Molybdenum: Effects on the Growth and Composition of Hevea

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Hevea seedlings were grown in sand at various supply levels of molybdenum, sulphate and calcium, or under molybdenum deficiency conditions with nitrate or nitrate and ammonium ions as a source of nitrogen. Effects were noted of molybdenum deficiency, which varied according to the levels of other applied nutrients, on growth and chemical composition. The deficiency may occur in Malaya but may be unaccompanied by well defined foliar symptoms.

THE IMPORTANCE OF MOLYBDENUM, as a plant nutrient, has become well established in recent years and spectacular crop yield responses to the addition of such small amounts as one ounce of molybdenum per acre are not unusual (ANDERSON 1956). The acidic nature and low lime status of local soils make it probable that molybdenum deficiency of both rubber trees and of leguminous cover plants occurs in Malaya. The occurrence of molybdenum deficiency has not been established with confidence although its presence is suspected in Johore and a claim has been made (SANDISON & HANLY 1954) of growth responses, to added molybdenum, by leguminous plants grown in that area. The research programme of the Soils Division of this Institute includes the artificial production, identification and cataloguing of the symptoms of each mineral nutrient deficiency; some of the results of these investigations were previously reported (BOLLE-JONES 1956a). An investigation of the effects of molybdenum deficiency on the growth and composition of *Hevea brasiliensis* comprised part of this programme. It was hoped that a description of the visual symptoms of molybdenum deficiency would facilitate the detection of the field occurrence of the deficiency and lead to remedial action.

The first part of this paper deals with an attempt to produce symptoms of molybdenum deficiency in rubber plants which were grown in sand and supplied with nitrogen either as nitrate alone or as a mixture of nitrate and ammonium salts. It was suspected that the requirement for molybdenum would be lower for plants supplied with nitrogen as ammonium salts than if given nitrate salts (HEWITT & MCCREADY 1956). It was also anticipated that the rubber plant would exhibit iron deficiency effects and not grow well on a high-nitrate source of nitrogen. It was therefore desirable to attempt to produce molybdenum deficiency in the absence of such a complicating factor and, for this reason, some of the rubber plants were supplied with a well tried nutrient solution which contained a large proportion of ammonium ions even though it was known that by doing so the requirement for molybdenum would probably be considerably reduced. Other plants were supplied with nitrogen as nitrate salts and were compared with those provided with ammonium ions. The experiment, although producing notable growth effects according to molybdenum status, was not entirely successful as the nature of the molybdenum deficiency symptoms was not established with certainty. A complicated syndrome arising from iron deficiency effects in the nitrate fed plants interfered with the interpretation of the visual responses while the cultural technique did not appear good enough to permit the appearance of molybdenum deficiency symptoms in plants fed with nitrogen, as ammonium salts. About

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one and a half years later another attempt was made to produce molybdenum deficiency in plants grown in a similarly designed experiment but with the modification that iron was supplied not as citrate but as the chelated ferric form of disodium ethylene-diamine-tetra-acetate. Unfortunately the use of this chelated form of iron induced zinc deficiency effects and had to be abandoned in favour of ferric citrate. A few of the preliminary results of this experiment are included in the present paper and serve to confirm the general conclusion that if nitrogen was partially supplied in the ammonium form the apparent requirement for molybdenum was much diminished and symptoms of the deficiency could not be readily produced.

The molybdenum uptake of plants may be governed by the amount of sulphate supplied to the roots (STOUT, MEAGHER, PEARSON & JOHNSON 1951); sulphur deficient plants possess a higher concentration of molybdenum in their laminae than do healthy plants (BOLLE-JONES 1956b). As, therefore, the accumulation of molybdenum by plants is affected by their sulphate status and as molybdenum is made more readily available to plants by the addition of lime (ANDERSON 1956) it was decided to investigate the effects and interactions of molybdenum, sulphate and calcium on the growth of the rubber plant. While there was evidence that sulphate ions may directly influence molybdenum uptake by roots and so affect the concentration of molybdenum in the plant there was no indication of any effect of (nutrient) calcium ions on the concentration of molybdenum within the plant. Both sulphur and calcium are noteworthy as mineral nutrients required by *Hevea brasiliensis* in relatively small amounts. Neither sulphur nor calcium deficiency has been authoritatively reported to occur in Malaya although a deficiency of calcium might well be expected in crops grown on the strongly acid Malayan soils. The factorial experiment, designed to investigate the interrelationship between molybdenum, sulphate and calcium, showed that large differences in growth could often occur between treatments without the concomitant development of visual symptoms of any deficiency. Symptoms, believed to be those of molybdenum deficiency, were also observed and found to be more severe as the supply of sulphate to the plants increased. The relative importance of the three nutrient elements in the nutrition of the plant was well illustrated.

Molybdenum Deficiency Effects as Affected by Source of Nitrogen Supply

EXPERIMENTAL

Tjirandji 1 selfed seedlings were grown in purified sand contained in pyrex glass vessels with and without molybdenum and at different levels of nitrate and ammonium supply. Each of the four nutrient treatments was carried out in duplicate and consisted of the following levels of molybdenum, nitrate nitrogen, and ammonium nitrogen (in milligram equivalents per litre).

	Mo (hexavalent)	NO_3^-	NH_4^+
– Mo(N)	0	8	0
Complete(N)	0.001	8	0
– Mo(Am)	0	5	3
Complete(Am)	0.001	5	3

The pyrex glass vessels used to contain the sand were of a two gallon capacity and were thoroughly acid washed and steamed before use. The central drainage hole of each freely drained vessel was covered by a thin glass wool layer which was overlain by an inverted watchglass; both glass wool and watchglass were thoroughly washed with hot, constant-boiling, hydrochloric acid.

The sand (particle size 0.5 to 1.5 mm) was purified by three successive hot washings with a mixture of hydrochloric (14% w/v) and oxalic (1% w/v) acids; each washing lasted seven hours and required a fresh acid charge. The sand was subsequently restored to neutrality by leaching with a molybdenum-free, potassium and sodium nitrate solution until immediately prior to the commencement of the experiment, when the respective nutrients were applied.

Water distilled from, and condensed in, pyrex glass stills was used to prepare the respective nutrient which possessed a basal composition of (in milligram equivalents per litre) SO_4^{--} 2.5 (N type) 7.5 (Am type), PO_4^{---} 3, K^+ 3, Mg^{++} 2.5, Ca^{++} 4, Na_+ 2 (N type) 1 (Am type), Fe^{+++} 1, Mn^{++} 0.02, BO_3^{---} 0.033, Cu^{++} 0.002, Zn^{++} 0.002, Al^{+++} 0.001, Ni^{++} 0.0002, Co^{++} 0.0002, Ga^{+++} 0.0002.

The salts of the macronutrient elements were cleansed of molybdenum by the coprecipitation of the molybdenum contaminant with copper, as the sulphide, according to the procedure described by HEWITT and BOLLE-JONES (1952) whose method for the preparation of molybdenum-free ferric citrate was also followed with but slight modification. This modification consisted of extracting the ether extracted ferric chloride with a solution of dithiol (1, 2-dimercapto-4-methyl-benzene) in amyl acetate before combining it with molybdenum-free citric acid to form ferric citrate. The salts of the micronutrient elements were all recrystallised twice from glass distilled water.

Eighteen seeds, each weighing more than five grams, were sown in each pyrex vessel on 4 January 1955. After a preliminary thinning out, complete plants were removed for analysis in April 1955, May, August, October, December 1955, May 1956. At the earlier samplings two or more seedlings were removed from each vessel at each sampling until at the final, May, sampling one seedling only remained. Seedlings taken from vessels within a treatment replicate were bulked together and apportioned into laminae, stems and roots each of which were separately analysed according to methods previously described (BOLLE-JONES 1954, BOLLE-JONES, MALLIKARJUNESWARA & RATNASINGAM 1957).

RESULTS

Visual and Growth Effects

Molybdenum deficient seedlings which were not supplied with ammonium ions (that is, the –Mo(N) plants) were smaller and paler than those given molybdenum. Unfortunately, iron deficiency symptoms in the upper part of the plants interfered with the true interpretation of the molybdenum deficiency effects. The nearest approach to a consistent effect was the tip or even marginal scorch which occurred in the midstem leaves. The molybdenum deficient seedlings which received a supply of ammonium ions (that is, the –Mo(Am) plants) were smaller and slightly less vigorous than those given molybdenum but did not show any distinctive symptoms which could be attributed to molybdenum deficiency.

Figure 1 illustrates the order of the differences obtained between seedlings grown in different treatments in respect of height, girth and dry weight per plant. The beneficial effect of added molybdenum on these values illustrated the importance of molybdenum in the nutrition of hevea. The height, girth and dry weight per plant values tended to be smaller for the molybdenum deficient plants which were not supplied with ammonium salts. A deficiency of molybdenum retarded growth more severely when nitrogen was supplied entirely as nitrate salts.

Chemical Composition of Plant

The molybdenum content per plant increased markedly with time for the complete nutrient plants; during the first part of the experiment, the molybdenum content per plant was greater in the plants not given ammonium ions (*Figure 1*). However towards the end of the experiment, when iron deficiency effects intervened, the molybdenum content of the Compt.(N) plants was less than for the Compt.(Am) plants.

Statistical examination of the variation of the concentration of molybdenum in laminae taken from different storey positions showed that there was no significant

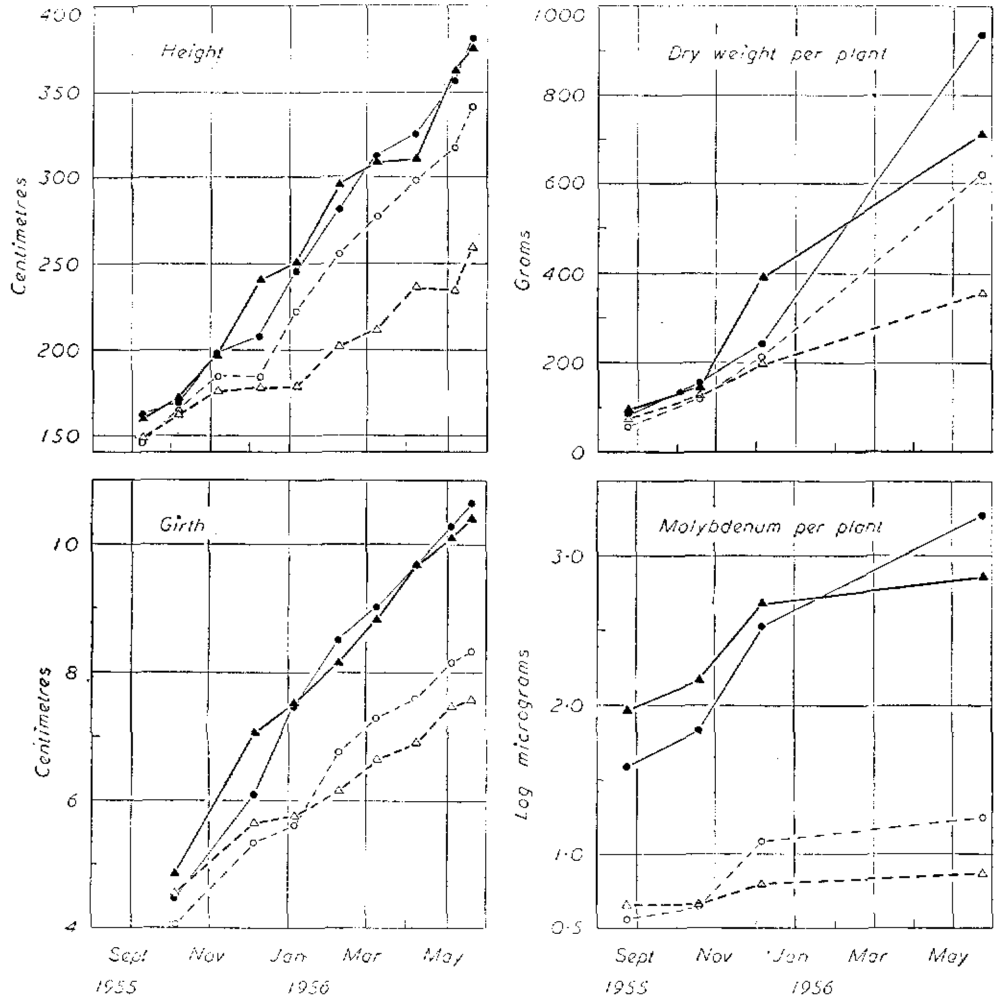

Figure 1. Height, stem girth, dry weight per plant and weight of molybdenum contained in each plant. Girth measured 7 cm above sand level; weight of molybdenum presented as logarithm of microgram value.
Compt.(*N*)▲ -Mo(*N*)△ Compt.(Am)● -Mo(Am)○

gradient, according to storey position, in any of the samplings examined. Neither was there established a significant interaction between treatment and storey position for any of the components which were determined by analysis. Consequently the results of the analyses of the laminae are presented as means without reference to storey positions. As the trends for each individual sampling were similar, it is unnecessary to present each separately. The mean values which are presented in Table 1, in respect of stems and roots as well as of laminae, are based on the analyses of plants sampled in August 1955, October, December 1955, May 1956.

The concentrations of molybdenum in the laminae and stems of the Compt.(N) plants were higher than for the Compt.(Am) plants; there were no marked differences between the concentrations found in the corresponding molybdenum deficient laminae and stems (Table 1). For both laminae and roots the absence of molybdenum from the nutrient significantly reduced the concentration of molybdenum in the tissue. It was noteworthy that, for ammonium-supplied plants, a deficiency of molybdenum did not reduce, significantly, the concentration of molybdenum in the stems. It appeared that severe visual symptoms of molybdenum deficiency would not be consistently obtained until the concentration of molybdenum in the dried laminae was well below 0.07 p.p.m., probably at a level of about 0.01 p.p.m. Unexpectedly, it was found that the concentration of molybdenum was much higher in the roots of the Compt.(Am) than in the roots of the Compt.(N) plants (Table 1). Thus the Compt.(Am) plants tended to accumulate a much higher concentration of molybdenum in their roots (4.66 p.p.m.) than in the laminae (0.29 p.p.m.) whereas the Compt.(N) plants, which were not given ammonium ions, possessed a roughly similar concentration in both roots (2.00 p.p.m.) and laminae (1.64 p.p.m.).

A lack of molybdenum depressed the concentration of chlorophyll in the laminae of the nitrate plants but did not affect the concentration found for the ammonium supplied plants (Table 1).

Variation in molybdenum status did not produce any significant effect on the concentrations of rubber found in the petioles, stems or roots (Table 1). However, there were indications that for the roots a deficiency of molybdenum tended to increase the concentration of rubber in the tissues.

The concentrations of total nitrogen in the laminae, stems and roots were greater in the plants supplied with ammonium ions than for those given nitrate nitrogen only (Table 1). Molybdenum deficiency did not effect the concentration of nitrogen in the roots but usually depressed that found in the laminae and stems.

Significant differences in phosphorus and calcium concentrations due to molybdenum level were only established in respect of the roots of the nitrate-fed plants; a deficiency of molybdenum reduced the concentrations of phosphorus and calcium (Table 1). Molybdenum level did not significantly affect the concentrations of magnesium, potassium or manganese in the tissues analysed. A lack of molybdenum generally reduced the iron concentration in the tissues, but only in respect of the laminae and roots of plants which were not fed with ammonium ions was this effect significant (Table 1).

DISCUSSION AND CONCLUSIONS

Molybdenum was thus shown to be an element required for the wellbeing of the rubber plant; its absence was better reflected, in terms of growth and other effects, if ammonium ions were omitted from the nutrients. A molybdenum deficient nutrient

TABLE 1. CHEMICAL COMPOSITION OF LAMINAE, STEMS, AND ROOTS

Expressed as % or p.p.m. of oven dried materials

Treatment	Mo p.p.m.	N %	P %	Mg %	K %	Ca %	Fe p.p.m.	Mn p.p.m.	Rubber* %	Chlorophyll %
					Laminae					
Complete (N)	1.64	4.26	0.30	0.28	1.83	0.76	124	42	1.29	0.83
-Mo (N)	0.07	3.86	0.31	0.30	2.04	0.79	110	44	1.38	0.75
Complete (Am)	0.29	4.82	0.50	0.24	1.25	0.49	144	48	1.12	1.14
-Mo (Am)	0.08	4.52	0.48	0.26	1.38	0.50	137	39	1.20	1.12
Min. 5% sig. diff.	0.200	0.151	0.041	0.045	0.223	0.098	11.6	9.7	0.133	0.060
					Stems					
Complete (N)	0.54	0.97	0.23	0.27	1.26	0.62	29	20	0.22	
-Mo (N)	0.03	0.89	0.24	0.27	1.24	0.62	26	22	0.28	
Complete (Am)	0.11	1.18	0.30	0.20	0.86	0.31	50	24	0.23	
-Mo (Am)	0.03	0.98	0.32	0.18	0.81	0.32	26	21	0.24	
Min. 5% sig. diff.	0.294	0.145	0.066	0.065	0.233	0.092	39.2	8.1	0.079	
					Roots					
Complete (N)	2.00	1.18	0.46	0.32	1.22	0.75	886	102	0.30	
-Mo (N)	0.02	1.15	0.34	0.29	1.02	0.51	402	71	0.36	
Complete (Am)	4.66	1.55	0.46	0.20	1.16	0.24	1374	16	0.31	
-Mo (Am)	0.04	1.55	0.44	0.20	1.18	0.22	1152	18	0.38	
Min. 5% sig. diff.	0.102	0.249	0.032	0.050	0.341	0.098	284.6	43.1	0.079	

*Rubber in laminae not given, but concentration in petioles.
N : plants received nitrate but no ammonium nitrogen.
Am : plants received both ammonium and nitrate nitrogen.

in which all nitrogen was given as nitrate grew plants whose concentrations of chlorophyll, nitrogen and iron in the laminae and of phosphorus, calcium and iron in the roots were each significantly reduced. The effects of molybdenum status on height, girth and dry weight per plant also illustrated the undoubted importance of molybdenum in the nutrition of hevea (*Figure 1*). It was unfortunate that the experiment did not lead to a more definite characterisation of the foliar symptoms of molybdenum deficiency other than the tip and marginal scorch.

To confirm and possibly amplify the findings of the experiment described above, another was started about one and a half years later and is still in progress. This later experiment differed from its predecessor in that the iron was supplied not as the citrate but as the ferric form of disodium ethylene-diamine-tetra-acetate, with the object of eliminating iron deficiency effects. Regrettably, the use of this chelated form of iron supply, at a third of the concentration given as citrate in the previous experiment, induced zinc deficiency effects. It was necessary to restore the application of ferric citrate at the rate of 1.0 milligram equivalents per litre in order to eliminate iron deficiency and other complicating effects from the plants not given ammonium ions. Seedlings were removed nine months after the initiation of this second experiment for analysis. The results of this analysis confirmed broadly the findings of the previous experiment with the additional information that the presence of ammonium ions in the nutrient, although decreasing the requirement for molybdenum, greatly increased the concentration of sulphur in the tissues. The concentration of molybdenum in the laminae of the molybdenum deficient nitrate fed plants became reduced to the order of 0.03 p.p.m., in terms of dry tissue, but indubitable symptoms of molybdenum deficiency were not noted nor recognised. The concentration of molybdenum within the tissues was lower for complete nutrient plants supplied with ammonium ions except in the case of roots where the nitrate-fed plants possessed the lower concentration. The dry weight per plant of the nitrate-fed seedlings was slightly greater than that of the seedlings supplied with ammonium ions; similar differences existed in regard to height and girth.

Molybdenum Status as Affected by Calcium and Sulphate Levels

EXPERIMENTAL

The relative importance of molybdenum, sulphur and calcium was evaluated in a factorial experiment in which Tjirandji 1 seedlings were grown in sand at each of the 18 combinations of three levels of molybdenum, three levels of sulphate and two of calcium. These levels represented the following concentrations (in milligram equivalents per litre).

Mo_1 ... 0.00001 MoO_4^{--}	S_1 ... 0.3 SO_4^{--}	Ca_1 ... 0.5 Ca^{++}
Mo_2 ... 0.001 MoO_4^{--}	S_2 ... 1.8 SO_4^{--}	Ca_2 ... 5.0 Ca^{++}
Mo_3 ... 0.1 MoO_4^{--}	S_3 ... 10.8 SO_4^{--}	

Each treatment was carried out in duplicate, thus giving a total of 36 plots; each plot contained 3 pots or vessels. Five-gallon clay pots, internally painted with bitumen, were used for the Mo_2 and Mo_3 treatments but the Mo_1 plants were grown in sixgallon pyrex vessels. The sand (particle size 0.5 to 1.2 mm) contained in the Mo_2 and Mo_3 pots was purified by a cold acid leaching (5% HCl, v/v) for a three day period. The Mo_1 pyrex vessels were filled with sand which was purified by a seven hour washing with a hot (100°C) hydrochloric (14% w/v) oxalic (1% w/v) acid mixture.

The nutrient solutions were prepared and purified in the same manner as for the experiment earlier described in this paper. For each treatment the following ionic concentrations were maintained (in milligram equivalents per litre): NH_4^+ 3.0, NO_3^- 5.0, PO_4^{---} 3.0, Mg^{++} 2.5, K^+ 3.0, Fe^{+++} 1.0, Mn^{++} 0.02, Cu^{++} 0.002, Zn^{++} 0.002, BO_3^{---} 0.033, Al^{+++} 0.001, Ni^{++} 0.0002, Co^{++} 0.0002, Ga^{+++} 0.0002. Variations in the sodium and chloride ionic concentrations with increased sulphate level were unavoidable but were kept to a minimum; these variations were not thought to be of great importance as a deficiency or toxicity level of either sodium or chloride was avoided.

Eighteen seeds, each weighing more than four grams, were sown in each pot or vessel on 23 April 1955. Plants were removed in August 1955, October 1955, January 1956, April, September 1956, January 1957; all plants taken from any one treatment replicate were bulked together before analysis. The chlorophyll, nitrogen, sulphur phosphorus, magnesium, potassium, calcium, manganese and molybdenum concentrations of the laminae, according to storey position, were determined. The concentration of rubber in the petioles was also determined as well as the total dry weight of each plant. Methods of analysis employed have been previously indicated.

It is unnecessary to present all the results obtained from this experiment here; instead, the values obtained for the September 1956 sampling are considered in some detail as they were representative of the main trends observed. Data obtained from other months are also discussed in the presentation of the results.

RESULTS

Visual and Growth Effects

Throughout the duration of the experiment, the Mo_1 plants possessed fewer leaflets and a smaller height, girth and total dry weight than the Mo_2 plants (*Figure 2*, Table 2). These significant effects of molybdenum were very pronounced in respect of the girth and dry weight values. The Mo_3 plants were consistently shorter than the Mo_2, but in respect of leaflet number, girth and dry weight there appeared little difference between the effects of the two levels. Calcium level did not significantly affect any of these measurements of growth but sulphate application was responsible for beneficial linear effects which were significant (Table 2). Thus the beneficial effect of molybdenum level on growth was almost entirely accounted for by the difference between the Mo_1 and Mo_2 levels whereas the increment in growth measurement values obtained by increasing sulphate from S_2 to S_3 was almost as great as that obtained for the difference between the S_1 and S_2 values (Table 2).

Visual symptoms due to the applied nutrient levels were slow to appear. A dull brownish green appearance of some of the midstem and lower leaves became apparent in the Mo_3S_1 and S_2 plants about a year after sowing. The brownish appearance was caused by a purple tinting of the leaves, which was well marked on the underside of the lamina. These symptoms were ascribed to molybdenum toxicity effects and were noted only in the Mo_3 plants. The symptoms persisted until September 1956 or later

TABLE 2. HEIGHT, STEM GIRTH AND DRY WEIGHT OF SEEDLINGS RECORDED IN JANUARY 1957

Girth measured 7 cm above sand level

Level	Mo_1	Mo_2	Mo_3	S.e.	Ca_1	Ca_2	S.e.	Mean	S.e.
				Height (cm)					
S_1	284	395	361		361	332		347	
S_2	306	419	408	±12.1	391	364	±9.9	378	±7.0
S_3	358	440	438		408	416		412	
Ca_1	321	426	413	±9.9				387	±5.7
Ca_2	311	410	392					371	
Mean	316	418	402	±7.0	387	371	±5.7		
				Girth (cm)					
S_1	9.4	13.0	13.1		12.4	11.2		11.8	
S_2	9.9	13.7	14.0	±0.38	12.8	12.3	±0.31	12.5	±0.22
S_3	11.3	14.6	15.4		13.6	14.0		13.8	
Ca_1	10.4	14.2	14.2	±0.31				12.9	±0.18
Ca_2	10.0	13.4	14.1					12.5	
Mean	10.2	13.8	14.2	±0.22	12.9	12.5	±0.18		
				Dry Weight per Plant (gm)					
S_1	559	1132	1120		1039	835		937	
S_2	688	1451	1339	±135	1204	1115	±110	1159	±78
S_3	881	1497	1633		1334	1340		1337	
Ca_1	747	1413	1418	±110				1192	±64
Ca_2	672	1308	1310					1097	
Mean	710	1360	1364	±78	1192	1097	±64		

but disappeared by the end of the year. It seemed, as was also apparent in the growth measurement values, that during the earlier part of the experiment the Mo_3 level exercised a mild toxic effect on growth but that later even this high level benefited growth, particularly when recorded as girth (Table 2).

In June 1956 the first indications of leaf symptoms believed to be those of molybdenum deficiency, were recorded. The tips and margins became scorched without any preliminary chlorosis of the leaf tissues. The scorched area which was thin, paper-like and fawn in colour could extend all around the lamina margin or it could be confined to the tip region with its base line running transversely across the lamina (*Figures 3* and *4*). The scorch was not necessarily preceded by a paling of the lamina and could occur in quite dark green leaflets. The location of the symptom-bearing leaves was difficult to classify. The leaflets were always fully expanded and the symptom usually occurred in the second or third storeys away from the top of the plant (*Figure 3*) but in many instances they extended even into the top storey leaflets. The symptoms were more frequent and more severe at the Ca_2 level and occurred in all the Mo_1 plants; they were first noted in the Mo_1S_3 treatments and took their most severe form in plants of those treatments.

Mild symptoms of sulphur deficiency were noted in the topmost leaves of the $Mo_3S_1Ca_1$ and $Mo_3S_1Ca_2$ plants in January 1957. They were not severe and did not appear in all plants of the affected treatment.

Chemical Composition Of Laminae

The distribution of molybdenum, in terms of concentration, between storeys seemed to vary according to the level of molybdenum applied in the nutrient (Table 3). The differences between the molybdenum concentrations of the top and second storey laminae of the Mo_1 plants were not significant but for most samplings the second storey laminae contained the lower concentration. The molybdenum concentration values for the Mo_1 plants were of the order of 0.05 to 0.20 p.p.m. of molybdenum in oven dried second storey laminae even in plants showing symptoms suspected to be those of molybdenum deficiency (Table 3); these values were higher than anticipated. For the Mo_2 plants there was every indication that the second storey laminae contained more molybdenum than those of the top storey. This effect was not significant if only the September values were considered but became so if the values for September and January were combined before statistical analysis (Table 3). The laminae of the lower storeys of Mo_3 plants contained much more molybdenum than those of the upper storeys and this effect was significant when data from the September and January samplings were combined prior to analysis (Table 3).

It was necessary for the statistical evaluation of the effect of treatment on molybdenum concentration to examine separately the values for each molybdenum level as the range covered would otherwise tend to obscure any statistically significant effect. Thus the second storey laminae of the Mo_3 plants sometimes contained as much as 1,000 p.p.m. of molybdenum whereas those of the Mo_1 plants contained as little as 0.05 p.p.m. Nevertheless certain consistent effects were detectable. Increased sulphate level consistently reduced the concentration of molybdenum in the laminae, but increased calcium level produced no effect (Table 3). The rubber plant showed itself able to accumulate large concentrations of molybdenum in the older laminae especially at the Mo_3S_1 levels, but accompanied by relatively mild visual indication of any toxicity effect.

One of the well known effects of molybdenum deficiency is the accumulation of nitrate in deficient laminae. To obtain further evidence that the symptoms observed in the Mo_1 plants were those of molybdenum deficiency, nitrate nitrogen was determined in the top storey laminae sampled in January 1957. The result (Table 4) clearly confirmed that nitrate nitrogen accumulated in the laminae of the Mo_1 plants and that a fivefold reduction in nitrate concentration occurred when the molybdenum level was increased from Mo_1 to Mo_2.

The remaining data discussed below were obtained from plants sampled in September 1956 and are presented as means of the top and second storey values, as examination of the individual samplings showed that the trends were similar for both storeys. The concentration of rubber in the petioles diminished, significantly, as the plant's molybdenum status improved (Table 4); the concentration of chlorophyll within the lamina was not affected by any of the applied treatments and is not given here.

Increased supplies of molybdenum significantly decreased the concentration of nitrogen, sulphur, phosphorus, magnesium and potassium in the laminae but increased that of manganese (Tables 5 and 6). Increased sulphate level increased the concentration of potassium in the laminae but decreased those of calcium and manganese (Table 6). The principal effect of increased calcium level, apart from increasing the concentration of calcium in the laminae, was to decrease that of magnesium (Table 6). Negative interactions between molybdenum and sulphate levels which affected the concentrations of sulphur, phosphorus and potassium in the laminae were also noted.

TABLE 3. CONCENTRATION OF MOLYBDENUM IN LAMINAE OF DIFFERENT STOREYS

Values given as p.p.m. Mo in oven dried laminae

Mo_1 plants, September 1956 sampling.

Treatment	Storey *top*	Storey *second*	Min. 5% sig. diff.
S_1 Ca_1	0.29	0.12	Between 2 treatment values of same storey 0.26
S_2 Ca_1	0.28	0.16	
S_3 Ca_1	0.10	0.16	
S_1 Ca_2	0.10	0.10	Between 2 different storey values of same treatment 0.23
S_2 Ca_2	0.24	0.06	
S_3 Ca_2	0.16	0.06	
Mean	0.20	0.11	Between overall mean storey values 0.14

Mo_2 plants, September 1956 and January 1957 samplings.

Treatment	Storey top	Storey second	Min. 5% sig. diff.
S_1 Ca_1	562	1011	Between 2 treatment values of same storey 496
S_2 Ca_1	168	410	
S_3 Ca_1	66	138	
S_1 Ca_2	527	1043	Between 2 different storey values of same treatment 374
S_2 Ca_2	201	508	
S_3 Ca_2	54	142	
Mean	263	542	Between overall mean storey values 112

Mo_3 plants, September 1956 and January 1957 samplings.

Treatment	Storey top	Storey second	Min. 5% sig. diff.
S_1 Ca_1	8.22	13.80	Between 2 treatment values of same storey 2.33
S_2 Ca_1	3.17	4.24	
S_3 Ca_1	1.74	2.34	
S_1 Ca_2	8.69	11.81	Between 2 different storey values of same treatment 2.46
S_2 Ca_2	3.90	5.56	
S_3 Ca_2	1.68	1.95	
Mean	4.56	6.62	Between overall mean storey values 1.30

TABLE 4. THE CONCENTRATION OF NITRATE NITROGEN IN TOP STOREY LAMINAE, SAMPLED IN JANUARY 1957, AND OF RUBBER IN TOP AND SECOND STOREY PETIOLES, SAMPLED IN SEPTEMBER 1956

Expressed as p.p.m. or % of oven dried materials

p.p.m. Nitrate – N

Level	Mo_1	Mo_2	Mo_3	S.e	Ca_1	Ca_2	S.e.	Mean	S.e.
S_1	584	77	80		299	195		247	
S_2	406	92	136	±94.6	235	188	±77.2	211	±54.6
S_3	638	128	137		286	316		301	
Ca_1	602	80	138	±77.2				273	±44.6
Ca_2	484	118	97					233	
Mean	543	99	117	±54.6	273	233	±44.6		

Rubber %

Level	Mo_1	Mo_2	Mo_3	S.e.	Ca_1	Ca_2	S.e.	Mean	S.e.
S_1	1.45	1.08	1.12		1.20	1.23		1.22	
S_2	1.68	1.05	1.05	±0.070	1.36	1.16	±0.057	1.26	±0.041
S_3	1.33	1.23	1.18		1.29	1.20		1.25	
Ca_1	1.57	1.13	1.15	±0.057				1.28	±0.033
Ca_2	1.40	1.11	1.08					1.20	
Mean	1.49	1.12	1.12	±0.041	1.28	1.20	±0.033		

GENERAL DISCUSSION

These investigations clearly indicate that a deficiency of molybdenum will markedly reduce the height, girth and dry weight of the rubber plant (*Figures* 1 and 2, Table 2). These effects appeared even when the plants were given a large proportion of their nitrogen supply as ammonium ions, suggesting that molybdenum was probably essential for some reaction other than one involved directly in nitrate reduction. Symptoms of molybdenum deficiency are usually recorded in plants supplied with nitrogen as nitrate and it is much more difficult to produce symptoms in the same plants when given part or all of their nitrogen as ammonium; it has been reported that a deficiency of molybdenum in tomato was not accompanied by symptoms when a non-nitrate source of nitrogen was supplied (HEWITT & MCCREADY 1956). However, other reports (VANSELOW & DATTA 1949) show that symptoms of molybdenum deficiency may be produced even in plants supplied with ammonium nitrogen.

The present results indicate that the rubber plant may also produce symptoms (*Figures* 3 and 4) when supplied with a molybdenum deficient nutrient (Mo_1) containing ammonium nitrogen. It was unfortunate that the interpretation of similar symptoms in plants not given ammonium nitrogen was rendered impracticable due to the occurrence of complicating iron deficiency effects. An anomalous factor regarding the present results was the occurrence of the supposed molybdenum deficiency symptoms in plants grown at the Mo_1 level (of the second experiment reported here) whereas no symptoms were observed in the leaves of the –Mo(Am) plants (of the first experiment) even though the concentrations of molybdenum in the laminae were

TABLE 5. CONCENTRATION OF NITROGEN, SULPHUR AND PHOSPHORUS IN TOP AND SECOND STOREY LAMINAE, SEPTEMBER 1956 SAMPLING

Expressed as % of oven dried materials

Level	Mo_1	Mo_2	Mo_3	S.e.	Ca_1	Ca_2	S.e.	Mean	S.e.
			Nitrogen %						
S_1	4.17	3.73	3.85		3.95	3.88		3.92	
S_2	4.17	3.78	3.71	±0.094	3.88	3.90	±0 77	3.89	±0.054
S_3	4.17	3.90	3.79		3.97	3.94		3.95	
Ca_1	4.16	3.77	3.87	±0.077				3.93	±0.044
Ca_2	4.17	3.84	3.70					3.90	
Mean	4.17	3.81	3.78	±0.054	3.93	3.90	±0.044		
			Sulphur %						
S_1	0.18	0.16	0.15		0.16	0.16		0.16	
S_2	0.25	0.21	0.21	±0.019	0.21	0.24	±0.016	0.22	±0.011
S_3	0.43	0.22	0.23		0.28	0.30		0.29	
Ca_1	0.28	0.19	0.18	±0.016				0.22	±0.009
Ca_2	0.29	0.20	0.21					0.23	
Mean	0.28	0.19	0.20	±0.011	0.22	0.23	±0.009		
			Phosphorus %						
S_1	0.46	0.30	0.27		0.37	0.32		0.34	
S_2	0.41	0.25	0.26	±0.022	0.31	0.30	±0.018	0.31	±0.013
S_3	0.54	0.26	0.25		0.37	0.33		0.35	
Ca_1	0.50	0.28	0.27	±0.018				0.35	±0.010
Ca_2	0.45	0.26	0.25					0.32	
Mean	0.47	0.27	0.26	±0.013	0.35	0.32	±0.010		

roughly of the same order (Tables 1 & 3). Differences in cultural conditions might account for this anomaly. Thus the Mo_1 plants were grown in the open air, exposed to full sunlight, whereas the –Mo(Am) plants were grown under light shade in the glasshouse; shading might well have tended to reduce the threshold deficiency concentration level for the appearance of molybdenum deficiency symptoms in the glasshouse grown plants.

The occurence of marginal leaf scorch was clearly correlated with a low molybdenum status as it was largely confined to the Mo_1 plants and increased in severity as the sulphate level increased. These leaves also accumulated nitrate nitrogen (Table 4) and this is one of the effects of molybdenum deficiency (ANDERSON 1956.) It is believed that these symptoms were a true manifestation of molybdenum deficiency but complete acceptance of this conclusion must be reserved owing to the rather high molybdenum concentration values recorded in the laminae of some of the plants which showed symptoms. Under these circumstances it is difficult to predict the concentration value for molybdenum in laminae at which symptoms of molybdenum deficiency would appear. The first experiment reported here would seem to indicate a threshold value of about 0.01 p.p.m. of molybdenum in dry laminae, but the second experiment indicates a value which would be closer to 0.10 p.p.m. However, there is sufficient evidence on which to base a method of dealing with leaf samples obtained in

TABLE 6. CONCENTRATION OF MAGNESIUM, POTASSIUM, CALCIUM AND MANGANESE IN TOP AND SECOND STOREY LAMINAE, SEPTEMBER 1956 SAMPLING

Expressed as % of oven dried materials

Level	Mo_1	Mo_2	Mo_3	S.e.	Ca_1	Ca_2	S.e.	Mean	S.e.
				Magnesium %					
S_1	0.32	0.24	0.23		0.29	0.25		0.27	
S_2	0.30	0.26	0.24	±0.017	0.30	0.24	±0.014	0.27	±0.010
S_3	0.34	0.22	0.23		0.30	0.23		0.26	
Ca_1	0.34	0.27	0.27	±0.014				0.29	±0.008
Ca_2	0.30	0.21	0.20					0.24	
Mean	0.32	0.24	0.24	±0.010	0.29	0.24	±0.008		
				Potassium %					
S_1	1.23	1.02	1.19		1.13	1.16		1.14	
S_2	1.10	1.00	0.97	±0.046	1.01	1.04	±0.038	1.02	±0.027
S_3	1.48	1.05	1.10		1.21	1.21		1.21	
Ca_1	1.29	1.02	1.04	±0.038				1.12	±0.022
Ca_2	1.25	1.02	1.14					1.14	
Mean	1.27	1.02	1.09	±0.027	1.12	1.14	±0.022		
				Calcium %					
S_1	0.49	0.44	0.47		0.26	0.67		0.47	
S_2	0.45	0.51	0.50	±0.027	0.26	0.72	±0.022	0.48	±0.015
S_3	0.31	0.46	0.39		0.24	0.54		0.39	
Ca_1	0.20	0.28	0.28	±0.022				0.25	±0.012
Ca_2	0.63	0.66	0.63					0.64	
Mean	0.42	0.47	0.45	±0.015	0.25	0.64	±0.012		
				Manganese p.p.m.					
S_1	57	76	74		69	69		69	
S_2	55	85	82	±5.3	67	81	±4.3	74	±3.0
S_3	36	75	66		62	56		59	
Ca_1	47	76	74	±4.3				66	±2.5
Ca_2	51	81	74					68	
Mean	49	79	74	±3.0	66	68	±2.5		

the course of advisory or extension work. Thus, any leaf sample which shows well defined marginal scorch, an absence of severe chlorosis and which possesses a concentration of molybdenum within the laminae of the order of 0.05 p.p.m. may be suspected of being molybdenum deficient. In such instances field application of molybdenum is warranted. Once the field occurrence of molybdenum deficiency has been established with confidence and growth responses to molybdenum application obtained it should become relatively simple to establish the threshold concentration of molybdenum in laminae.

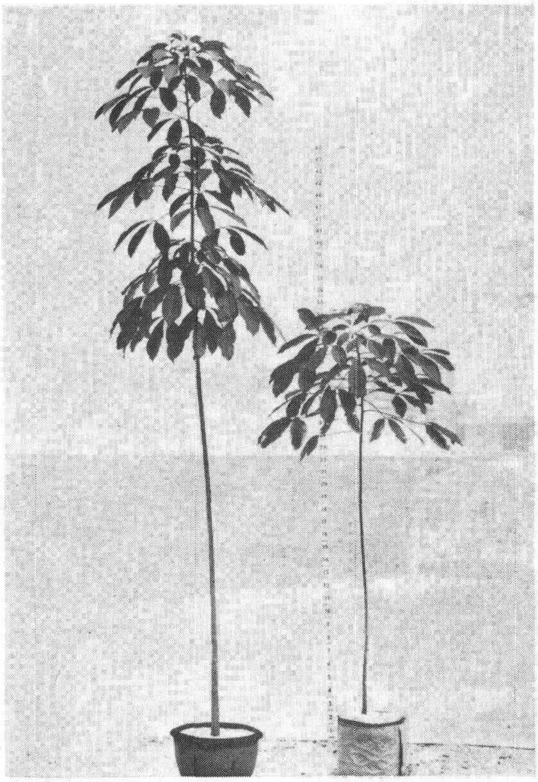

Figure 2. $Mo_2S_1Ca_2$ left, $Mo_1S_1Ca_2$ right. Note retardative effect of molybdenum deficiency on growth and occurrence of scorch in lowermost leaves of Mo_1 plant. Mo_2 seedling about 11 feet tall. September 1956.

It was beyond doubt that the tissues of the shoots of rubber plants supplied with a complete nutrient which contained ammonium nitrogen contained a smaller concentration of molybdenum than plants which did not receive ammonium nitrogen (Table 1). However, this did not necessarily imply that the absolute requirement for molybdenum was smaller as the roots of the Compt.(Am) plants contained a higher concentration of molybdenum than those of the Compt.(N) plants. For the earlier part of the experiment it was true that the weight of molybdenum contained per plant was higher in the Compt.(N) plants but this difference diminished with time and became reversed as the Compt.(Am) plants outstripped their nitrate fed counterparts (*Figure 1*). Under conditions of molybdenum deficiency the concentration of molybdenum in the laminae of both ammonium and nitrate fed plants fell to about the same level (Table 1) but the plants not given ammonium nitrogen became inferior in terms of height, girth and dry weight (*Figure 1*). Some effects on chemical composition also showed that the effect of deficient molybdenum supply was more acutely felt in the absence of a supply of ammonium nitrogen. Thus, as also recorded by HEWITT and MCCREADY (1956), a deficiency of molybdenum reduced the concentration of chlorophyll in the laminae when nitrogen was supplied as nitrate but not if given ammonium nitrogen (Table 1). The concentrations of iron in the laminae and of phosphorus, calcium and iron in the roots were significantly reduced in the –Mo(N) plants but not in the – Mo(Am) plants (Table 1).

Increased sulphate level diminished the concentration of molybdenum in the laminae (Table 3) and fully confirmed the initial finding of STOUT et alii (1951) who showed that sulphate application depressed molybdenum uptake. Heavy sulphate application, especially under acid soil conditions, may therefore be conducive to the production of molybdenum deficiency effects. The present work shows that these effects need not necessarily be represented as visual symptoms but may take the form of reduced growth. Accordingly it is credible that under Malayan conditions there may be instances of retarded growth which are caused by a lack of molybdenum. This is a possibility which should be investigated under field conditions.

Increased calcium supply seemed to increase the severity of the supposed molybdenum deficiency symptoms and there were indications that the concentration of molybdenum in the Mo_1Ca_2 laminae was less than for the Mo_1Ca_1 plants (Table 3). However, this difference was far short of being significant and was certainly not applicable to the Mo_2 and Mo_3 plants. It did not seem that calcium had a profound effect on the molybdenum nutrition of the rubber plant although its effect may well have been greater if ammonium ions had not been supplied in the nutrient. The beneficial effect of lime on molybdenum uptake from soils must be attributed to its effect (presumably one of pH) on the availability of molybdenum in the soil and not to any specific effect of the calcium ions. It has also been reported (WARRINGTON 1950) that, in solution culture, the amount of applied calcium did not affect the plant's response to molybdenum.

It is usually supposed that there is some form of antagonism between molybdenum and manganese (MULDER 1954) so that applied manganese would depress molybdenum uptake. The present work shows that, unlike most of the other mineral nutrients determined, the concentration of manganese in the laminae increased as the molybdenum supply increased (Table 6). However, this effect may only be of significance when ammonium nitrogen is supplied in the nutrient (Table 1).

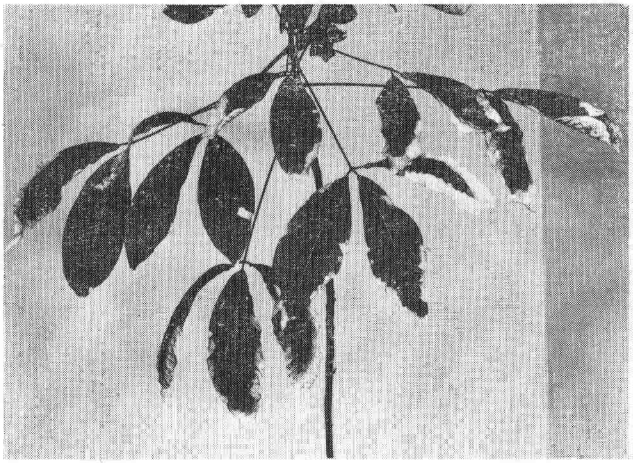

Figure 3. Leaves of second storey of $Mo_1S_1Ca_2$ plant. Note marginal scorch believed due to molybdenum deficiency. January 1957.

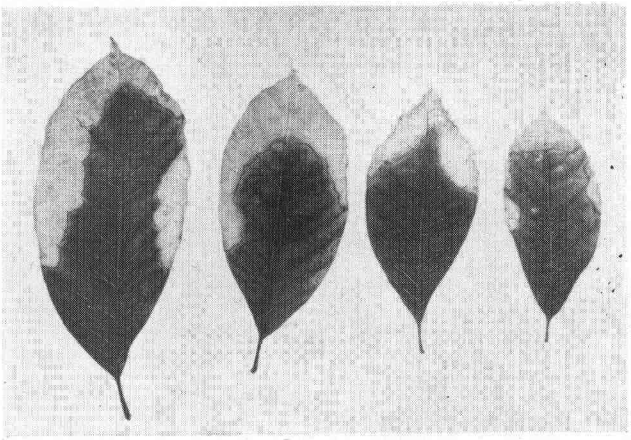

Figure 4. Leaflets of $Mo_1S_3Ca_2$ plant. Note severe marginal and tip scorch and relatively slight mottling. September 1956.

SUMMARY

Rubber seedlings, in sand culture, were supplied with molybdenum deficient nutrients in which the nitrogen was supplied as nitrate or as a mixture of nitrate and ammonium ions. Other seedlings were grown at different levels of molybdenum, sulphate and calcium.

Seedlings deprived of molybdenum or given a very low level of supply were shorter, of smaller girth and dry weight, and developed a marginal or tip scorch in the midstem and upper laminae. Increased sulphate level improved their growth despite the usually lowered molybdenum concentration in the laminae.

Molybdenum deficient plants, given nitrogen as nitrate ions, possessed smaller concentrations of chlorophyll and iron in the laminae and of phosphorus, calcium and iron in the roots, than those given molybdenum. Complete nutrient seedlings supplied with ammonium nitrogen contained a greater concentration of molybdenum in their roots and lower concentrations in their stems and laminae than those given nitrate only.

Although not established beyond doubt, it appeared that under conditions of molybdenum deficiency the topmost storey of laminae contained a higher concentration of molybdenum than the second storey in which the symptoms often first appeared. At normal and high levels of molybdenum supply the older laminae possessed a higher concentration than the younger ones. Mild symptoms of molybdenum toxicity were observed in the older leaves at the highest level of molybdenum supply.

Increased molybdenum supply diminished the concentration of nitrate nitrogen within the laminae as well as the concentrations of sulphur, phosphorus, magnesium and potassium, but increased that of manganese.

Increased sulphate supply diminished the concentration of molybdenum in the laminae; calcium had no significant effect but appeared to accentuate the severity of the symptoms of molybdenum deficiency.

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