Effects of Trace Elements on Hevea Brasiliensis Seedlings Grown in the Nursery

J. A. VARLEY*

Two trace element experiments are described. The first, an unreplicated exploratory trial involving five trace elements, was designed for obtaining sufficient information to enable the second experiment, a fully replicated 3^4 factorial, to be laid down. In the exploratory trial it was possible to characterise boron toxicity and to determine, by leaf analysis, an approximate level at which the first syndrome of this deficiency was observed.

The main experiment showed that the poor response to major nutrient application of rubber seedlings growing on well and leached coarse granitic soil under conditions of good husbandry and drainage was not due to trace element deficiencies.

Trials in Indonesia and Malaya (GRANTHAM, 1924, 1927, 1930, VAN HEUSDEN AND VOLLEMA, 1931, VOLLEMA, 1931, HAINES AND GUEST, 1936) resulted in the early emphasis on rubber fertilising being placed on nitrogen, supplemented on poor soils with phosphate and potash. Recognition of a widespread need for magnesium subsequently led to the incorporation of magnesium in the recommended fertiliser mixtures for Malaya (RUBBER RESEARCH INSTITUTE OF MALAYA, 1963). Field syndromes of iron and manganese deficiency are now recognised, suspected calcium, copper, molybdenum and zinc deficiencies have been reported and the possible development of boron deficiency has been anticipated (SHORROCKS, 1964a).

Five factorial fertiliser trials laid down by the Guthrie Corporation to determine the N, P, K and Mg needs of rubber seedlings on different soils showed that the growth, vigour and response levels were so satisfactory that interfering deficiencies of other nutrients seemed unlikely. In a sixth trial on Tampin series soil (OWEN, 1951), however, growth was poor and responses were negligible.

* Of Chemara Research Station, Seremban, Malaysia. Present address: Tropical Soils Analysis Unit, Land Resources Division, Directorate of Overseas Surveys, Ministry of Agriculture, Fisheries and Food, S.E. Region, Coley Park, Reading, Berkshire.

The present experiments were designed to determine whether the major nutrients were ineffective in this last trial because micronutrient deficiencies limited growth and, if so, to obtain information on application rates.

EXPERIMENTAL

The investigation started with an exploratory study to determine which elements warranted further examination and the rates at which they should be used, and to investigate possible toxicities. Boron, copper, manganese, molybdenum and zinc were selected for the exploratory study. The exploratory work led to the further investigation of boron, copper, manganese and molybdenum in five replicates of a 3^4 factorial experiment.

Copper, manganese and zinc were applied as their sulphates, boron as sodium metaborate (borax) and molybdenum as sodium molybdate. The salts were applied directly to the ground at each planting point and gently mixed with the soil.

Site

The exploratory trial was conducted on a coarse-grained granite-derived soil at the Chemara Research Station, near Seremban, in Malaysia.

The factorial trials were on estate and some miles away that had previously carried old seedling rubber. The rubber was felled and

COMMUNICATION 435

stumped, with minimum soil disturbance, and the cleared area clean weeded by hand, and then sprayed with Simazine. Two sites some 500 yards apart both on Rengam/Tampin association soil, were used.

Lay-out

For both the exploratory trial and the main experiment micro plots were used. In the exploratory trial, each plot had ten points per treatment, with two seedlings per point.

The five replicates of the main experiment were located, two on one site and three on the other. Each plot contained five points of two plants per point. Points were spaced 3 ft apart within plots, with 6 ft between plots. Bunds were raised between plots and the site was protected by storm drains from surface water movement. There were about one hundred additional plots evenly distributed over the five replicates, enabling only plots carrying a full stand of ten seedlings to be used for the experiment.

In both the exploratory trial and the main experiment, seed germination techniques were similar. Tjir 1 selfed seeds were germinated in a sand bed and rogued for abnormalities. Three good six-day-old seedlings were planted at each point, and after one month one seedling was removed, so that there was an even stand of two seedlings per point.

Major Element Manuring

In the exploratory trial, a mixture of ammonium sulphate, double superphosphate, muriate of potash and kieserite containing 8% N, 14% P_2O_5, 6% K_2O and 2.4% MgO was applied at the following rates and intervals:

1 month after planting — 1 oz per point
2 months after planting — 1 oz per point
4 months after planting — 2 oz per point
6 months after planting — 2 oz per point

Some three and a half months after planting, slight magnesium deficiency developed throughout the experiment. Applying ½ oz of kieserite per point, along with the four-month major element fertiliser dressing, rapidly cured the condition.

In the main experiment, the intervals were somewhat different.

1 month after planting — 1 oz per point
2 months after planting — 1 oz per point
7 months after planting — 2 oz per point

The reason for the long interval between the second and third application of major element manures was a five-month period of drought. During this time, surface application of fertilisers would have been ineffective.

Minor Element Manuring

The minor elements in the exploratory trial were applied individually in three applications, after three, six and eight months. The forms used, and the amounts applied are detailed in *Table 1, Experiment 1*. After the first round of manuring with borax, toxicity symptoms developed at Levels 3 and 4. For this reason, the amounts of borax applied in the second round of trace element manuring were not increased.

In the factorial experiment, the rates of application (see *Table 1, Experiment 2*) were kept at levels indicated by the exploratory trial as being non-toxic. Only one application of the minor element manures was applied, two months after planting.

Measurements

The height of each plant and its diameter 2 in. above the collar were measured and leaf samples were taken for analysis, seven and ten months after planting in the exploratory trial and eleven months after planting in the factorial experiment.

In both investigations the bottom two leaves from the second hardened whorl were the leaves taken for analysis (CHAPMAN, 1941). The factorial experiment analysis were on bulk leaf samples from all five replicates, the amount collected being insufficient for the replicates to be analysed separately.

Analytical Methods

Nitrogen was determined by a micro Kjeldahl digestion using a selenium catalyst followed by distillation and titration of the ammonia. The other major elements and manganese were determined on a nitric acid extract of the plant ash, phosphorus as the yellow molybdenum-vanadate complex, potassium by flame photometer, calcium and magnesium by

TABLE 1. TYPE, RATE* AND TIME OF APPLICATION OF THE TRACE ELEMENT MANURES IN THE TWO TRACE ELEMENT EXPERIMENTS

Expt. 1. Trace Element Exploratory Trial

Fertiliser	% Element	3 months after planting Amount (g)					6 months after planting Amount (g)					8 months after planting Amount (g)				
		Level 0	1	2	3	4	Level 0	1	2	3	4	Level 0	1	2	3	4
$Na_2B_4O_7.5H_2O$ (Fertiliser borax)	15% B	0	2	4	8	16	0	2	4	8	16	0	4	8	16	32
$CuSO_4.5H_2O$	25% Cu	0	2	4	8	16	0	4	8	16	32	0	8	16	32	64
$MnSO_3.H_2O$	32% Mn	0	4	8	16	32	0	8	16	32	64	0	16	32	64	128
$Na_2MoO_4.2H_2O$	40% Mo	0	1	2	4	8	0	2	4	8	16	0	4	8	16	32
$ZnSO_4.7H_2O$	22% Zn	0	2	4	8	16	0	4	8	16	32	0	8	16	32	64

*per point of two seedlings

Expt. 2. 3^4 Factorial Trace Element Experiment

Fertiliser	% Element	2 months after planting Amount (g)		
		Level 0	1	2
$Na_2B_4O_7.5H_2O$	15% B	0	2	4
$CuSO_4.5H_2O$	25% Cu	0	2	4
$MnSO_4.H_2O$	32% Mn	0	4	8
$Na_2MoO_4.2H_2O$	40% Mo	0	1	2

E.D.T.A. titration after removing interfering phosphate by precipitation with zirconium nitrate, and manganese as permanganate produced in the cold by sodium bismuthate. Boron was determined in concentrated sulphuric acid with carmine red. Copper and molybdenum were determined on a single nitric/perchloric acid digest, copper by zinc 00-di-isopropyl phosphorodithizonate in carbon tetrachloride and molybdenum as the green dithiol complex after removing copper and co-precipitating with iron using cupferron. Zinc was determined on a separate nitric/perchloric acid digest. After digestion, the heavy metals were complexed with potassium cyanide, the zinc cyanide was destroyed using chloral hydrate and the zinc determined colorimetrically with dithizone.

The results are presented in *Tables 2–5*.

DISCUSSION

Effect of Boron

The highest levels of boron application in the exploratory trial rapidly induced boron toxicity. Its syndrome has previously been described and in fact has been reported on young rubber growing on Tampin series soil (RUBBER RESEARCH INSTITUTE OF MALAYA, 1964). The first symptom, which appeared about one week after the trace element manure application, was an interveinal chlorosis which developed very rapidly over one or two days and at first markedly resembled severe manganese deficiency. This was followed by necrosis, first of the leaf tips, and later the leaf margins. In the most severe cases, occurring at Level 4 (16 g borax per point), the plants become

TABLE 2. HEIGHT, DIAMETER AND CHEMICAL ANALYSIS AT 7 MONTHS AFTER PLANTING
(Average of 20 plants)

(a) BORON

Level	Height (cm)	Diameter (mm)	B p.p.m.	K %
0	154.4	16.2	14	1.08
1	174.0	18.4	59	1.25
2	171.1	17.9	92	1.29
3	156.5	16.9	177	1.41
4	127.8	14.0	242	1.56

(b) COPPER

Level	Height (cm)	Diameter (mm)	Cu p.p.m.
0	144.0	17.3	8.8
1	152.7	18.0	9.0
2	144.5	17.2	8.6
3	162.8	17.8	8.6
4	125.2	15.2	10.2

(c) MANGANESE

Level	Height (cm)	Diameter (mm)	Mn p.p.m.
0	152.4	17.0	39
1	153.7	16.5	174
2	164.6	18.1	165
3	160.3	16.9	159
4	154.4	16.3	216

(d) MOLYBDENUM

Level	Height (cm)	Diameter (mm)	Mo p.p.m.
0	166.9	18.4	0.3
1	155.2	17.9	1.7
2	163.3	18.2	1.7
3	165.9	18.3	4.9
4	152.4	17.5	13.0

(e) ZINC

Level	Height (cm)	Diameter (mm)	Zn p.p.m.
0	147.3	17.4	25
1	142.5	15.6	49
2	146.6	16.3	52
3	145.0	17.6	44
4	147.6	16.6	77

completely defoliated. Some of the plants recovered, but in others the terminal bud was killed. A number developed a chronic boron toxicity: though no new leaves emerged, the stems and bud remained green for 2–3 months before dying.

One of the twenty plants receiving Level 2 (4 g borax per plant) developed visible toxicity, whereas at Level 3 (8 g borax per point), nineteen were affected. It is concluded that the maximum permissible concentration of boron in the leaf, above which toxicity and stunting of growth occurs in rubber seed-

TABLE 3. HEIGHT, DIAMETER AND CHEMICAL ANALYSIS AT 10 MONTHS AFTER PLANTING
(Average of 20 plants)

(a) BORON

Level	Height (cm)	Diameter (mm)	B p.p.m.	K %
0	231.4	24.0	16	0.88
1	252.5	26.6	70	1.00
2	251.5	25.2	90	1.00
3	217.4	22.9	168	1.19
4	158.8	16.3	243	1.48

(b) COPPER

Level	Height (cm)	Diameter (mm)	Cu p.p.m.
0	209.6	23.1	7.4
1	222.5	25.1	7.6
2	221.5	24.6	8.3
3	241.0	25.9	8.2
4	191.0	22.8	9.3

(c) MANGANESE

Level	Height (cm)	Diameter (mm)	Mn p.p.m.
0	221.7	24.7	50
1	227.1	24.5	105
2	242.6	26.6	94
3	240.8	25.4	109
4	232.2	24.7	247

(d) MOLYBDENUM

Level	Height (cm)	Diameter (mm)	Mo p.p.m.
0	236.0	26.0	0.2
1	224.8	24.9	3.9
2	235.5	25.3	5.7
3	233.2	25.3	7.6
4	224.3	24.3	9.0

(e) ZINC

Level	Height (cm)	Diameter (mm)	Zn p.p.m.
0	212.9	26.6	24
1	214.9	23.9	37
2	220.7	24.5	35
3	228.6	26.2	38
4	218.7	24.0	57

lings, is slightly lower than the value obtained from Level 2 of the first sampling, that is, 80–90 p.p.m. boron in dry leaf material.

Within one month of applying the boron fertiliser in the factorial experiment, a large number of the plots receiving the highest level (4 g borax per point) showed the initial toxicity syndrome. The mean level of 87 p.p.m. leaf boron found in plants on these plots agrees well with the 80–90 p.p.m. estimate from the exploratory trial.

These concentrations are on a par with the leaf lamina B concentration with which

TABLE 4. EFFECT OF BORON AND COPPER ON THE HEIGHT, DIAMETER AND CHEMICAL ANALYSIS OF RUBBER LEAVES FROM THE FIRST HARDENED WHORL

(Mean of five replicates)

Element	Treatment	Mean per tree		Elements expressed on leaf dry matter									
		Height (cm)	Diameter (mm)	Ash %	N %	P %	K %	Mg %	Ca %	B p.p.m.	Cu p.p.m.	Mn p.p.m.	Mo p.p.m.
Boron	B0	286.3	32.4	4.76	3.85	0.235	1.052	0.230	0.813	23	5.9	180	1.42
	B1	281.7	31.3	4.71	3.86	0.240	1.075	0.221	0.734	56	5.9	170	1.53
	B2	277.6	30.7	4.62	3.90	0.245	1.101	0.226	0.700	87	6.1	166	1.78
	s.d.	±1.831	±0.195	±0.0348	±0.0162	±0.00116	±0.00805	±0.00230	±0.0128	±1.09	±0.005	±3.84	±0.0767
	B lin.	***	***	**	*	***	***	N.S	***	***	N.S	*	**
	B curv.	N.S	N.S	N.S	N.S	N.S	N.S	*	N.S	N.S	N.S	N.S	N.S
Copper	Cu0	280.7	31.6	4.73	3.86	0.242	1.075	0.228	0.761	57	4.4	172	1.80
	Cu1	284.0	31.6	4.72	3.87	0.239	1.071	0.227	0.756	54	6.5	177	1.62
	Cu2	280.4	31.3	4.66	3.88	0.239	1.082	0.223	0.729	54	6.9	166	1.31
	s.d.	±1.831	±0.195	±0.0348	±0.0162	±0.00116	±0.00805	±0.00230	±0.0128	±1.09	±0.005	±3.84	±0.0767
	Cu lin.	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	***	N.S	***
	Cu curv.	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	***	N.S	N.S

TABLE 5. EFFECT OF MANGANESE AND MOLYBDENUM ON THE HEIGHT, DIAMETER AND CHEMICAL ANALYSIS OF RUBBER LEAVES FROM THE FIRST HARDENED WHORL

(Mean of five replicates)

Element	Treatment	Mean per tree		Elements expressed on leaf dry matter									
		Height (cm)	Diameter (mm)	Ash %	N %	P %	K %	Mg %	Ca %	B p.p.m.	Cu p.p.m.	Mn p.p.m.	Mo p.p.m.
Manganese	Mn0	280.4	31.4	4.65	3.86	0.240	1.077	0.223	0.737	54	6.0	101	1.66
	Mn1	284.0	31.5	4.74	3.88	0.240	1.082	0.227	0.760	57	5.8	183	1.56
	Mn2	280.7	31.4	4.70	3.87	0.241	1.069	0.227	0.750	55	6.0	230	1.52
	s.d.	±1.831	±0.195	±0.0348	±0.0162	±0.00116	±0.00805	±0.00230	±0.0128	±1.09	±0.005	±3.84	±0.0767
	Mn lin.	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	***	N.S
	Mn curv.	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	***	N.S
Molybdenum	Mo0	283.0	31.6	4.70	3.85	0.241	1.081	0.223	0.761	56	5.9	172	0.26
	Mo1	280.4	31.4	4.71	3.87	0.239	1.073	0.227	0.733	56	5.9	171	1.59
	Mo2	281.9	31.5	4.69	3.89	0.241	1.074	0.228	0.753	54	6.0	171	2.88
	s.d.	±1.831	±0.195	±0.0348	±0.0162	±0.00116	±0.00805	±0.00230	±0.0128	±1.09	±0.005	±3.84	±0.0767
	Mo lin.	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	***
	Mo curv.	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

SHORROCKS (1964b) associates the onset of toxicity in the older leaves of twelve-month-old seedlings and of field plantings, are equal to or lower than the concentrations that BOLLE-JONES (1954) reported for the top and second whorl of leaves on healthy four-month-old Tjir 1 seedlings grown in sand culture with a complete nutrient solution, and are well below the concentrations he reported for the second whorl at five and a half months.

In the exploratory trial, there was some response (11% increase in the height and diameter) to non-toxic levels of borax. On average, this corresponded to an increase in leaf boron from 15 to 65 p.p.m. This effect was not found in the factorial experiment where both the height and the diameter of the plants were significantly reduced as leaf boron concentration increased. However, there was a difference in leaf boron levels in the *B0* plots between the experiments, indicating that 15 p.p.m. boron in leaf dry matter may be sub-optimal and 23 p.p.m. sufficient.

Borax application significantly increased leaf boron. There were positive correlations between leaf boron and leaf potassium, phosphorus and molybdenum in the factorial experiment, probably because the smallness of the leaves on the boron-damaged plants led to a concentration of the other elements in them.

Calcium was depressed significantly in step with increases in boron application.

Effect of Manganese

Again, in the exploratory trial, height and diameter were increased by 11%, leaf manganese correspondingly increasing from 45 to 130 p.p.m. SHORROCKS AND WATSON (1961) associated the manganese deficiency syndrome in *Hevea* with leaf manganese concentrations less than 50 p.p.m.

At the highest concentration measured, 247 p.p.m. leaf manganese, there was no sign of toxicity even though the manganese fertiliser applications involved is extremely high (224 g per point at ten months compared with the standard recommendation of less than 30 g per tree for one- to three-year-old rubber).

In the factorial experiment, manganese did not increase plant growth significantly, although leaf manganese concentration had been significantly increased.

Effect of Copper

Although the copper sulphate applications significantly increased leaf copper, they did not increase plant growth. In the exploratory trial, no copper toxicity syndrome developed but growth appeared to be reduced at the highest levels tested.

There was a significant depression of leaf molybdenum concentrations after heavy manuring with copper sulphate.

Effect of Molybdenum

Leaf molybdenum concentrations were increased by the sodium molybdate application, the effect being significant in the factorial experiment. In neither experiment was plant growth increased. The leaf molybdenum concentrations are well above the 0.05 p.p.m. limit of adequacy set by BOLLE-JONES (1957a).

In the exploratory trial, there were no signs of molybdenum toxicity although leaf molybdenum concentrations reached 13 p.p.m.

Effect of Zinc

Zinc sulphate application, tested only in the exploratory trial, did not increase growth. The leaf zinc content, when zinc had not been applied, was above the 15–16 p.p.m. that BOLLE-JONES (1957b) had found as the sharp lower limit of zinc adequacy in the leaves of Tjir 1 seedlings up to thirteen months old grown in sand culture. At the highest levels tested (112 g per point in ten months sustaining 77 p.p.m. zinc in leaf dry matter) growth was not impaired.

CONCLUSIONS

Properly nurtured *Hevea* seedlings growing on well-drained granitic soils, are unlikely to suffer from minor element deficiencies. This may not be true for bigger trees, for which, in particular, soil manganese reserves may be insufficient.

The experiment investigated growth where micro nutrient availability proved to be adequate or excessive: where these nutrients were withheld, the concentrations of boron, manganese, molybdenum and zinc in the leaves are higher than those that have been established in sand culture studies as adequate for young rubber seedlings.

Very heavy dressings of trace elements other than boron would be required to induce toxicity in the nursery. Toxicity develops where leaf boron concentration attains 80–90 p.p.m.

ACKNOWLEDGEMENT

The author thanks Mr I. Watson and Mr E. Bellis for their many helpful suggestions during the preparation of this paper, and Mr I. Watson for his valuable statistical work. Thanks are also due to the Directors of Chemara Plantations Limited for permission to publish this paper.

Chemara Research Station
Seremban
Malaysia

October 1968

REFERENCES

BOLLE-JONES, E.W. (1954) Nutrition of *Hevea brasiliensis* II. Effect of nutrient deficiencies on growth, chlorophyll, rubber and mineral contents of Tjirandji 1 seedlings. *J. Rubb. Res. Inst. Malaya*, **14**, 209.

BOLLE-JONES, E.W. (1957a) Molybdenum: effects on the growth and composition of *Hevea. J. Rubb. Res. Inst. Malaya*, **15(3)**, 141.

BOLLE-JONES, E.W. (1957b) Zinc: effects on the growth and composition of *Hevea. J. Rubb. Res. Inst. Malaya*, **15(3)**, 159.

CHAPMAN, G.W. (1941) Leaf analysis and plant nutrition. *Soil Sci.*, **52**, 63.

GRANTHAM, J. (1924) Manurial experiments on *Hevea. Archf Rubbercult. Ned.-Indië*, **8(8)**, 501.

GRANTHAM, J. (1927) Manurial experiments on *Hevea* II. *Archf Rubbercult. Ned.-Indië*, **11(10)**, 465.

GRANTHAM, J. (1930) Manurial experiments on *Hevea* III. *Archf Rubbercult. Ned-Indië*, **14(9)**, 345.

HAINES, W.B. AND GUEST, E. (1936) Recent experiments on manuring *Hevea* and their bearing on estate practice. *Emp. J. exp. Agric.*, **4(16)**, 300.

OWEN, G. (1951) A provisional classification of Malayan soils. *J. Soil Sci.*, **2**, 20.

RUBBER RESEARCH INSTITUTE OF MALAYA (1963) Revised manuring programme for young replantings. *Plrs' Bull. Rubb. Res. Inst. Malaya No.67*, 79.

RUBBER RESEARCH INSTITUTE OF MALAYA (1964) Boron toxicity in rubber. *Plrs' Bull. Rubb. Res. Inst. Malaya No. 73*, 76.

SHORROCKS, V.M. (1964a) *Mineral Deficiencies in* Hevea *and Associated Cover Plants.* Kuala Lumpur: Rubber Research Institute of Malaya.

SHORROCKS, V.M. (1964b) Boron toxicity in *Hevea brasiliensis. Nature, Lond.*, **204**, 599.

SHORROCKS, V.M. AND WATSON, G.A. (1961) Manganese deficiency on *Hevea*: the effect of soil application of manganese sulphate on the manganese status of the tree. *J. Rubb. Res. Inst. Malaya*, **17**, 19.

VAN HEUSDEN, W.C. AND VOLLEMA, J.S. (1931) Results of some manuring experiments carried out on the Government Rubber Estate 'Serpong'. *Archf Rubbercult. Ned.-Indië*, **15(3)**, 140.

VOLLEMA, J.S. (1931) Manuring experiments with *Hevea* in West Java. *Archf Rubbercult. Ned.-Indië*, **15(10)**, 569.