Plant and Soil V, no 3 August 1954

THE PRODUCTION OF COPPER, ZINC AND MOLYBDENUM DEFICIENCIES IN CROP PLANTS GROWN IN SAND CULTURE WITH SPECIAL REFERENCE TO SOME EFFECTS OF WATER SUPPLY AND SEED RESERVES

by E. J. HEWITT and E. W. BOLLE-JONES *)

Agricultural Research Council, Unit of Plant Nutrition (Micro-nutrients),
Long Ashton Research Station, University of Bristol

and PEGGY MILES

National Agricultural Advisory Service,
Kirton Experimental Husbandry Farm, Boston, Lincs.

Sand culture methods of general application to the study of micronutrient deficiencies in crops on a large scale have been described recently [4]). Experience has shown that provision of sufficient water of suitable purity for the study of copper, zinc or molybdenum deficiencies may present technical difficulties in large scale experiments when reliance is placed on glass stills. The mineral reserves in large seeds [13]) [18]) [22]) [24]) may delay the onset of acute deficiency symptoms in some crops. The importance of these points is considered in this paper. A preliminary note on effects of seed reserves has already appeared [11]).

METHODS

Culture methods.

The general methods and details of purification procedures used in this work have been fully described elsewhere [4]) and are only outlined here.

Plants were grown from seed in 1 gallon pyrex glass containers provided with a central drainage outlet. The outlet was covered by

---

*) Present address: — The Rubber Research Institute, Kuala Lumpur, Malaya.

glass wool and a watch glass to retain the sand but allowed free drainage. All glassware was cleaned with detergent, strong acid and steaming.

Sand was purified as described previously. In 1950 and 1951 two-day and three-day treatments with acid were used and compared; in 1952 two days with acid and 1 day with alkali were allowed for copper and zinc deficiencies. For molybdenum deficiency treatments the sand was treated for 3 days with acid and 1 day with alkali.

In 1950 water was obtained by distillation from pyrex glass stills of tap water already distilled from tinned copper stills with silver condensers. This once glass distilled water was compared with water purified by three other methods; water distilled twice from glass, water distilled from tinned copper stills with pyrex glass condensers and rain water purified by ion exchange resins. In 1952 once glass distilled water and purified rain water were compared. The resins used were Permutit Zeocarb 215 and Deacidite E resins followed by IMAC C11 cation resin in 1951 and by Permutit Biodeminrolit FF mixed-bed resin in 1952, contained in a pyrex glass column measuring 75 cm × 12.5 cm and holding about 15 l of resin. The rate of flow was 6–10 l/hour. The resin treated water was also used for the whole of the experimental work on seed reserves in 1952.

The nutrient solutions were prepared from suitably purified stock solutions [4]. The main constituents were provided at the following concentrations as me/l NO_3^- 15; PO_4^{---} 4; Ca^{++} 10; K^+ 5; Mg^{++} 3; Fe^{+++} 0.3. Micronutrients were given as stated previously, *loc. cit.*

Plant material.

The crops grown during the 3 years were — tomato (Market king), oat (Star), wheat (Atlee), maize (Golden bantam), red clover (Dorset marl), alsike clover, lucerne (Provence), runner bean (Scarlet emperor), dwarf bean (Masterpiece), broad bean (Longpod), pea (Onward), sugar beet (Webbs No. 2), swede (Purple top).

Commercial seed was used in all years for each crop. In addition, seed was saved in 1951 from peas, broad, runner and dwarf beans grown in sand cultures given complete nutrient solutions or solutions deficient in copper, zinc and molybdenum respectively and re-sown in cultures given these respective treatments in 1952 for comparison with fresh commercial seed of the varieties used.

Analytical methods.

Samples of foliage were collected at the end of each year's experiment, dried in a steam oven and redried before analysis. Seeds were sampled from the air-dried mature seeds saved for sowing again or stored from the previous year. Dried material was digested with nitric and perchloric acids and evaporated to dryness. The residue was dissolved in boiling water, filtered and made to a convenient volume. Copper and zinc were determined in the same aliquot by a method based on that of Piper [17]) using purified dithizone [16]) but modified to include the addition of thiosulphate to increase the specificity of the method for zinc [1]) [21]).

Molybdenum was determined in a separate aliquot of the acid digest solution by the method of Piper and Beckwith [19]) slightly modified for use with 8 ml 1 cm absorption cells and with omission of the dithizone extraction stage since no interference due to copper was found with our material.

Layout.

Treatments were replicated in randomised sub-blocks, in which the respective deficiency or complete nutrient treatments were segregated to avoid risks of contamination by splashing during the application of the solutions. In order to ensure as far as possible that comparisons between the use of commercial and "sand culture" seed were made under similar conditions plants were grown from both the commercial and the respective "deficiency" seeds in the *same* pots and were thus independent of any variations in nutrient supply or environment.

RESULTS

Effects of sand treatment.

Visual comparison and yield data in Table I showed there was no appreciable increase in the severity of copper and zinc deficiencies in 1951 when the sand was treated for 3 days with acid instead of 2 days. Results for zinc deficiency in 1952 (Table II) were outstandingly and consistently more severe than those obtained previously. This was tentatively attributed to the quality of the sand and possibly to the inclusion of the alkaline treatment which was already considered advantageous for production of molybdenum deficiency [4]). There was no effect on severity of copper deficiency.

TABLE I

Yields g dry weight per plant of crops grown in 1951 water supply test

Crop and source of water supply	Sand treatment	Copper deficiency		Zinc deficiency		Crop and source of water supply	Molybdenum deficiency	
		Dry wt. g	% of control	Dry wt. g	% of control		Dry wt. g	% of control
Tomato:						*Tomato:*		
Metal stills	2 days	14.9	24.2	21.7	35.7	1 × glass distilled	16.6	27.4
	3 ,,	13.4	22.2	24.2	38.8	Resin treated rain water. .	21.3	34.8
						Red Clover:		
1 × glass distilled	2 days	2.3	3.8	14.8	24.2	1 × glass distilled	4.0	34.9
	3 ,,	1.6	2·7	14.5	24.0	Resin treated rain water. .	3.1	25.2
2 × glass distilled	2 days	1.9	3.2	14.0	23.0			
	3 ,,	1.4	2.3	20.6*	33.8			
						Sugar beet:		
Resin treated rain water.	2 days	3.3	5.4	16.7	27.6	1 × glass distilled	4.0	7.2
	3 ,,	1.2	1.9	17.0	28.1	Resin treated rain water. .	7.6	13.5
Red clover:						*Lettuce:*		
Metal stills	2 days	6.5	56.2	8.4	72.4	1 × glass distilled	0.28	1.3
	3 ,,	8.0	69.7	8.8	75.6	Resin treated rain water. .	0.15	0.7
						Carrot:		
1 × glass distilled	2 days	0.21	1.7	4.3*	37.2	1 × glass distilled	2.6	—
	3 ,,	0.32	2.6	1.8	15.6	Resin treated rain water. .	1.3	—
						Swede:		
2 × glass distilled	2 days	0.16	1.3	4.8*	41.5	1 × glass distilled	23.4	—
	3 ,,	0.20	1.7	7.5*	64.8	Resin treated rain water. .	24.3	—
						Radish:		
Resin treated rain water	2 days	0.46	3.9	2.8	24.2	1 × glass distilled	21.5	—
	3 ,,	0.68	5.9	3.1	26.4	Resin treated rain water. .	25.3	—

*) Probably contaminated by condensation or paint from roof.

TABLE II

Yields g dry weight per plant; crops grown in 1952 water supply test

Crop	Copper deficiency		Zinc deficiency		Molybdenum deficiency		Complete nutrient
	Glass distilled water	Resin treated rain water	Glass distilled water	Resin treated rain water	Glass distilled water	Resin treated rain water	Resin treated rain water
Tomato							
44 days	0.23	0.24	0.08	0.07	0.43	0.36	2.26
90 days	1.25	1.02	0.30	0.35	1.12	1.42	81.5
Red clover							
44 days			0.09	0.05	0.11	0.13	0.22
90 days			0.06	0.05	0.43	0.75	7.00
Wheat							
86 days	0.60	0.60					9.13

Effects of water supplies.

The yields of crops grown in the water supplies tests are shown in Tables I and II. The data were confirmed by visual observations of the crops in each test. It is evident that the provision of pyrex glass condensers is not an effective safeguard against serious heavy metal contamination in water obtained from tinned copper stills, and that the extent of the contamination is sufficient to interfere seriously with experiments on copper and zinc deficiencies. On the other hand, rainwater purified by ion-exchange resins may compare satisfactorily with glass distilled water and attains the exacting standards necessary for the production of severe deficiencies of copper, zinc and molybdenum whilst providing the copious supplies required for large scale methods. A single charge of resin provided over 15000 l of consistently satisfactory water in 5 months, at rates of flow up to 10 l/hour.

There was a slight advantage for glass distillation in 1951 compared with use of the IMAC C11 resin. The advantage disappeared or was reversed later in the season as regards molybdenum content, when judged either by changes recorded visually or by yields in the respective treatments. In 1952 results with water treated with the Biodeminrolit mixed bed resin were comparable to those of the glass distilled water for all three deficiencies. The Biodeminrolit was concluded to be more suitable for the present purpose than the IMAC C11 resin. Comparative effects are illustrated in Plate I.

Although not listed here, severe molybdenum deficiency was produced in lettuce, cauliflower and Brussels sprouts grown throughout the test, with solutions made up in the Biodeminrolit treated rain water and effects were as severe as those previously recorded 9) 10).

TABLE III

Yields g dry weight per plant of crops grown in copper and zinc deficient sand cultures in 1950 and 1951 experiments.

Crop		Copper deficiency		Zinc deficiency	
		dry wt.	% of control	dry wt.	% of control
Tomato	1950	1.5	1.0	19.5	12.6
Swede	1950	4.9	4.5	5.1	4.7
Sugar beet	1950	7.1	6.8	32.1	31.0
Oat	1950	1.9	24.1	3.6	46.2
Alsike clover	1950	2.0	5.4	1.6	4.3
,, ,,	1951	0.19	1.6	5.0	42.0
Lucerne	1951	0.15	1.4	5.6	53.7

Visual symptoms.

Systematic notes on effects of copper and zinc deficiencies have already been given for many of the crops 7) 8). Only the more important general effects and fresh observations are therefore presented here. Characteristic examples are shown in Plates III, IV and V.

Copper deficiency: The relation of leaf age to incidence of symptoms depended on the particular crop. Young leaves or the growing point were principally affected in wheat, oat, broad bean and lucerne. Profuse tillering or branching occurred in this group. Old leaves were more sensitive in dwarf bean, tomato, clovers and swede. Foliage was often dull olive green, or grey-green, but was abnormally dark blue-green in tomato and lucerne. Tomato plants grown with slightly more copper obtained from the water from metal stills showed pronounced yellow and purple tints of older leaves and the upcurling of leaf margins associated with severe deficiency was absent. Characteristic necrotic patterns along major veins developed in dwarf and runner bean leaflets. Effects of "white tip" disease in oats do not require description; wheat, however, showed in addition, a characteristic coiling of the chlorotic or pale rolled leaf tips, which was often reversed midway in a manner suggestive of the tendrils of certain climbing plants.

Most plants of the large seeded legumes produced a few seeds but peas, which produced normal sized pods, were entirely devoid of seed. Flowering was prevented in the smaller seeded clovers, tomato and cereals.

Zinc deficiency: Many crops showed effects suggestive of acute phosphorus deficiency. This was especially so for maize, swede, clovers, pea and the beans. Foliage became dull olive green and faded. Purple or bronze tints were produced in maize, beans and clovers. "Little leaf" effects due to reduction in the breadth of the lamina appeared in the upper leaflets of broad bean and in leaflets of runner bean when severe deficiency was produced in 1952. Crinkling or wavy outlines of leaf margins developed in runner bean and in clovers which also became involute at the apex. The leaflets of runner bean and tomato showed marked asymmetry. They tended to become concave on the distal margin of the lateral leaflets of runner bean or of any leaflets of tomato. Runner bean also showed a characteristic spotting in the form of numerous small, rounded, dark green areas against a more general chlorotic background. Leaflets tended to remain folded in the clovers and in 1952 sudden breakdown and death of red clover seedlings was common. This was apparently associated with the growth of saprophytic fungi in the dying seedlings and was preceded by necrotic spotting of young leaves, and collapse of petioles. Leaflets of tomato plants curled downwards and sometimes completely round. Cell contents diffused out to the exterior of the leaves and collected in the hollows where saprophytic organisms were able to grow. A similar escape of cell contents which appeared as clear brown fluid that dried out as a white deposit occurred in maize and also in oats grown in 1953. Various types of necrosis were also common. These included effects resembling "grey speck" caused by lack of manganese in oat, breakdown in main and lateral petioles or major veins in tomato and necrosis of undersides of leaf veins in dwarf bean. As in copper deficiency the age of leaf affected was not consistent. Thus younger leaves were more sensitive in broad bean whereas older leaves were first involved in peas and tomato.

Molybdenum deficiency: Symptoms of severe molybdenum deficiency which developed in lettuce, tomato, red clover, cauliflower, Brussels sprouts and others grown as test crops to study the effects of water supplies have already been described [9]) [10]).

The following effects observed in the leguminous crops apply mainly to plants raised from impoverished seed. In pea and runner bean they showed as a uniform grey-green or yellow chlorosis followed by sudden wilting and withering of the older leaves. Flowering was suppressed and plants died prematurely. Broad bean and dwarf bean were less sensitive but sudden wilting and drying out of large areas of leaflets occurred in dwarf bean and blackened scorching of older leaves occurred in broad bean; this was preceded by sunken intervenal areas which were wilted and grey-green in colour. Symptoms usually developed suddenly, often overnight, as recorded by Meagher *et al.* [13]).

Effects of seed reserves.

Removal of cotyledons from dwarf and broad bean immediately after emergence of the shoot in 1951 resulted initially in reduced growth (Table IV). This effect persisted in broad bean in control plants but was not maintained in dwarf beans. Comparison of the growth of similarly excised plants in the deficiency treatments showed an additional response in each treatment but this was least

TABLE IV

Relation of seed origin to yields, g dry weight per plant, of large seeded leguminous crops grown in copper, zinc or molybdenum deficient sand cultures

Crop and seed origin		Treatment			
		Copper deficiency	Zinc deficiency	Molybdenum deficiency	Complete nutrient
Runner bean	1951 commercial source	19.0	19.5	77.3	97.5
	1952 commercial source	49.8	39.8	147.3	117.8
	1952 saved from 1951 sand culture	12.2	12.5	22.8	—
Dwarf bean	1951 entire seed commercial source	10.8	16.3	46.0	58.2
	1951 cotyledons excised	7.0	12.2	33.0	55.5
	1952 commercial source	31.0	11.1	118.3	73.1
	1952 saved from 1951 sand culture	1.1	4.6	32.3	31.9
Broad bean	1951 entire seed commercial source	12.1	7.6	50.7	54.1
	1951 cotyledons excised	1.5	5.5	34.7	40.1
	1952 commercial source	46.7	26.8	55.6	66.2
	1952 saved from 1951 sand cultures	2.9	12.5	25.6	50.0
Pea	1951 commercial source	7.3	4.3	24.4	19.9
	1952 commercial source	—	7.2	55.6	26.1
	1952 saved from 1951 sand culture	—	4.6	25.6	23.0

noticeable for molybdenum deficiency. The effect was most consistent for dwarf beans but the greatest depression was obtained with copper deficiency in broad beans. The visual effects of these excision tests were evident in dwarf bean after about 7 days in all treatments. No visual symptoms were produced in the molybdenum deficient plants, but copper and zinc deficiencies were accentuated.

The effects of using impoverished seed in 1952 were, however, far more striking than those of cotyledon excision (Table IV). The plants raised from seed grown in complete nutrient sand cultures the previous year were normal and not visibly distinguishable from those raised in the same pot from commercial seed in the control treatment with complete nutrient. Yields were, however, smaller and the effect varied with the crop. Plants raised from deficient seed were strikingly reduced in growth and showed far more severe symptoms in all respective deficiency treatments when compared with plants in the same pot, grown from commercial seed. The contrast was greatest for molybdenum deficiency where symptoms were only seen visually in such impoverished plants. Runner bean and pea were most sensitive visually whilst dwarf bean and broad bean did not respond until much later in the experiment. In terms of yields, however, peas were the least responsive (Table IV).

Copper deficient seeds were next in order of contrast but the reserves of commercially raised seed were not sufficient entirely to prevent the appearance of deficiency symptoms in 1952 as was found for molybdenum. Runner bean and broad bean seemed most sensitive to the difference. Zinc deficiency was least influenced by the status of seed reserves but the effect was still evident both in yield (Table IV) and in the time required for visual symptoms to develop. Runner bean and broad bean again appeared to be the most sensitive to the difference in origin of seed. Examples of the effects of seed reserves are shown in Plate II and Plate IIId.

Analytical data.

Tables V and VI show the copper, zinc and molybdenum contents of seeds, and of foliage of plants derived from seed of different nutrient status. Tables of significance are given in Table VII. The concentrations of these elements and the total amount per seed shown in Table V varied considerably between different years for commercial seed or between commercial seed and that obtained

TABLE V

Relation of seed origin to copper, zinc and molybdenum content of the seed of large seeded leguminous crops

Crop	Seed origin *)	Copper		Zinc		Molybdenum	
		ppm	μg/seed	ppm	μg/seed	ppm	μg/seed
Runner bean	C 1951	5.11	3.53	8.17	5.69	0.52	0.36
	C 1952	2.29	2.39	16.9	17.7	0.19	0.20
	S complete nutrient	4.75	2.93	29.2	18.0	2.33	1.44
	S deficiency treatments	less than 0.051	less than 0.013	11.5	3.89	0.28	0.11
Broad bean	C 1951	8.59	8.48	14.2	14.1	1.14	1.13
	C 1952	8.10	16.8	22.5	46.8	3.64	7.54
	S deficiency treatments	—	—	—	—	0.13	0.14
Dwarf bean	C 1951	3.74	1.83	31.8	15.4	2.32	1.21
	C 1952	4.50	2.40	25.8	13.8	6.11	3.25
	S complete nutrient	8.31	3.99	41.7	20.1	5.95	2.87
	S deficiency treatments	less than 0.037	less than 0.010	8.15	3.87	0.11	0.04
Pea	C 1951	8.26	2.58	7.34	2.30	0.91	0.28
	C 1952	4.17	1.20	24.2	6.04	0.57	0.16
	S complete nutrient	2.89	0.80	16.9	4.68	3.02	0.83
	S deficiency treatments	—	—	11.5	3.72	0.24	0.07

*) C Commercial seed for the year shown.
S Seed saved from plants grown in respective sand cultures.

from plants grown in complete nutrient sand cultures. Still greater differences were found when seed raised from complete nutrient sand cultures was compared with that obtained from deficiency treatments. The nutrient status of such deficient seed was usually also drastically reduced when compared with seed of commercial origin. The relative decrease was greatest for copper in the instances where material was available for analysis and least for zinc. The molybdenum content of commercial broad bean and dwarf bean seed was apparently adequate for the normal growth of plants produced from them.

The analysis of foliage samples given in Table VI showed that the use of deficient seed saved from sand cultures did not have any great or consistent effect on the content of the deficient element in

TABLE VI

Relation of treatment and seed origin to copper, zinc and molybdenum content of foliage of large seeded leguminous crops grown in sand cultures

Treatment		Copper deficiency	Zinc deficiency	Molybdenum deficiency	Complete nutrient		
Crop	Seed origin *)	ppm Cu	ppm Zn	ppm Mo	ppm Cu	Zn	Mo
Runner bean	C 1951	4.1	22.6	0.045	8.0	28.9	1.4
	C 1952	2.3	27.5	0.025	5.7	25.0	1.2
	S	3.1	25.4	0.030	—	—	—
Broad bean	C 1951	5.5	15.3	0.41	8.7	41.2	2.3
	C 1951 cotyledons excised	0.4	6.2	0.27	3.5	32.0	2.4
	C 1952	2.6	9.4	0.029	9.1	30.7	—
	S	3.9	3.8	0.085	9.0	18.4	0.85
Dwarf bean	C 1951	3.3	22.5	0.067	4.8	43.3	1.5
	C 1952	1.9	13.0	0.11	6.8	16.3	0.50
	S	6.0	8.9	0.058	12.1	17.0	0.76
Pea	C 1951	6.7	15.5	0.060	11.6	46.0	2.0
	C 1952	—	12.9	below 0.01	7.8	11.7	0.48
	S	—	21.7	below 0.01	23.0	26.5	0.39

*) C Commercial seed for year shown.
S Seed saved from plants grown in respective sand cultures.
Plants produced from C 1952 and S seed were grown adjacent in the same containers in each respective treatment.

the leaves when compared with material obtained from commercial seed and no differences were significant. The effect of using deficient seed was shown in the yield data and was therefore reflected mainly in *total content* of the element rather than in its concentration in the plants. On the other hand, deficiency treatments usually resulted in reduced concentrations of the respective elements compared with effects of the complete nutrient treatment irrespective of the origin of the seed, or the fact that plants were grown from the two sources of seed in the same container. There were occasional varietal differences summarised in the tables of significance (Table VI).

The relative decrease in concentration due to deficiency conditions was generally least for zinc and greatest for molybdenum although the latter was present in the least amount. Thus, the effect of deficiency treatments on nutrient content of foliage differed from that on the accumulation of nutrients in seed, as regards the *relative* effects for the different elements.

TABLE VII

Tables of significance of treatment effects for analytical data on copper, zinc and molybdenum in leaves and seeds of large seeded leguminous crops grown in sand culture

1951 Harvest: leaves from commercial seed			
Effect of Cu deficiency on content	5%		
,, Zn ,, ,,	5%		
,, Mo ,, ,,	1%		
,, varieties × treatments	1%		
,, varieties (Mo) on content	0.1%		
,, varieties (Cu) ,,	5%		
Variety × treatment interactions were tentatively ascribed to the higher molybdenum content of broad beans and the higher copper content of pea and broad beans.			
1952 Harvest: leaves from commercial or "sand culture" seed			
Effect of Cu deficiency on content	1%		
,, Zn ,, ,,	n.s.	block replicate values varied widely	
,, Mo ,, ,,	5%		
,, seed origin ,,	n.s.		
Origin of seeds × varieties	n.s.		
,, seeds × treatments	n.s.		
Varieties × treatments	n.s.		
1951/52 Seeds obtained commercially or saved from sand culture plants; for data in terms of μg metal per seed			
(i)		*(ii)*	
Effect on Cu: Commercial seed *v.* control saved seed:	n.s.	Control *v.* deficiency saved seed:	—
,, ,, Zn: Commercial seed *v.* control saved seed:	n.s.	Control *v.* deficiency saved: seed	$F = 4.77$ $n = 2$
,, ,, Mo: Commercial seed *v.* control saved seed:	n.s.	Control *v.* deficiency saved seed:	$F = 7.05$ $n = 2$
,, of season: 1951/52	n.s.	Not significant at 5% owing to limited number of degrees of freedom	
Effect of varieties: Commercial seed:	5%;	attributed to the high concentration in broad bean and the low concentration in pea seeds.	
,, ,, varieties: Saved seed:	1%;	attributed to low content of pea seed.	
Interaction metals × varieties	1%;	ascribed to high Mo content in dwarf bean and generally low content in pea seeds.	
Differences between Cu, Zn and Mo concentrations significant at 0.1% in each set; Zn > Cu > Mo.			

The concentrations of the respective element found in the dried foliage of plants and the levels present in the complete nutrient solution permit an approximate estimate of the relative ratios of these values for each element as follows: Cu, 50–350; Zn, 180–700;

Mo, 8–45. The differences between the concentrations of copper, zinc and molybdenum in the foliage were significant at 0.1%. Corresponding ratios deduced for deficiency conditions when the respective treatments provided probably 0.001 ppm or less of Cu or Zn and 0.00001 ppm Mo in the nutrient solution in 1952 range as follows: Cu 2000–7000; Zn, 4000–27000; Mo, 1000–40000. The values for copper and zinc have maintained similar proportions but the ratio for molybdenum has notably increased to a similar level. The somewhat high ratios for zinc deficiency were not considered to be due to contamination of the cultures as identical treatments produced exceptionally severe effects in tomato and were also rapidly lethal for red clover, but all ratios would be inflated by the contribution from the seed.

DISCUSSION

The striking contrast between the effects of zinc deficiency seen in 1950 and 1951 and those recorded in 1952 suggests either that alkali-soluble zincates may occur in the sand or that successive batches differed markedly in available zinc content.

The water supply tests show beyond reasonable doubt that ion-exchange resins can be used to purify rain water to a degree comparable to distillation in all-glass apparatus. The former method provides a high output with little attention and should greatly assist in the maintenance of large scale culture experiments. The extent of molybdenum removal by the cation resin used in 1951 was less than that achieved in 1952. The average values found by *A. niger* 5) assay were 0.000013 and 0.000008 ppm respectively as compared with an initial value of 0.0005 ppm. That the cation resin was so effective suggests either that reduction of molybdate occurred at the resin surface or that lower valency compounds of molybdenum were present in the rain water. The reduction of vanadium on ion-exchange resins has been shown recently 20).

The results demonstrating the importance of seed reserves in determining the type of response to all three deficiencies and especially of molybdenum entirely confirm those of Meagher *et al.* 13). Seed reserves will thus affect the uniformity of responses in experiments designed to study these deficiencies. The results also support the conclusion of Wilson 24) on the origin and incidence of "scald" in beans and explain our earlier failures 6) 10) to obtain marked effects from molybdenum deficiency in sand cultures with such large seeded species. Removal of the cotyledons was tested also in this earlier work without effect. Results in the present work suggest that this procedure is not very effective and the seed reserves are therefore presumably located in, or rapidly translocated to the plumule and radicle during the first 5–8 days of germination. Bertrand 2) recorded up to 50 ppm molybdenum in the embryos of bean seeds. A few instances were recorded (Table IV) in which differences between yields obtained from plants grown in successive years from commercial seed exceeded those found between plants grown from commercial or sand culture seed in 1952. Although comparisons between results obtained in different years are not strictly valid it is noteworthy that in the deficiency treatments these relative changes in yields often reflected the variations in the total content of the respective elements in the seed. Similar changes, though less in proportion, occurred with plants given the complete nutrient treatments. Yields obtained with molybdenum deficiency in 1952 were mainly greater than those recorded for plants given the complete nutrient treatment when grown from commercial seed and the molybdenum level in the complete nutrient may have exceeded at some stage the optimum concentration for these species already provided with apparently adequate reserves in the seed.

No explanation is possible at present for the slight reduction in growth of plants in the complete nutrient treatment as the result of using seed saved from previous cultures. The relative effects of the deficiency treatments were, however, much greater than this latter effect and left no doubt about the importance of seed reserves in the incidence of copper, zinc and molybdenum deficiencies in these large seeded species.

Leaf morphological effects produced by zinc deficiency in tomato, runner bean and broad bean were consistent with those already known to occur in other crops. The asymmetrical unilateral curvature in tomato and runner bean leaflets recalled the "sickle leaf" symptoms in cacao recently identified as zinc deficiency by Greenwood and Hayfron 3).

Millikan 14) 15) also observed the characteristic notching of subterranean clover leaflets deficient in zinc; leaflets of red or alsike clovers grown in our experiments were not noticably thickened but no anatomical studies were made on microscopic structure. There is general agreement with Millikan [14]) and others, *loc. cit.* on the greater tolerance of lucerne to zinc deficiency, and confirmation of the great sensitivity of this species to copper deficiency and of the characteristic "die-back" of apical growth preceded by necrosis and dark blue-green foliage, but not of the epinastic effects noted by Millikan.

The exudation observed in zinc deficient tomato and maize, and also in oat (unpublished work) suggests that the cell membranes may have deteriorated with consequent loss of their capacity to retain cell solutes.

The analytical results show that the concentration of zinc may not always provide a reliable guide to a deficiency status and suggest that the level of 0.002 me/l (0.065 ppm) given in the complete nutrient solution is still below the threshold for optimum growth of these crops. This view would be consistent with conclusions derived from a recent survey of literature on this point [4]) and with the results of Wilson [23]) who concluded that loblolly pine requires a minimum of 0.1 ppm Zn for normal growth. The lack of correspondence between depleted content in the seed of the three elements examined, and values found in foliage of deficient plants is also noteworthy. The sharp reduction in copper content of depleted seed suggests that these organs may reflect more sensitively the copper status of some crops than their foliage. Seeds were also relatively sensitive to changes in molybdenum status but were least sensitive to zinc.

Our results with large seeded legumes did not show the marked increase in copper content of the deficient plants reported by Millikan [14]) for the clovers, whilst the decreases in zinc content in deficient plants were generally less than those found by Millikan and were only notable in broad bean. The molybdenum content and range in dwarf bean seed compared closely with the values found by Johnson *et al.* [12]) but normal pea seeds analysed by us contained only one fourth to one sixth of the molybdenum content of commercially produced seed examined by Johnson *et al.* The data suggest that their seeds were larger as well as richer in molybdenum than ours.

SUMMARY

(1) A method is described for the production of acute deficiencies of copper, zinc or molybdenum in large scale sand cultures in which an adequate and satisfactory supply of water was obtained by treating rain water with ion-exchange resins. Results compared favourably with the use of glass distilled water.

(2) Visual symptoms of copper, zinc and molybdenum deficiencies in large seeded leguminous crops, and in other species are summarised.

(3) Seed reserves played an important part in the incidence of copper and molybdenum deficiencies and to a lesser extent of zinc deficiency in pea, runner bean, dwarf bean and broad bean.

(4) The "deficient" seed was obtained by harvesting seed from plants grown in the respective deficiency sand cultures, and was compared with seed obtained similarly from complete nutrient sand cultures or commercially from field-grown plants.

(5) Seed reserves of molybdenum were usually sufficient for a complete generation but reserves of copper and zinc were not adequate for the whole copper or zinc requirements of the plants.

(6) Analytical data showed a marked decrease in the copper or molybdenum content of "deficient" seed, and a less striking decrease in zinc.

(7) Foliage of deficient plants showed decreased content of copper and molybdenum and often of zinc compared with plants given the complete nutrient treatment, but use of "deficient" seed only decreased yields without further appreciably decreasing the mineral content of the foliage.

ACKNOWLEDGMENTS

We wish to thank Mr. G. M. Clarke, B.Sc. for statistical evaluation of the analytical data, and Mr. G. H. Jones, A.R.P.S. for the photography. The analytical method for copper and zinc was investigated and standardised by Mr. B. A. Notton whose assistance is gratefully acknowledged.

Received 26 November, 1953.

REFERENCES

1) "Analysis of aluminium and its alloys" p. 33. British Aluminium Company, London (1941).

2) Bertrand, D., Contribution a l'étude de la diffusion du molybdène. Compt. Rend. **208**, 2024–2026 (1939).

3) Greenwood, M. and Hayfron, R. J., Iron and zinc deficiencies in cacao in the Gold Coast. Empire J. Exp. Agr. **19**, 73 (1951).

4) Hewitt, E. J., "Sand and water culture methods used in the study of plant nutrition". Commonwealth Bur. Hort. Plantation Crops, Tech. Commun. no 22 (1952).

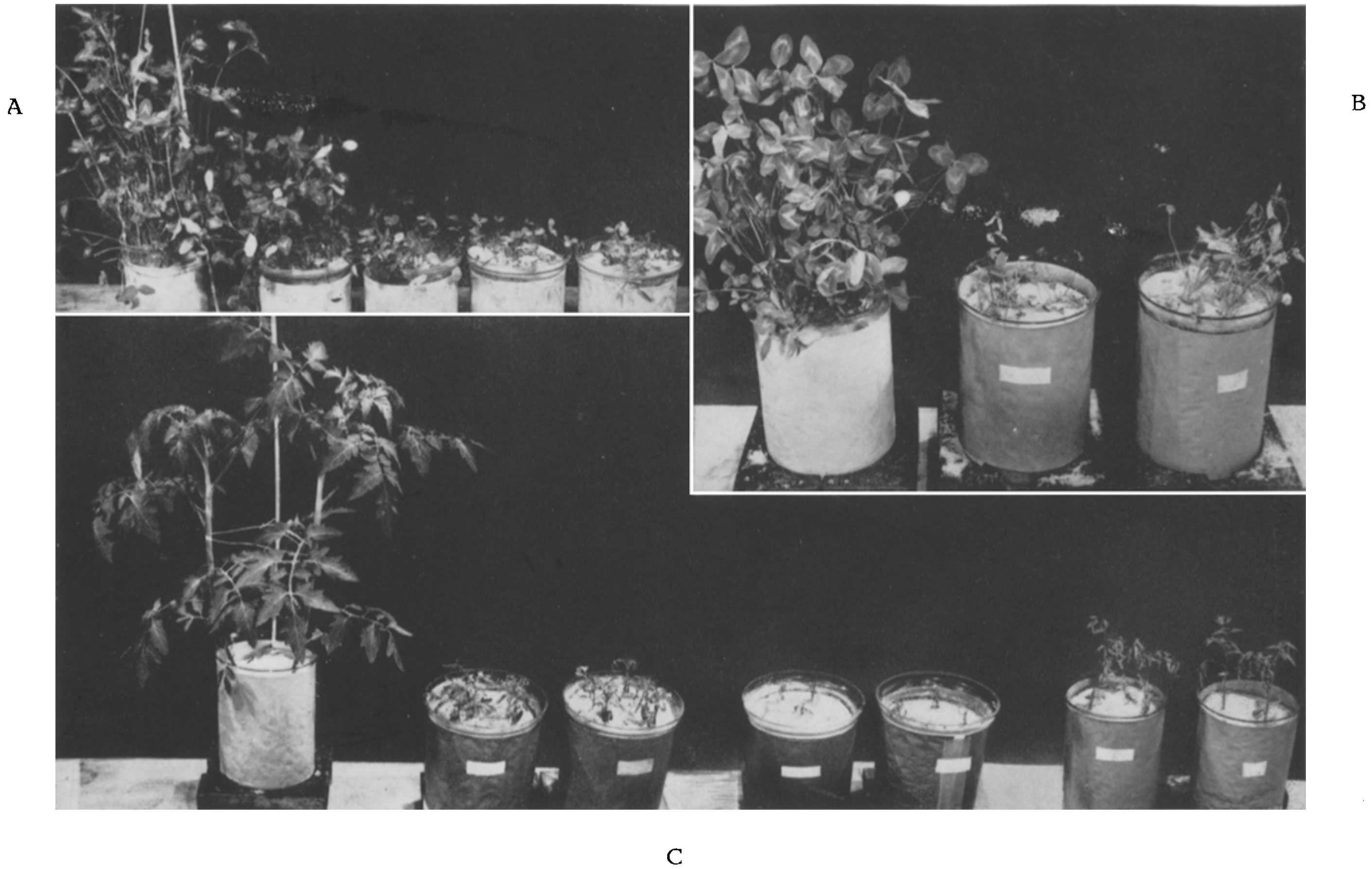
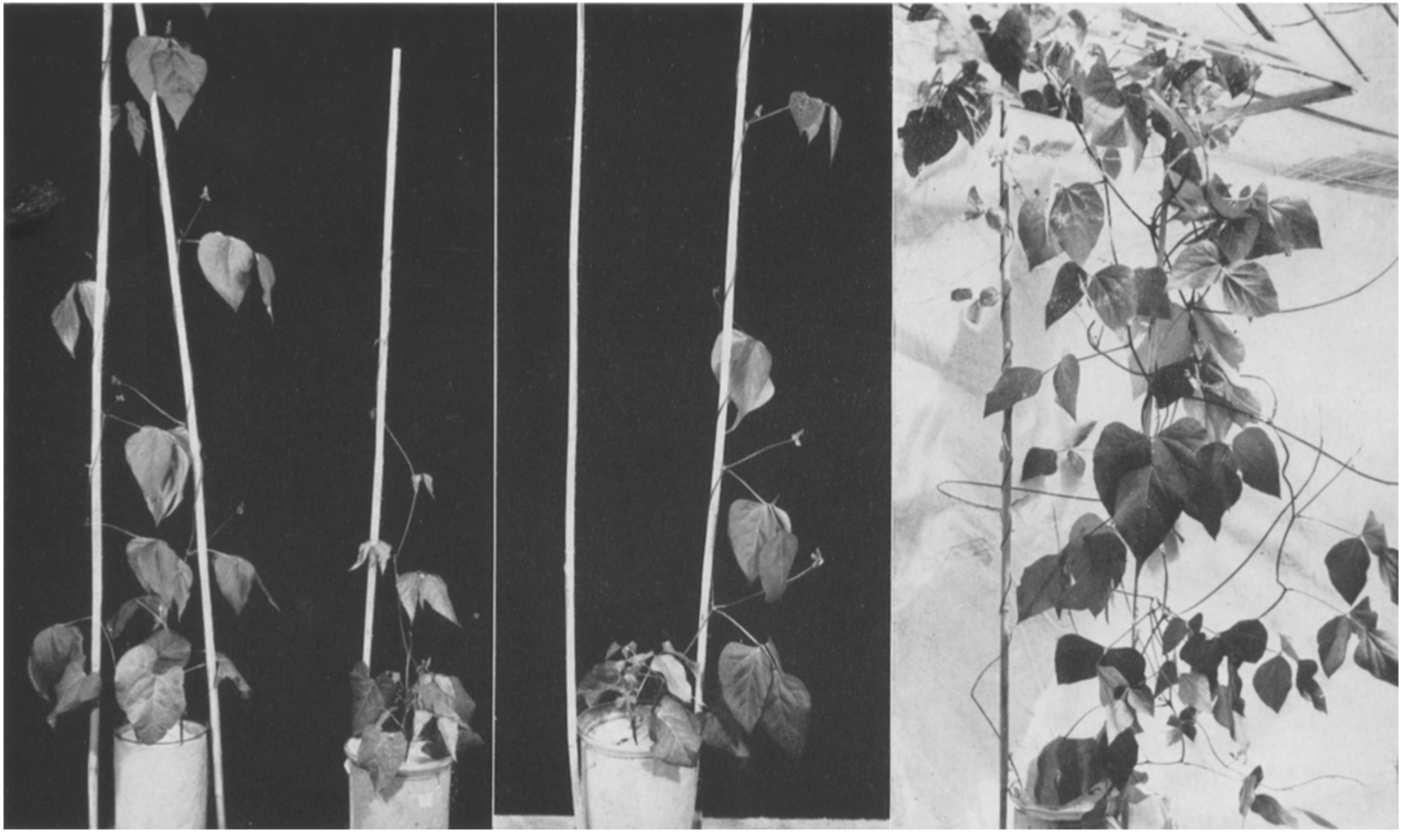


Plate I



A B C

Plate II

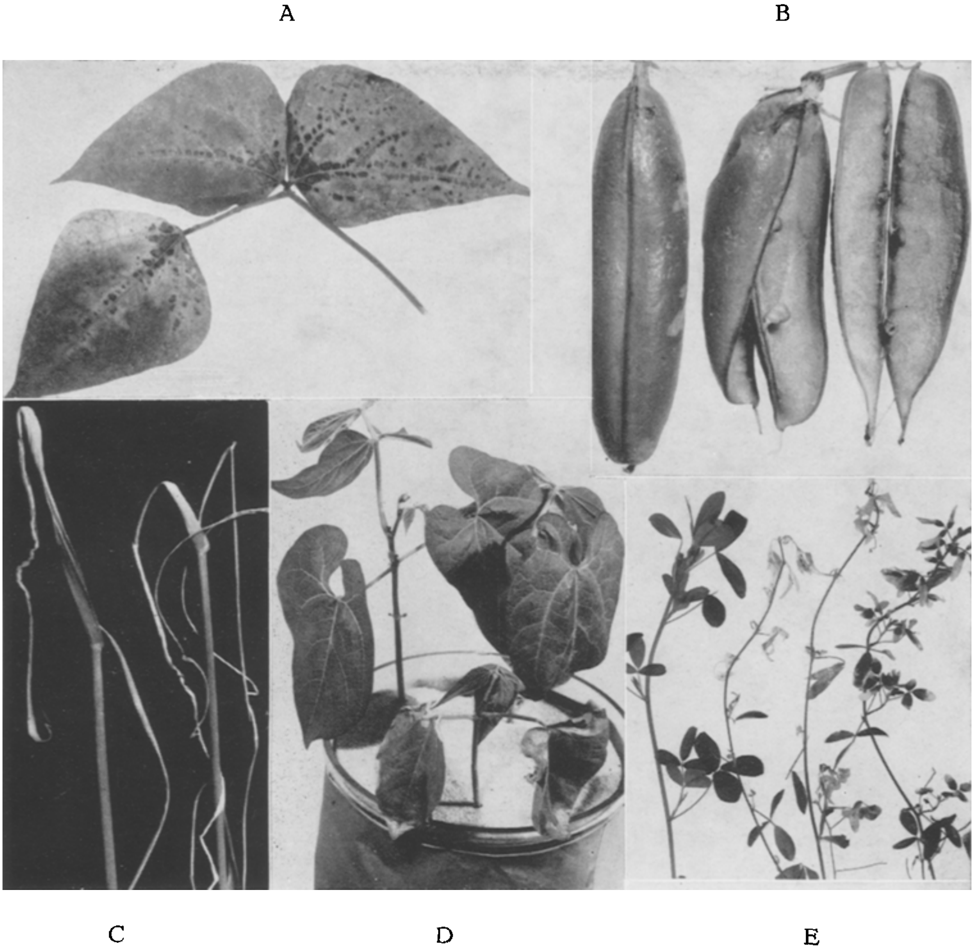


Plate III

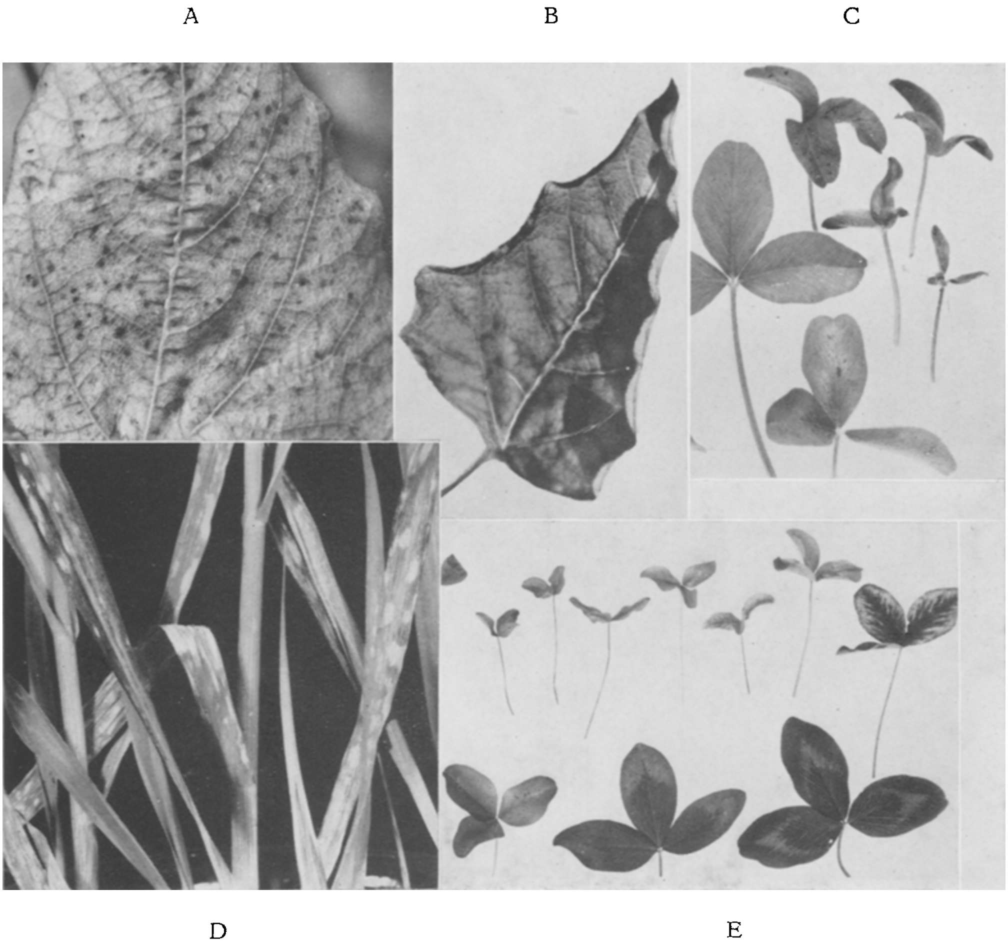


Plate IV

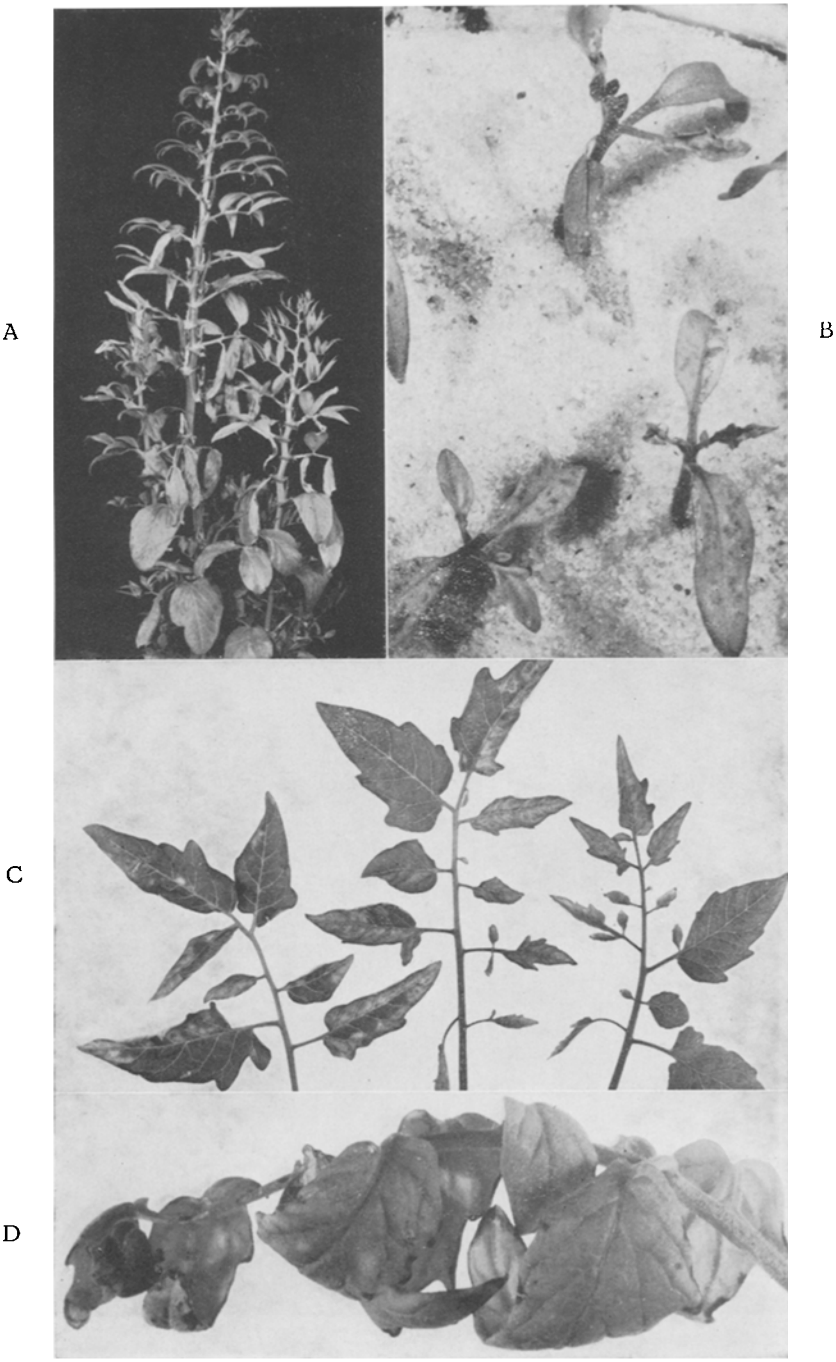


Plate V

5) Hewitt, E. J. and Hallas, D. G., The use of *Aspergillus niger* (Van Teigh.) M Strain as a test organism in the study of molybdenum as a plant nutrient. Plant and Soil **3**, 366 (1951).
6) Hewitt, E. J. and Jones, E. W., Molybdenum as a plant nutrient. The effects of molybdenum deficiency on some vegetables, cereals and forage crops. Long Ashton Research Sta. Ann. Rep. 1948, 81 (1949).
7) Hewitt, E. J. and Jones, E. W., The effect of zinc and copper deficiencies on crop plants grown in sand culture. Long Ashton Research Sta. Ann. Rep. 1950, 56.
8) Hewitt, E. J. and Bolle–Jones, E. W., The effects of zinc and copper deficiencies on crop plants grown in sand culture. Long Ashton Research Sta. Ann. Rep. 1951, 58 (1952).
9) Hewitt, E. J. and Bolle–Jones, E. W., Molybdenum as a plant nutrient. I. The influence of molybdenum on the growth of some brassica crops in sand culture. J. Hort. Sci. **27**, 245 (1952).
10) Hewitt, E. J. and Bolle–Jones, E. W., Molybdenum as a plant nutrient. II. The effects of molybdenum deficiency on some horticultural and agricultural crop plants grown in sand culture. J. Hort. Sci. **27**, 257 (1952).
11) Hewitt, E. J. and Miles, P., The effects of seed reserves on the production of deficiencies of copper, zinc and molybdenum in some large seeded leguminous crops. Long Ashton Ann. Rep. 1952, 199 (1953).
12) Johnson, C. M., Pearson, G. A. and Stout, P. R., Molybdenum nutrition of crop plants. II. Plant and soil factors concerned with molybdenum deficiencies in crop plants. Plant and Soil **4**, 178 (1952).
13) Meagher, W. R., Johnson, C. M. and Stout P. R., Molybdenum requirements of leguminous crops supplied with fixed nitrogen. Plant Physiol. **27**, 223 (1952).
14) Millikan, C. R., Relative effects of zinc and copper deficiencies on lucerne and subterranean clover. Australian J. Biol. Sci. **6**, 164 (1953).
15) Millikan, C. R., Subterranean clover; symptoms of nutritional deficiencies. J. Agr. Victoria (Australia) May 1953, 215 (1953).
16) Parks, R. Q., Hood, S. L. and Hurwitz, C., Quantitative micro-determination of 12 elements in plant tissues. Ind. Eng. Chem. Anal. Ed. **15**, 529 (1943)
17) Piper, C. S., "Soil and plant analysis" 1st Ed. Univ. Adelaide (1942).
18) Piper, C. S., Manganese deficiency in oats. Nature **153**, 197 (1944).
19) Piper, C. S. and Beckwith, R. S., A new method for the determination of small amounts of molybdenum. J. Soc. Chem. Ind. (London) **67**, 374 (1948).
20) Salmon, J. E. and Tetze, H. R., Separation of quadrivalent and quinquevalent vanadium from phosphoric acid by ion-exchange resins. J. Chem. Soc. (London) 2324 (1952).
21) Sandell, E. B., "Colorimetric determination of traces of metals" 1st Ed. Interscience, New York 1944, p. 454.
22) Virtanen, A. I., The use of seeds with low content of trace elements in studies on essentiability of micronutrients. Plant Physiol. **28**, 323 (1953).
23) Wilson, C. C., The response of two species of pine to various levels of nutritional zinc. Science **117**, 231 (1953).
24) Wilson, R. D., Molybdenum in relation to the "scald" disease of beans. Australian J. Sci. Research **11**, 209 (1949).

EXPLANATION OF PLATES

Plate I. Comparison of effects of different methods of water treatment on the production of copper, zinc or molybdenum deficiency in sand culture.

a. 1951 copper deficiency series in red clover; from left: complete nutrient, copper deficiency using metal distilled water, resin purified rain water, once glass- and twice glass-distilled water.

b. 1952 molybdenum deficiency series in red clover; left; complete nutrient; centre: molybdenum deficiency using resin purified rain water; right: using once glass distilled water.

c. 1952 tomato series; extreme left: complete nutrient; 1st pair: copper deficiency; 2nd pair: zinc deficiency; 3rd pair molybdenum deficiency. In each pair the pots given resin purified water are on the left and those given glass distilled water are on the right.

Plate II. Effects of seed reserves on growth of runner bean in sand cultures.

a. Left hand pot: Complete nutrient; saved seed on left, commercial seed on right. Right hand pot: zinc deficiency; commercial seed at rear slightly to left, plant climbing; saved seed at front and not climbing.

b. Copper deficiency; saved seed in front on left, plant not climbing; commercial seed at rear on right, plant climbing.

c. Molybdenum deficiency; saved seed on left, weak, chlorotic and scorched; commercial seed on right, vigorous growth.

Plate III. Copper deficiency.

a. Necrosis pattern in dwarf bean leaves.

b. Seed abortion after pod development in pea. Left hand shows apparently normal external habit of similar pods shown opened.

c. Characteristic coiling of chlorotic apical leaves of wheat.

d. Effect of seed reserves of dwarf bean. Foreground plant grown from copper deficient seed with collapse of prophylls; background plants grown from commercial seed.

e. Terminal shoot "die-back" and sudden withering from top of leaflets in lucerne.

Plate IV. Zinc deficiency symptoms.

a. Part of runner bean leaflet showing scattered rounded dark green areas in chlorotic tissue.

b. Runner bean leaflet showing pronounced marginal waving.

c. Alsike clover; pronounced marginal waving and folding of leaflets with involute leaf tips, also general chlorosis.

d. Oat; non-pathogenic lesions on older leaves showing a resemblance to manganese deficiency, plants remarkably free from mildew attack.

e. Red clover; fading and chlorotic patterns, margins of leaflets wavy (lower right from complete nutrient).

Plate V. Zinc deficiency symptoms.

a. Broad bean; characteristic conical habit, shortened internodes, narrow downcurved leaflets towards apex, and chlorosis.

b. Severe deficiency in young tomato seedlings, necrosis and collapse of first leaves.

c. "Little leaf" effect in tomato leaflets and unilateral curvature or asymmetry.

d. Coiling effect in tomato leaflets with marginal necrosis.