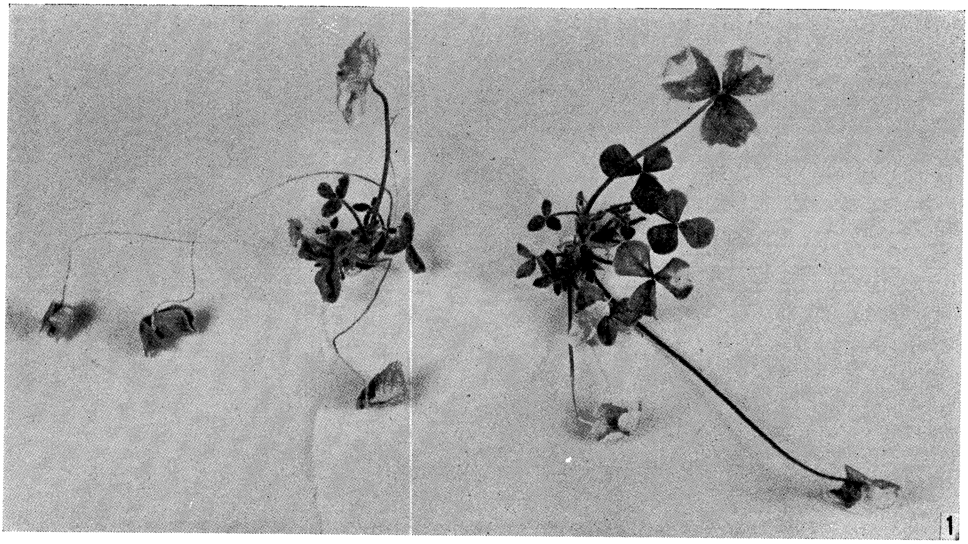
Fig. 1.—Middle leaves from lucerne plant grown in a zinc-deficient nutrient solution. The marginal spots are bronze-coloured at first, but later develop a white centre (right).

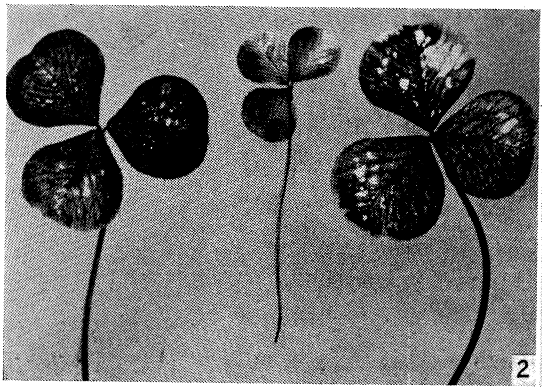
Fig. 2.—Zinc-deficient lucerne leaves showing numerous bronze spots on the upper surface.

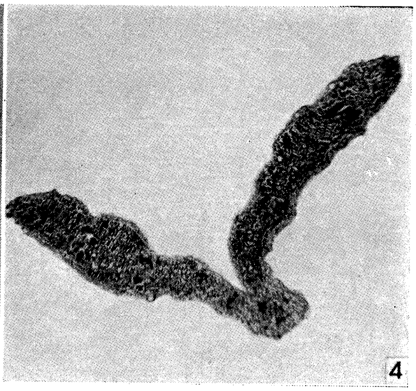
Fig. 3.—Left, normal leaf. Right, "little" leaves from base of zinc-deficient lucerne plant, showing their abnormal shape.

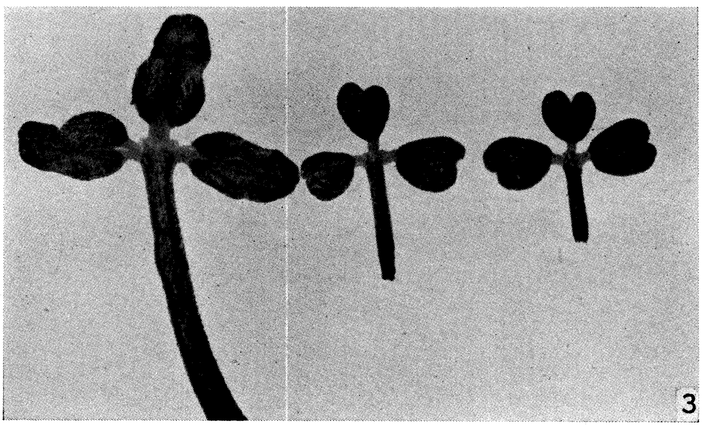
Fig. 4.—Severe reduction in size of leaves of secondary shoots from the base of a zinc-deficient lucerne plant. The leaves at the top of the plant were also smaller and much stiffer than normal, and their margins were inrolled upwards.

Zn AND Cu DEFICIENCIES ON LUCERNE AND SUBTERRANEAN CLOVER

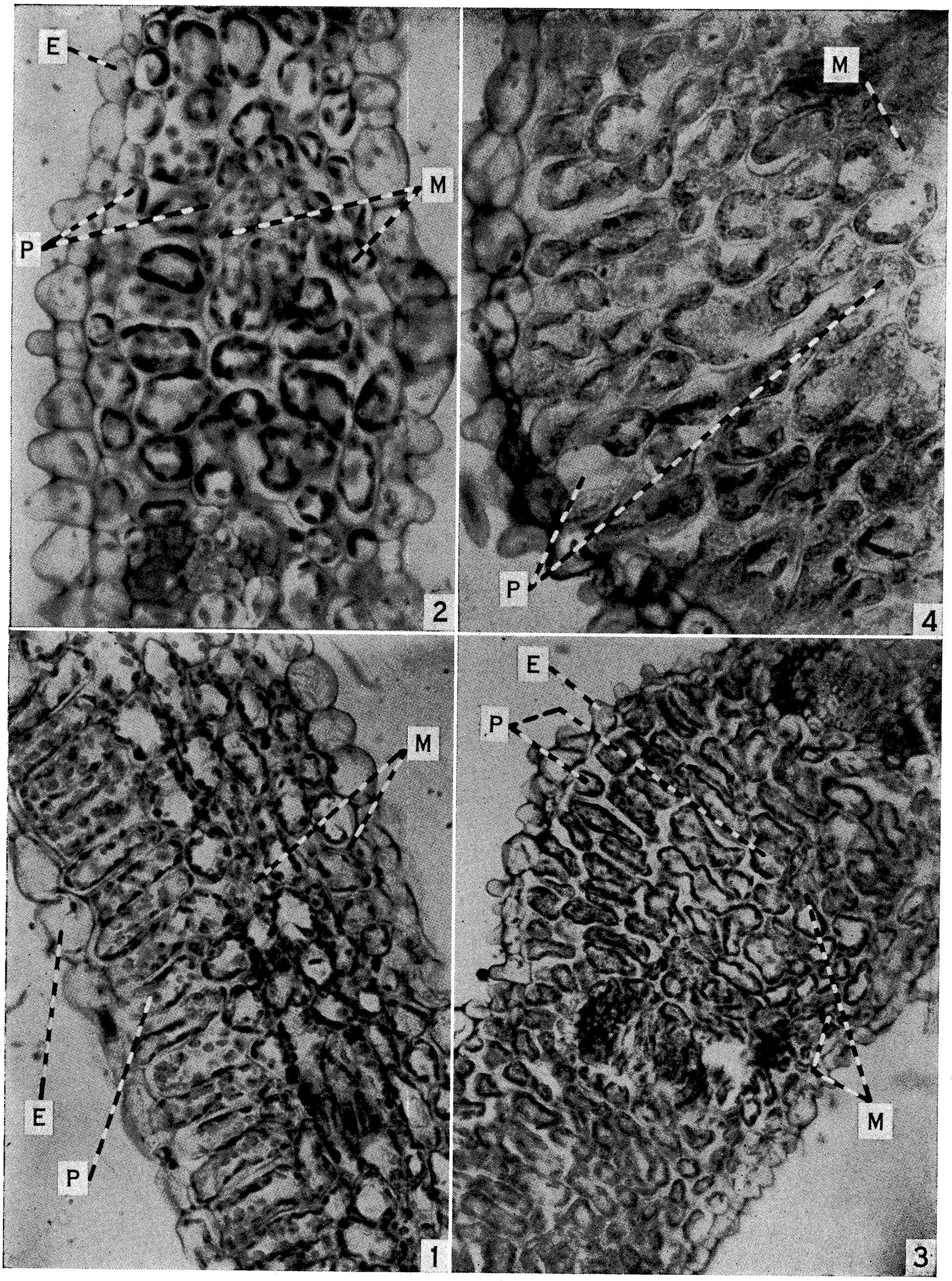


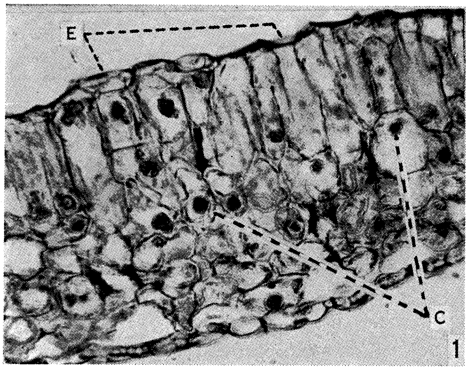






Zn AND Cu DEFICIENCIES ON LUCERNE AND SUBTERRANEAN CLOVER

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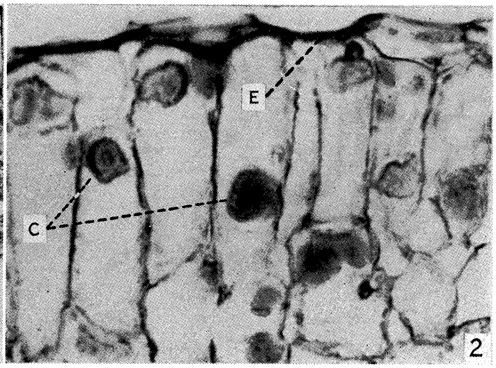
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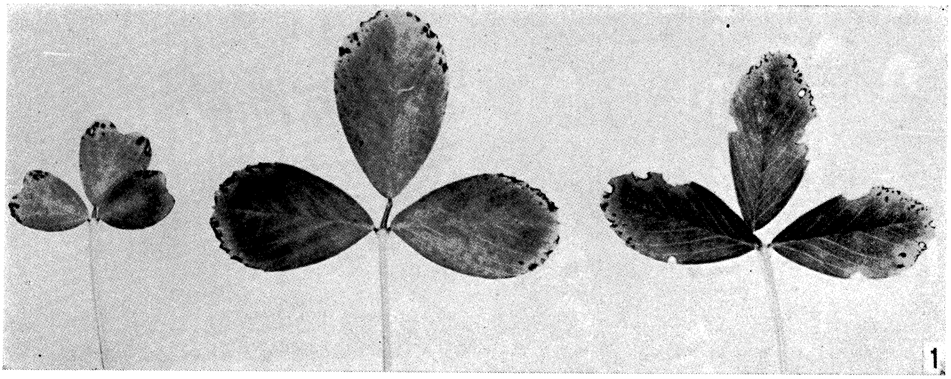
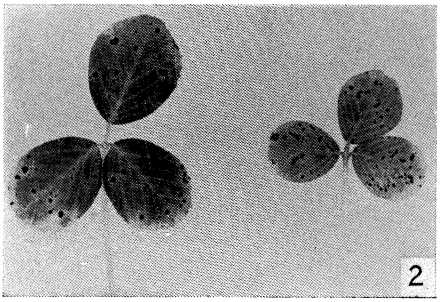
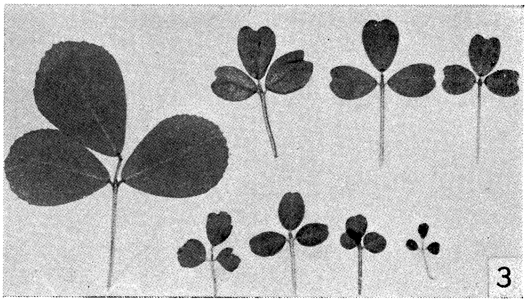
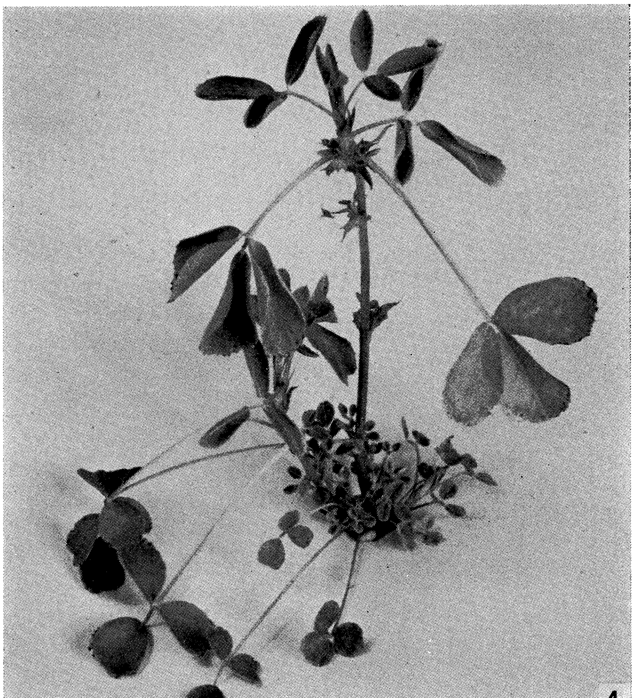
Zn AND Cu DEFICIENCIES ON LUCERNE AND SUBTERRANEAN CLOVER

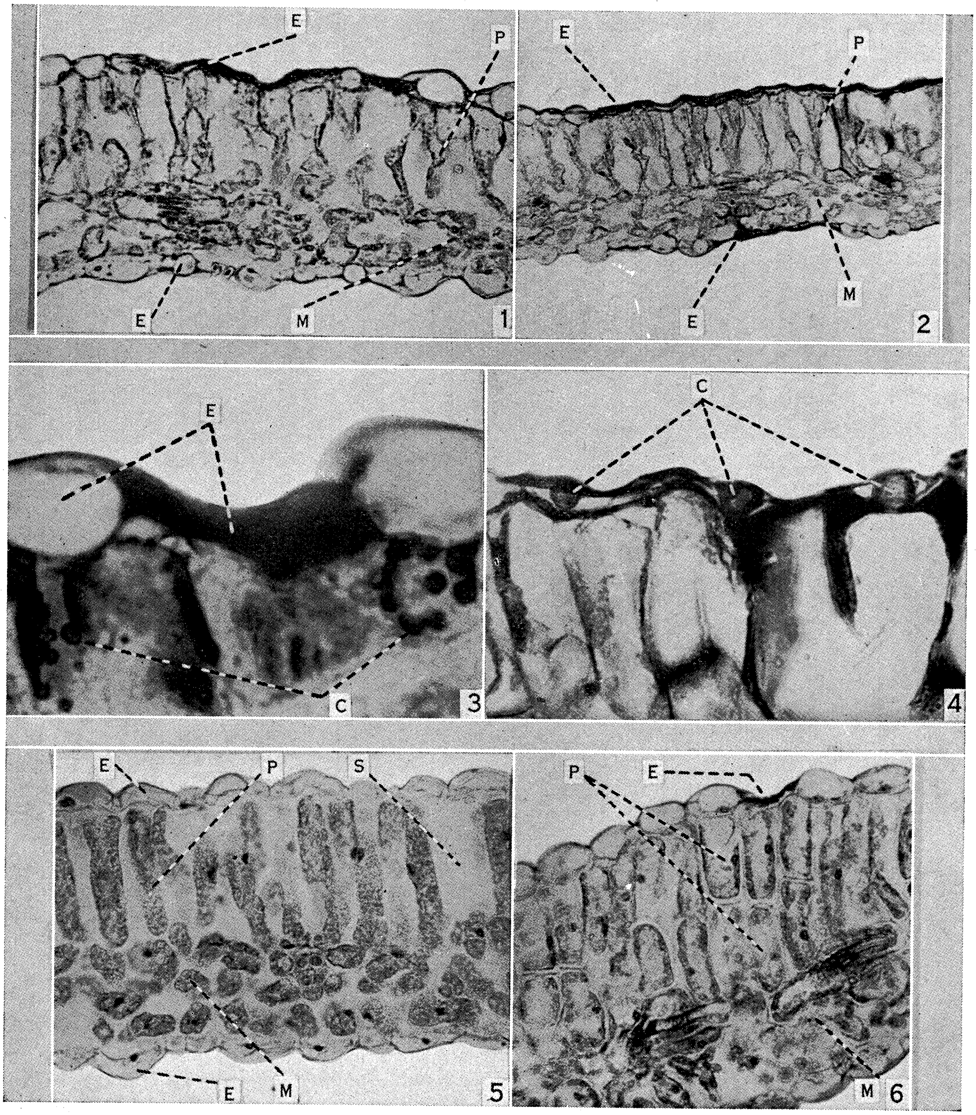








Zn AND Cu DEFICIENCIES ON LUCERNE AND SUBTERRANEAN CLOVER



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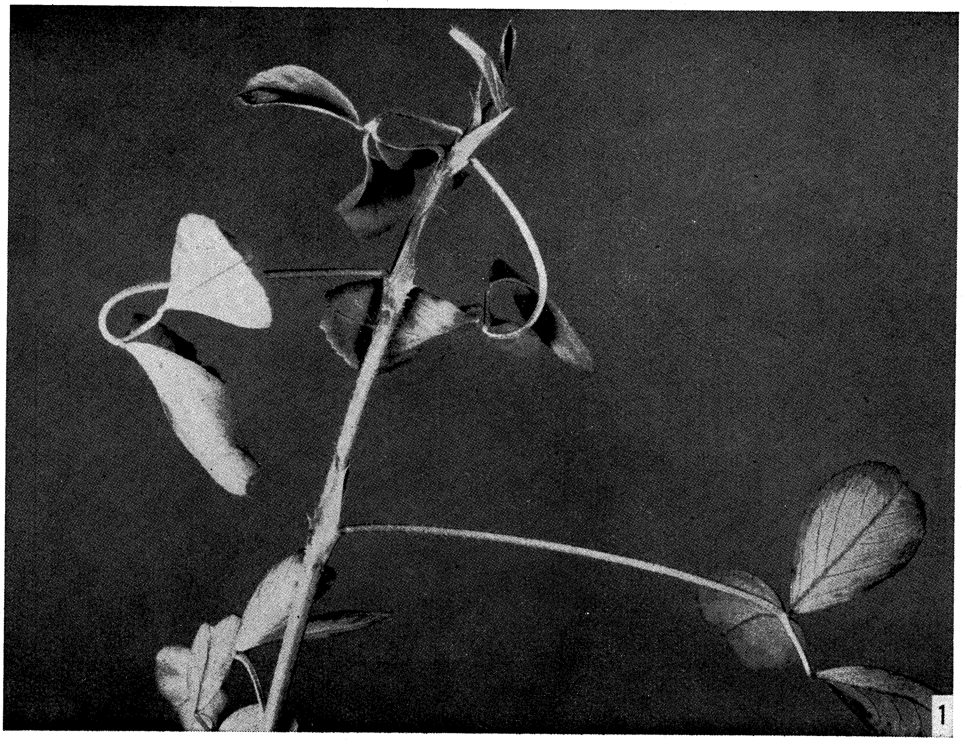
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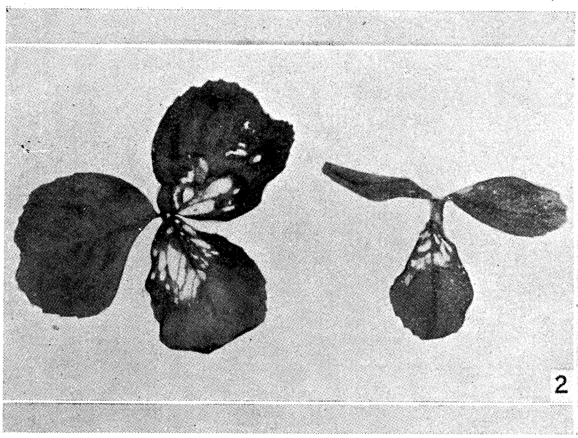
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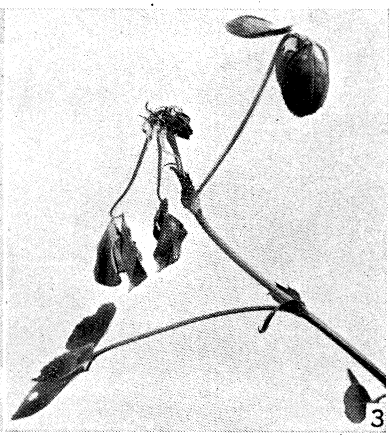
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Zn AND Cu DEFICIENCIES ON LUCERNE AND SUBTERRANEAN CLOVER

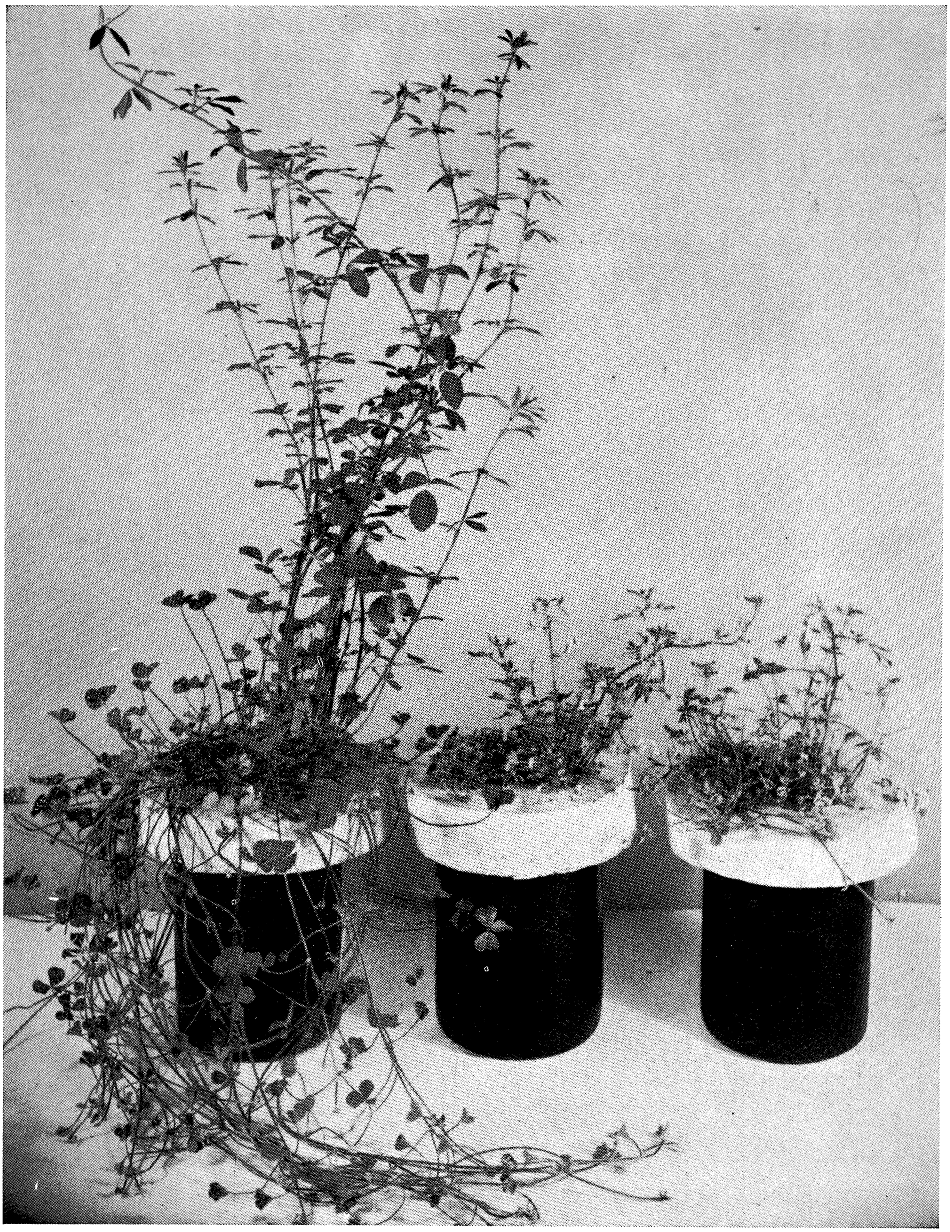

PLATE 5

Figs. 1 and 2.—Sections through bronze spots on zinc-deficient lucerne leaves, similar to those shown in Plate 4, Figure 2. The spots are caused by a collapse of the cells of the upper epidermis (Fig. 1). The underlying cells appear normal at first. Later necrosis of the lower epidermis also occurs (Fig. 2). *E*, epidermis; *P*, palisade cells; *M*, mesophyll cells.

Figs. 3 and 4.—Sections through bronze spots on zinc-deficient lucerne leaves similar to those shown in Plate 4, Figure 2. Figure 3 shows numerous small coacervates in cells adjacent to the necrotic upper epidermis, and Figure 4 larger coacervates in the necrotic cells of the upper epidermis. *E*, epidermis; *C*, coacervates.

Figs. 5 and 6.—Sections through lucerne leaves. Figure 5, normal leaf showing well-defined columnar-shaped cells of the palisade layer with large intercellular spaces. Figure 6, zinc-deficient "little" leaf. Disorganization of the palisade layer is evident in the subdivision of the cells and the marked reduction in the intercellular spaces. This probably accounts for the stiff feel of these leaves. *E*, epidermis; *P*, palisade cells; *M*, mesophyll cells; *S*, intercellular space.

PLATE 6

Fig. 1.—First symptom of copper deficiency in lucerne, consisting of a marked epinasty of the petioles of the youngest leaves.

Fig. 2.—Light grey to whitish-coloured necrotic spotting of the younger leaves of copper-deficient lucerne plants. The necrosis usually appears at the base of the leaflet.

Fig. 3.—Death of the top of a copper-deficient lucerne plant following the epinasty of the petioles and white necrosis of the leaflets.

PLATE 7

Portion of the water culture series in which subterranean clover and lucerne were grown together in the same pots. Left to right; complete solution, zinc deficiency, copper deficiency.