A NUTRITIONAL STUDY OF HEVEA RUBBER SEEDLINGS

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BIOGRAPHICAL SKETCH

Edilberto Camacho Vargas was born in Heredia, Costa Rica. His elementary and secondary education was completed in his home town. He received a Teacher's degree from the Escuela Normal de Costa Rica.

Up to 1943 he worked for the Ministry of Education of Costa Rica as a School Teacher, School Principal, and Inspector of Schools. He has been working at the U. S. Department of Agriculture Rubber Experiment Station from 1944 to date.

He was in the United States in 1946 and 1947, trip made under the auspices of the U. S. Department of Agriculture. In the United States he received a training in Extension Service and attended some courses at Cornell University.

He entered the Graduate School of the Inter-American Institute of Agricultural Sciences in September 1953.

He is married to Gladys Cooper G. and their family at present consist of one daughter and three sons.

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A NUTRITIONAL STUDY OF HEVEA RUBBER SEEDLINGS

Rubber has a preponderant place among the commodities of the world. Its use in industry is ever expanding and its consumption has shown a steady upward trend in the last four decades. Bugbee (6) gives the following figures on the world consumption of rubber:

Year	Tons of rubber	Increase
1910	99,000	
1920	277,000	200 %
1930	709,000	140 %
1940	1,100,000	55 %
1950	2,285,000	105 %

Based on above record he has estimated that some 3,500,000 tons of rubber will be needed by 1963.

The great economic importance of the natural rubber crop may be judged by the production of over 1,700,000 tons in 1953, or 65% of total rubber consumed most of which is produced in the Far East by countries for which rubber is almost their sole source of income.

Dijkman (9) says that "in its social aspects as well as the economic aspects, the rubber industry, largely through its extensive educational programs, has had a tremendous impact upon the lives of the Indonesian people."

Seibert (21) believes that natural rubber, a newcomer among the necessities of our advancing civilization, holds and will continue to hold a leading position among the commodities of the world.

Natural rubber is produced by the Hevea brasiliensis tree, The genus is a native of South America and belongs to the family of the

Euphorbiaceae. And even though this tree has been in cultivation on plantations and small farms in the Far East for over 3/4 of a century little experimental work has been done on its nutrition.

Wright (33) has indicated that a wide variety of fertilizers were being used in rubber plantings prior to 1910 and that the two recommended formulae contained nitrogen, phosphate and potassium, but the experimental evidence supporting these fertilizers was very limited. Grantham (11) reports a marked increase in rubber yield from applications of nitrogen, and a retarding effect from superphosphate on white Sumatra soil. Flint (10) reports that an experiment carried out gave the following increases in girth of rubber trees: organic fertilizer 10.9%, nitrogen alone 4.5%, complete inorganic 3.5%; nitrogen plus phosphate showed a depression in rate of girth and phosphate plus potassium had no effect. Murray (14) found that nitrogen checked deterioration of mature trees and produce yield increases in young trees. Phosphate plays an important role in the nutrition of immature trees up to seven years of age. On white Sumatra soils applications of potassium and phosphate to mature trees has given negative results, and there is some evidence that superphosphate depresses yield in the presence of nitrogen and the absence of potassium. Akhurst (2) reports that early measurements show that rubber trees receiving a complete inorganic fertilizer yielded significantly better than those on any other treatment (N alone, NK, PK). The Rubber Research Institute of Malaya (26) reported that trees of various clones showing chlorosis and leaf scorch were injected with N, P, and K. Leaf injections were not satisfactory, in their opinion, because of the presence of latex in the leaf tissues. When the solutions were introduced in the trunk of the plants the best results were obtained with K. The foliage became green and healthy with no trace of yellowing and scorch, and vigorous new growth was produced in branches which had exhibited die-back of twigs. In a fertilizing experiment in which N, NP and NPK were used, Haines (12) found that the effect of NPK was significantly greater than that of other fertilizers. In evaluating the effects upon girth of NP, NK and NPK, Bertrand (5) concludes that in his soil there is a relative poor response to mixtures lacking potash. Akhurst (1) points out that a good number of manuring experiments on virgin jungle land of different soil types have indicated that little or no manuring is required by rubber plants during the first six months after planting. According to Whelan (31) the experience gained in Ceylon in a good number of years leads to the conclusion that some form of manuring is necessary on most soils if the yield is to be maintained. This however is not supported by critical field tests. In an experiment to determine the fertilizing requirements of mature rubber (trees 24 years of age when the experiment was initiated) Whelan and de Silva (32) found that the results for the years 1937 to 1942 showed: 1) yield from the NPK treatment was apparently lower than that from N alone; 2) fertilization had no effect or girth increase or bark renewal and 3) the NP treatment showed a relatively poor yield suggesting a depressing effect of P when added to N in the absence of K. In the next two years of this experiment, the results as presented by Whelan (30) show a significant yield response by N, NK and NPK, and no response to NP. N and NK had no response on girth while NP and NPK had a highly significant effect on girth. Fertilization of nursery seedlings shortly after planting to within three months of budding, as reported by Whelan (29) resulted in an increase in the number of takes from 41% in the minus nitrogen plot to 79% in the plot in which nitrogen was applied, while P and K had no effect. C. C. T. Sharp (20) found a group of trees with poor foliage, which had not responded to applications of N, P and K, and were thought to be deficient in a minor element. Using the injection technique, Zinc, Nickel, Copper, Manganese, Iron, Cobalt, Molibdenum, Boron and Magnesium were administered to the trees. At the end of 18 months no improvement had been noted and the experiment was abandoned. In the discussion of the results of a good number of fertilizing experiments Smith (24) indicates that K and P applied jointly appeared to be harmful to young budded trees. The results are based on girth measurements for two years. He thinks that the negative response is presumably due to K. Akhurst (3) found that the heights of trees at one year from planting showed a much greater response to phosphate than appeared in the girth taken at a later date. He says his experiments demonstrated, as the most important fact, the marked effect of P in advancing maturity of young rubber. According to Dijkman (9) rubber centers in the Indonesian Archipielago, Malaya and Ceylon have confirmed that phosphates are most necessary for the optimum growth of young rubber. He says that P deficiency has been observed to severely stagnate growth. Discussing the results of a manuring experiment which has been carried out for twelve years, de Silva (23) points out that the better grown trees are in P plots. The trees have been in tap for seven years and during the first six of those years the effect of P on the yield was maintained at a significant level. Constable (7) says that according to Russell the effect of N is to make larger, softer and darker green leaves.

In excess, this results in a very thin-walled leaf which is most susceptible to fungous diseases and to drought. According to Rhines, Mc Gavack and Linke (17) the use of N has become a common practice but the use of mineral fertilizers has been rather limited and of doubtful value. The Planters Bulletin of the R. R. I. M. (16) points out that P deficiency has a marked retarding effect on growth, while Mg is not necessarily associated with a marked retarding effect on young rubber plants, even though leaves may become readily chlorotic.

The Rubber Research Board (18) reports on fertilizing experiments carried out in the East in mature and in young rubber trees. After fertilizing the mature rubber for 12 years and the young trees for 10 years they obtained results which varied from year to year. In the case of mature rubber, during the first few years of the experiment N alone seemed to be the most economic fertilizer but in the last few years NPK seemed to prove best. When P was added to N in the absence of K it was observed that there was a decrease in yield. In the case of young trees it was observed that the plots which had the better responses were those treated with P. It is concluded that the principal effect of P on young trees is the bringing of those trees into tap at an earlier date. The Rubber Research Scheme (15) states that the little evidence available suggests that P is essential during the growing phase and that its effect diminishes as the trees reach maturity.

Verslagen Proefstations (27) reports that Hevea rubber plants in sand cultures deprived of N, P, or S were drastically reduced in height and girth; the deficiency of these elements being exhibited earlier than that of K, Ca or Mg.

C. C. T. Sharp (21-22) reports on a pot culture experiment which was started in June 1938, using budded stumps. The treatments were the following: Complete nutrient solution, minus nitrogen, minus phosphorus, minus potassium, minus calcium, minus magnesium and minus trace elements (Mn, Zn, Cu and B). Yellowing and mild scorch was observed in all treatments and was suspected to be due to a shortage of potassium. Deficiency leaf symptoms were soon observed in the minus N and minus Mg treatments. P deficiency was observed by the seventh month of the treatment. At the end of 6 months the plants in the minus Ca, minus Mg and complete nutrient solution had made the best growth. Plants in minus K and P treatments were next and the ones in the minus N treatment were the smallest of all. The best growth occurred in the plants in the minus Ca treatment.

Rhines, McGavack and Linke (17) carried out a nutritional experiment using Hevea seedlings. From the results obtained they conclude that: 1) P, K and Mg are the minerals whose deficiency is more likely to retard growth; 2) absence of S severely retards growth, but a very small amount of it (0.8 p.p.m.) is adequate for normal growth; 3) no retardation of growth was caused by the absence of Ca; 4) an adequate available iron supply is highly essential; 5) the experiment failed to associate any great importance with the use of Zn, B or Mn; and 6), maximum growth on culture solutions takes place at a pH of 4.5 to 5.5.

It is apparent that the work carried out to date on nutrition of Hevea does not give satisfactory answers to questions like the following:

1) What is the effect on the growth of Hevea rubber plants,

deficient in each one of the macro and micro-elements which are considered essential for the normal development of plants?

2) What is the effect on the rubber content of the different parts of the Hevea plant the deficient in each one of the essential elements?

3) What are the characteristic symptoms of the deficiency of each one of the essential elements in young Hevea seedlings?

4) Which of the deficiencies are likely to show up first and have the most deleterious effect on the development of young seedlings?

Materials and Methods:

The plants were grown in sand culture in the greenhouse at the U.S.D.A. Rubber Plant Field Station, Turrialba, Costa Rica. A translucent plastic, with the trade name Resolite, was used in the construction of the greenhouse. This material gives only about 30% light transmission as measured on a Weston foot-candle meter model 614. Ventilation in the greenhouse was achieved by means of an open cupola at the comb and an 18" opening in the side under the overhanging eaves, plus a 30" side opening under the bench. Despite the ventilation, on bright sunny, windless days the temperature ranged up to ten degrees Fahrenheit higher inside the house than outside and the ralative humidity was correspondingly lower inside the house.

The sand used in the experiment was crushed screened quartz obtained from a pottery factory in San Jose, Costa Rica. The quartz stone was imported from Mexico and crushed and screened to 1-3 mm in size. The sand to be used in the minus K, P, N, Mg, S and the full nutrient cultures was washed with tap water until the wash water was perfectly clear and then further washed with demineralized water. THe sand for the minus Ca, B, Fe, Mn, Cu and Zn cultures was washed with tap water until the wash water was clear and then covered with 8% HCl plus 1% oxalic acid solution. After 8 days the HCl oxalic acid was removed and the sand rinsed several times with demineralized water and 4.5% NaOH added to completely cover the sand. After 3 days the NaOH was drained off and the sand was rinsed many times with demineralized water.

Following the cleansing treatment 5.5 Kgs. of sand were placed in each glazed culture pot. The pot was 18 cm. in diameter and 24 cm deep with a 1/2" hole in the side, 1/4 inch from the bottom. A pyrex glass tube was bent and placed through a cork in the hole for drainage purposes. A wooden cover was sawed to fit each pot. The under side of the cover was painted with a black non-toxic asphalt compound. Three 5/8 holes were drilled in the cover to accomodate the plants. The pots were separated into four pots per treatment and the sand in each pot rinsed with the appropriate culture solution to be used in the experiment, until the pH of the drained solution was the same as the pH of the freshly prepared solutions.

The seeds were obtained from the U.S.D.A. Rubber Station at Marfranc, Haiti. They were collected from a monoclone block of GA-608, and gave very uniform germination in washed quartz sand. The seedlings were left in the flats of washed quartz sand until they were about 12" in height before being transferred to the culture pots. The endosperm and cotyledons were not removed from the seedling until the time of transferring at age of two months. By this time most of the endosperm had been utilized by the young plants. At the time of transfer the plants were very uniform and in excellent condition as judged by their leaf color. They had developed the first flush of growth and were just starting the second flush. Figures 1 and 2 show the plants two weeks after potting. Figure 3 shows the plants some 14 weeks after potting.

Figure 1: Plants 2 weeks after transferring to the pots.

Figure 2: Another view of the plants two weeks after transplanting.

Figure 3: Plants some 14 weeks after transplanting.

The nutrient solutions.- .

Chemically pure salts were used and the solutions were prepared in the following manner:

Stock Solutions

Solution Symbol		Compound	Conc.	Nº grams into 1000 cc Dist. H_2O.
A		$Ca(NO_3)_2.4H_2O$	M/1	236
B		KNO_3	M/1	101
C		$MgSO_4.7H_2O$	M/1	246.5
D		KH_2PO_4	M/1	136
E		$Ca(H_2PO_4)_2H_2O$	M/100	2.52
F		K_2SO_4	M/2	87.12
G		$CaSO_4.2H_2O$	M/100	1.45
H		$MgCl_2.6H_2O$	M/1	203.4
I		Fe Tartrate	---	5.0
J	£	H_3BO_3	---	0.6
K	£	$CuSO_4 . 5H_2O$	---	0.05
L	£	$MnCl_2 . 4H_2O$	---	0.4
M	£	$ZnSO_4$	---	0.05

£ For the minor elements B, Cu, Mn, and Zn the concentration used was that of Haas and Reed's A-Z solution.

Nutrient Solutions

		A	B	C	D	E	F	G	H	I	J	K	L	M	H_2O
1	Full Nutrient	21	20	8	4					2	2	2	2	2	3937
2	-Potassium	30		8		200				2	2	2	2	2	3752
3	-Phosphorus	30		8			40			2	2	2	2	2	3912
4	-Calcium		60	8	4					2	2	2	2	2	3918
5	-Nitrogen			2		200	40	800		2	2	2	2	2	2948
6	-Magnesium	20	20		4		20			2	2	2	2	2	3926
7	-Sulfur	20	20		4				8	2	2	2	2	2	3938
8	-Iron	20	20	8	4					-	2	2	2	2	3940
9	-Boron	20	20	8	4					2	-	2	2	2	3940
10	-Manganese	20	20	8	4					2	2	2	-	2	3940
11	-Copper	20	20	8	4					2	2	-	2	2	3940
12	-Zinc	20	20	8	4					2	2	2	2	-	3940

The pH of the freshly prepared solutions were the following:

Solution			pH
1	full nutrient		5.00
2	minus	K	4.10
3	"	P	5.25
4	"	Ca	5.00
5	"	N	4.50
6	"	Mg	4.90
7	"	S	4.90
8	"	Fe	4.82
9	"	B	4.98
10	"	Mn	4.90
11	"	Cu	4.85
12	"	Zn	4.78

During the first three months, fresh nutrient solutions were prepared twice a month and from there on once every two weeks, four liters of each nutrient solution being prepared each time. Half a liter of solution was put into each pot every day in the morning and drained in the afternoon. Iron (stock solution I) was added to the nutrient solutions, except in the case of the minus Fe treatment, every 3 or 4 days. When the amount of nutrient solution was not enough for the half liter required by each pot, demineralized water was added to the nutrient solution so as to make the two liters needed for the four pots.

Demineralized water was used for the experiment. This water was obtained by the use of a Barnstead Bantan demineralizer, model BD. The 50-gallon tanks were installed for collection and storage of rain water. All parts used for this purpose were coated with black non-toxic asphalt paint. The rain water flows from the tanks to the demineralizer by gravity, through glass tubing. The quality of water obtained is excellent, with only 0.5 to 1 p.p.m. mineral impurities.

All the glassware used for the experiment was made of Pirex glass. Twenty liter carboys were used to collect and store the demineralized water. The nutrient solutions were prepared in 4-liter Erlenmeyer flasks,and these covered with a box made of wood and cardboard especially designed to cover the flasks and prevent the entrance of light. 500-ml. flasks were used to collect the drained solutions, one for each pot. Brown paper bags were used to keep the solutions from being exposed to the light.

Some six weeks after the experiment was initiated some tiny white insects were found causing slight damage to the plants especially at the young growing points.. The insects that attacked our plants are believed to be the mites described by Steinmann (25) as causing damage to young Hevea leaves.

With the exception of the plants in the minus N treatment all plants developed normally up to the end of the sixth or eighth week of the treatment. The first deficiency symptoms were noticed by the eighth week. They appeared in plants of the following treatments: minus K, minus Mg, minus Fe and minus Mn. Plants in the first three treatments exhibited some chlorosis and leaf tip scorching, and those in the minus Mn treatment exhibited a retarded growth. By the tenth week one plant of the minus B treatment was showing deficient growth and the plants in the minus Ca treatment showed leaf tip scorching. By the fifteenth week deficiency symptoms were noticeable in plants of the minus S, minus Cu and minus Zn treatments. At no time deficiency symptoms have been noticed in plants of the minus P treatment and these plants made very good growth up to the time of harvesting.

Yellowing of plants in all treatments, and mild leaf-tip scorch in some, was observed by the 15th week. One of the control plants (full nutrient solution treatment) showed a marked chlorosis. This yellowing was thought to be associated to the poor light transmission in the greenhouse and its high temperature, and later was suspected to be caused by an insuficient supply of nitrogen. When Dr. P. Alvim saw these plants indicated that in his opinion the yellowing was due to an iron deficiency and indicated that ferric tartrate often is unsatisfactory for pot culture work. Dr. Alvim suggested the use of Ferro-Green, an iron chelate, instead of ferric tartrate. To make the trial one of the pots with control plants was selected which had two of its three plants showing a pronounced chlorosis. After 15 days of use of Ferro-Greene the three plants in the pot showed some change in the color of the leaves and appearance of the plant and by the 20th or 25th day they had a marked improvement. On June first we substituted ferric tartrate by Ferro-Greene as the source of iron for all the plants in all treatments and a favorable response was observed in about two weeks. To apply the Ferro-Greene, 6 grams were dissolved in 100 cc of water and 1 cc of this solution added to 1 liter of nutrient solution every 3 or 4 days.

By the end of the 21th week mostly all treatments were showing distinct leaf deficiency symptoms and it was decided to make mineral foliar applications. The salts and concentrations used were the following:

Minus K plants	KCl	200 p.p.m.
" K "	KSO_4	200 p.p.m.
" P "	NaH_2PO_4	200 p.p.m.
" Ca "	$CaCl_2$	200 p.p.m.
" Ca "	$CaSO_4$	200 p.p.m.
" N "	NH_3NO_2	100 p.p.m.
" Mg "	$MgCl_2$	200 p.p.m.
" Mg "	$MgSO_4$	200 p.p.m.
" S "	$MgSO_4$	200 p.p.m.
" S "	Na_2SO_4	200 p.p.m.
" Fe "	$FeCl_3$	25 p.p.m.
" Fe "	$FeSO_4$	25 p.p.m.
" B "	H_3BO_3	25 p.p.m.
" Mn "	$MnCl_2$	25 p.p.m.
" Mn "	$MnSO_4$	25 p.p.m.
" Cu "	$CuCl_2$	10 p.p.m.
" Cu "	$CuSO_4$	10 p.p.m.
" Zn "	$ZnSO_4$	20 p.p.m.

Each salt was applied in three different manners: a) the salt solution was applied to an area of a leaf without any previous treatment; b) an area of a second leaf was gently rubbed with carburandum and the solution applied on this area; on a third leaf the Roach method, as explained by Alvim (4) was used with some slight modifications. Instead of glass tubes, the tubes used were cut from drinking straws which makes them lighter and cheaper. The treated leaves were inspected regularly for a period of twenty days and at no time correction of the deficiency symptoms was observed in any of them. No apparent harm was observed, except some slight damage caused by the rubbing with the carburandum.

A description of the plants of each of the various treatments follows:

Minus K:

The growth of the plants was apparently normal during the first 6 weeks. By the 8th and tenth week the pre-treatment leaves exhibited scorched and dying tips, the plants were becoming chlorotic and showed some retarded growth. After 12 weeks all the older leaves had scorched tips up to 2 cm., and some scorching was taking place in the outer margins of some leaves. As time went by more leaves showed soorched margins, necrotic areas appeared and chlorosis was more pronounced. The new young leaves were dark green in color but smaller in size. There has been a pronounced stunting in the plants of this treatment and a continuous defoliation.

Figures 4 and 5 in page 23 show leaves and plants of the minus K treatment.

Minus P:

The growth of these plants have been excellent judging by the dark green color of the leaves, and height and appearance of the whole plants. No distinct symptoms have been noticed at any time. A few deformed leaves have been found in some of the plants but it is doubtful that it was caused by the deficiency of phosphorus. There has been little defoliation. The roots of these plants seemed to be in very good condition.

Figure 6 in page 24 shows plants of the minus P treatment. No leaves were observed showing characteristic deficiency symptoms in plants of this treatment.

Minus Ca:

Growth of plants in this treatment seemed normal during the first 8 weeks. After ten weeks the leaf tips were dying in some plants. At 17 weeks growth stopped. The third flush of growth was very short. The fourth flush started but the growing point was scorched in 8 of the 12 plants. The leaves exhibited a marked chlorosis and they were reduced in size, Growing points were scorched after 17 weeks in all plants and there has been a dying back of the stems which in some cases has killed the entire upper part of the plants, in some cases to a short distance from the base. The plants in this treatment have practically lost all their leaves. 2/3 of the harvested plants had tops and roots dead.

Figures 7 and 8 in page 25 show leaves and plants of the minus Ca treatment.

Minus N:

This appeared to be the most drastic of the treatments. The plants did not make any growth and the leaves were reduced in size, had scorched tips and exhibited marked chlorosis and abundant necrotic spots especially around the edges. The plants shed their leaves soon after the treatment was started. On April first we started adding to the nutrient solution of these plants the equivalent of 12.5% the nitrogen given to the plants of the control group. A favorable reaction took place almost immediately; the leaves turned green and the terminal bud started to grow. Nitrogen feeding was stopped on June 21. Very soon the leaves started turning yellowish. The plants still look all right at 28 weeks but no growth was noticed on the terminal bud. Heavy defoliation took place since the early stages of the treatment. When harvested, the roots of these plants appeared to be in very good condition.

Figures 9 and 10 in page 26 show leaves and plants of the minus N treatment.

Minus Mg:

The first leaf symptoms appeared some eight weeks after the treatment was started. Pre-treatment leaves mottled with dying tips. Interveinal tissue pale yellow while midrib and main veins dark green. As time passed, chlorotic leaves became of a pale yellow color and then shed, leaving the lower part of the plants leafless.

Growth has been greatly retarded. 30% of the plants show die back of the stem, and lateral buds growing. New leaves are small in size and pale. There was considerably defoliation of the plants in this treatment. When the plants were harvested it was found that many roots were dead.

Figures 11 and 12 in page 27 show leaves and plants of the minus Mg treatment.

Minus S:

The growth has, in general, been good. The pre-treatment leaves have kept their dark green color. There has been some tip scorching of the leaves and in such cases the tip has curled upwards over the upper surface of the leaf. Some of the new leaves show chlorosis similar to that of plants in the minus magnesium treatment, that is, midrib and main veins dark green while interveinal spaces are of a light yellow color. The difference being that in the case of the minus Mg treatment chlorosis appears on the old leaves while in the case of the minus S treatment the old leaves are green and chlorosis appears on the new leaves. The leaves showing chlorosis are of a smaller size than normal leaves. There has not been much defoliation of these plants. The roots were found in very good condition, with lots of root hairs.

Figures 13 and 14 in page 28 show leaves and plants of the minus S treatment.

Minus Fe:

Leaf chlorosis was noticed on the new leaves some eight weeks after the treatment was started. From then on all new leaves were considerably reduced in size and exhibited a pronounced chlorosis. Many of the newly developed leaves were almost white and transparent. Scorching of meristematic tissues also occurred and the main stems kept dying back. By 26th week 100% of the plants had considerable die back of main stems, and half of them had developed branches from lateral buds. At this time, the pre-treatment leaves had their dark green color. The treatment has been very drastic and as a result they are in a very poor condition of growth and will in all probability die if kept longer without supplying iron. At the time of harvesting roots were found in very poor condition especially in the cases of plants without leaves.

Figures 15 and 16 in page 29 show leaves and plants of the minus Fe treatment.

Minus B:

Plants stunted. Retardation of growth was apparent some 15 weeks after the treatment was started. 75% of the plants with meristematic tissues dying back, but not as pronounced as in the case of the minus Ca and minus Fe treatments. New leaves seem to have normal size although they show some chlorosis. Pre-treatment leaves still dark green in color. There has been some defoliation especially from the upper part of the stem. The symptoms developed are somewhat similar to those of the minus Ca treatment. Many of the roots were found dead, especially those of plants with very poor tops. The top and secondary roots were thick but with few hairs.

Figure 17 in page 30 shows plants of the minus B treatment. No leaves were observed showing a characteristic deficiency symptom in plants of this treatment.

Minus Mn:

Plants stunted. Retarded development was obvious from the 8th week of the treatment, with growth of the lateral buds. Terminal growth was restricted and 40% of the plants are dying back. There has been, however, some growth on the diameter of the stems. Old leaves still dark in color while new ones are small in size and showing a chlorosis comparable to certain degree to the chlorosis of the plants in the minus S treatment. By the 25th week some leaves had a chlorosis similar to that of plants in the minus Fe treatment. There has been considerably defoliation especially from those plants in which die back has occurred. Top roots were thin, few root hairs. The roots, especially the secondary ones, were found dying back.

Figures 18 and 19 in page 31 show leaves and plants of the minus Mn treatment.

Minus Cu:

The growth was satisfactory up to the end of the 20th week. No new flushes of growth have developed since the 25th week. In 30% of the plants the terminal bud has died and in the rest it has been dormant for a good period of time without sprouting. All the leaves at the end of the stems shed when they were still small and young. The size and color of leaves seem normal. A few show a chlorosis of a special pattern, somewhat alike to that of the minus S and minus Mg deficiencies with some variations. When the chlorosis was advanced leaves turned to a dark bronze color. Some peculiar dark round spots, about 2-3 ml in diameter, appeared in a line along each side of the midrib in some leaves, some 15 weeks after the treatment was started. The roots were found in fair condition. The secondary roots were thick and dying back of tops corresponded to dying back of top and secondary roots.

Figures 20 and 21 in page 32 show leaves and plants of the minus Cu treatment.

Minus Zn:

Fairly good growth. Four plants show some die back of the stem. Old leaves dark green. New leaves have normal size, some of them somewhat pale in color. There has not been found a general and characteristic leaf or stem symptom. However some leaves developed some round necrotic spots, about 1 cm in diameter scattered on different areas of the leaves. These necrotic spots resemble to those found in some leaves of the plants in the minus Cu treatment, but those were smaller and were present in all cases along the midrib and very close to it. These spots have also some similarity to those described by John (13) and reported as being caused by a Colletotrichum.

Tap and secondary roots thick with few root hairs, cork tissue of roots black, cracking and new tissue developing.

Figures 22 and 23 in page 33 show leaves and plants of the minus Zn treatment.

Figure 24 in page 34 shows plants of all the treatments.

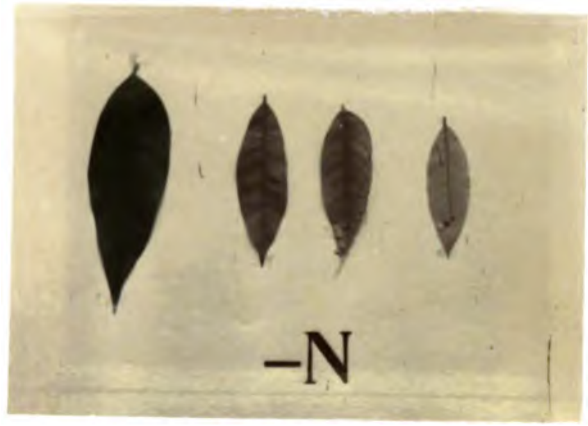

Figure 9: Leaves from plants in the minus N treatment. First leaf at right is from a plant in the full nutrient treatment.

Figure 10: Pot at right with plants grown in full nutrient treatment. Pot at left with plants grown in the minus N treatment.

Figure 24: Plants of all the treatments.

Table 1: Average weight and height of plants at harvest.

Treatment		Fresh weight Roots gms.	Fresh weight Tops gms.	Dry weight Roots gms.	Dry weight Tops gms.	Height mm
Full nutrient		19.70	33.27	4.32	11.43	472
Minus	K	9.88	18.50	2.32	6.32	445
"	P	20.67	31.43	5.18	10.83	520
"	Ca	12.17	10.50	1.78	2.90	345
"	N	19.25	16.17	4.52	5.75	410
"	Mg	11.08	16.92	1.93	3.58	402
"	S	27.08	32.0	6.27	10.95	487
"	Fe	10.80	15.80	2.52	4.32	338
"	B	16.50	17.93	4.80	7.92	390
"	Mn	10.12	13.03	2.37	4.72	255
"	Cu	17.75	23.85	5.15	8.90	393
"	Zn	17.33	23.83	5.38	9.12	408

Table 2: Miligrams dry rubber per gram of tissue.

Treatment		Content of dry rubber		
		Roots	Stems	Leaves
Full Nutrient		20.8	41.7	16.7
Minus	N	19.2	49.2	18.3
"	Mg	15.0	37.5	22.5
"	P	19.2	40.0	13.3
"	Ca	19.2	38.3	13.3
"	Zn	16.7	36.7	10.8
"	S	16.7	40.0	16.7
"	Mn	18.3	25.8	12.5
"	K	15.8	33.3	12.5
"	Cu	20.0	36.7	12.5
"	Fe	16.7	31.7	16.7
"	B	16.7	38.3	12.5

Method used for the extraction of rubber

A modified Traub method of rubber extraction was used in the rubber determinations.

The Hevea material was dried for 24 hours at 80°C., and ground in a Wiley mill with a 60 mesh screen. Samples of 300 mg. of powder were weighed and placed in centrifuge tubes with 25 ml of acetone added. The tubes were placed in a steam bath and allowed to boil for 15 minutes. The material was centrifuged and the acetone discarded. Another 25 ml of acetone were added and boiled again for 10 minutes, centrifuged and the acetone discarded. The powder was allowed to dry in the oven at 60°C for 40 to 60 minutes. 25 ml of 1% tetrachloroacetic benzene solvent were added, and the tubes allowed to boil for 30 minutes. The material was centrifuged and the extract collected in 25 ml volumetric flasks, made to volume and mixed. 5 ml of this last extract were placed in colorimeter tubes and 10 ml acidified ethanol added. The tubes were inverted three times and the turbidity read in a Coleman Model 8 colorimeter. Table 2 shows the contents of rubber in the different parts of the plants.

DISCUSSION

The results obtained give satisfactory answers to the four questions raised in the introduction, concerning the effect of the essential elements in the development of Hevea rubber seedlings.

Deficiency symptoms were observed in all treatments except in the minus P and minus S treatments which were not very marked. The plants in these two treatments developed apparently normal and the average height reached was greater than those of the full nutrient treatment (control plants). The minus P plants were 10% higher than the control plants and the minus S plants 3% higher than the controls. There was not any appreciable difference in the weights of the tops of these plants in comparison with the control plants. The tops of the minus P plants had 95% the weight of control plants and the minus S plants had 96% the weight of the controls. On the other hand the roots of the minus P were 20% greater in dry weight than those of the controls and the roots of the minus S plants were 45% heavier than those of the controls. We know of no explanation for the excessive growth of these roots, which tend to indicate a slight mineral contamination of the solutions. However, the conditions under which the experiment was carried out minimize the possibilities of such contamination. The demineralized water used in the preparation of the nutrient solutions was of excellent quality, with only 0.5 to 1 ppm mineral impurities; the glassware used for the preparation and handling of the solutions was made of Pyrex glass, the salts used for the solutions were chemically pure and the pots used were glazed pots which should yield no P or S. One possible source of contamination could be the quartz sand used for these two treatments, which was not acid treated. It is possible that young Hevea seedlings require these so called macro elements, in very small amounts. This was suggested by Rhines et al (15) who found that 0.6 ppm P and 0.8 ppm S were adequate for normal growth of Hevea seedlings. It is also possible that the Hevea seed has an amount of P and S sufficient for the requirements of the young plant for a period of seven or eight months after germination. The endosperm and cotyledons of these plants were removed when the plants were transferred to the pots, some eight weeks after germination, and by this time most of the endosperm had been utilized by the young seedlings. One remote possibility could be the substitution of other elements for these two in the various compounds, in which they enter. The results of these two treatments suggest that for similar experiments with Hevea, the sand should be acid treated and the cotyledons removed at a much earlier stage if a quick response to the deficiency of these minerals is expected.

There was ample confirmation of the great importance of N to the nutrition of a plant. The plants in this treatment simply did not grow at all after transplanting. They would have died had not a small amount of N been added to the solution. When 12.5% of the amount of N that was being fed to the full nutrient plants was added, they responded rapidly and only after reducing the N again to 0% did we get N deficiency showing. From this we can conclude that young Hevea seedlings require small amounts of N as compared to other species of plants.

The striking results obtained in the minus Ca treatment reveals the great importance of this mineral. First the leaves exhibited some chlorosis and reduction in size, possibly when the supply of Ca was getting low, and then the plants suddenly stopped their growth and die-back of the stems started. By this time it is probable that all the Ca in the endosperm had been utilized. Since Ca is a constituent of the cell walls no new cells are laid down when it becomes limiting and consequently growth stops. Meristematic tissues are the first affected because Ca does not migrate within the plant. In the pot-culture work reported by Sharp (21-22) it is mentioned that the plants in the minus Ca treatment grew very well. Such results are explained by the fact that the sand used for the work was not acid treated and no distilled water was used for the preparation of nutrient solutions. There was undoubtedly a source of Ca for the plants in this treatment. Rhines et al (17) also obtained good growth of their Hevea seedlings in the Minus Ca treatment. This suggests that these plants also found a source of Ca, probably in the sand or in the pots used. It is possible that a low amount of Ca is sufficient for the needs of young Hevea plants and consequently in experiments in which the availability of this element is not properly controlled the plants will not show the symptoms of the deficiency and will grow satisfactorily. The minus Ca plants only reached 73% of the height of the controls. Their roots only had 41% the weight of roots of the controls, and their tops only had 25% the weight of the top of the controls.

The low weight of the plants in the minus Mg treatment indicates that his element is of great importance in the development of Hevea seedlings. Mg deficient plants reached 85% the height of control plants. In weight the roots were 45% of the weight of the roots of the controls and the tops were 31% of the weight of the tops of the controls.

Since Mg is a constituent of chlorophyll, Mg deficient plants will be reduced in their photosynthetic ability and thus be restricted in their growth. There may be other causes of stunting of growth in Mg deficient plants also.

The plants in the minus Fe and minus Mn treatment were among the lowest in weight and height. The minus Fe plants had 72% the height of the controls, their roots had 58% the weight of controls and their tops 38% of the controls. The minus Mn plants reached 54% the height of the controls, their roots were 55% of the controls and their tops 41% of the controls. Since these two elements are recognized as indirectly essential for the formation of chlorophyll, it is obvious that their absence is a limiting factor for the photosynthesis and the growth of the plant is retarded.

The plants of the minus K treatment compare with the controls in the following manner: height 94%, weight of roots 54% and weight of top 55%. The great difference in weight between the control plants and those in the minus K treatment reveals that K is of great importance in the development of Hevea seedlings. These figures emphasize the suggestion of Curtis and Clark (8) about K being indirectly concerned in photosynthesis. If lack of K results in a reduction of photosynthesis it is obvious that a reduction in growth will also take place.

The plants in the minus B, minus Cu and minus Zn treatments compare with the control plants as follows: <u>Minus Zn</u>: height 86%, root weight 124%, top weight 80%. <u>Minus Cu</u>: height 83%, root weight 119%, and top weight 78%. <u>Minus B</u>: height 83%, root weight 111% and top weight 69%. These figures show that these three elements were limiting the development of the aerial parts of the plants but not the roots which in all three cases were greater than the roots of the control plants. The roots in the plants of these treatments, especially the ones of the minus Zn treatment were very thick.

The analysis of roots, stems and leaves for rubber contents show some interesting figures. In the minus N plants the stems had 4.9% rubber per unit of weight and the leaves 1.8%, while the control plants had 4.2% in the stems and 1.7% in the leaves. The minus N plants had leaves thinner than those in the control plants which would result in a larger area per unit of weight which could in part explain the higher concentration of rubber in the leaves of the minus N leaves. In the case of the stems only the bark was analyzed and probably again there was a thinner cell wall and hence more cell contents per unit area or per unit weight than the stems of the control plants. When the leaves were analyzed the highest concentration of rubber was found in the minus Mg treatment with 2.3% compared with 1.7% in the controls. The lowest percentages corresponded to the following treatments: minus K with 1.3%, minus Mn with 1.3%, minus Cu with 1.3%, minus B with 1.3%, minus Fe with 1.2% and minus Zn with 1.1%. The minus Mg treatment presents an interesting case in that the leaves of the plants in this treatment had the higher concentration of rubber among all the treatments, 2.3% compared with 1.7% in the control plants, while the roots had the lowest concentration among all the treatments with 1.5% compared with 2.1% in the controls. As to concentration of rubber in the stems the plants in the minus Mg treatment had an intermediate place with 3.8% compared to 4.2% of the controls. The treatment with the lowest concentration of rubber in the stems of the plants was the minus Mn treatment with 2.6%. With the exception of the cases mentioned above, there were not marked differences in the concentration of rubber in the roots, the leaves and the stems of the plants of the different treatments. There was a tendency for the contentration of rubber in the control plants to be higher than in most of the other treatments but the differences were not great. We do not have enough evidence to conclude that the differences found between treatments can be adscribed to the deficiency of each one of the various elements. It is considered that more work is needed to establish the correlation between the mineral deficiencies and the amount of rubber present in the various tissues of the plants. It was clearly demonstrated, however, that contrary to what other workers have found, that the stem was the tissue with the higher percentage of rubber per unit of area. The average percentage of rubber was as follows: stems 3.7%, roots 1.8% and leaves 1.4%.

No apparent results were obtained from the foliar applications of salts to the plants of the various treatments. This could not be due to impermeability of the cuticle since some of the leaves were rubbed with carburandum previous to the application of the solution and this would result in the braking of the cuticle as evidenced by the slight damage caused to some leaves by the treatment with the carburandum. The fact that there was no correction of the deficiencies suggests that the solutions did not penetrate the cell of the leaves or if the salts did penetrate, they were in an unavailable form or rapidly converted to an unavailable form by some action within the leaf tissue.

SUMMARY

1. No apparent damage was caused to the Hevea seedlings by the deficiency of P and S during the nine months of the experiment. The roots of the plants in this treatments were much bigger than the roots of the control plants, and their tops almost as big as those of the control plants.

2. N, Ca and Fe were the elements whose deficiency cause the greatest damage to the plants. There was complete stunting of the plants in the minus N treatment. Considerable die-back of stems was observed in the plants of the minus Ca and minus Fe treatments and complete defoliation occurred in several of the plants in these two treatments.

3. The deficiency of K and of Mn caused a great reduction in the dry weight of the plants. The plants in the minus B, minus Cu and minus Zn treatments had tops somewhat smaller than the tops of the control plants, but their roots were considerably bigger than the roots of the full nutrient plants.

4. No correction of deficiencies resulted from the foliar applications of single salts.

5. Fe tartrate was unsatisfactory as the source of iron and it was necessary to substitute Ferro-Greene, an iron chelate, which proved very satisfactory. Adequate available Fe supply was demonstrated to be highly essential for the development of Hevea seedlings.

6. The stems were found to have the highest percentage of rubber as compared to the roots and leaves. It was not possible to establish a correlation between mineral deficiencies and percentage of rubber.

COMPENDIO

Este trabajo se llevó a cabo en la Estación Experimental de Hule, en Turrialba, Costa Rica, de Octubre de 1953 a Julio de 1954.

Se deseaba producir plántulas de Hevea con deficiencias minerales conocidas y estudiar el efecto de esas deficiencias en el desarrollo de las plántulas, su peso seco y su contenido de hule.

Tratamientos: Un grupo de plantas recibió una solución nutritiva completa y otros grupos recibieron soluciones en que K, P, Ca, N, Mg, S, Fe, B, Mn, Cu y Zn estaban ausentes. La arena para el tratamiento con menos Ca y elementos menores se trató con ácido clorhídrico. Se usó agua demineralizada y frascos de vidrio Pirex para la preparación de soluciones nutritivas. Las plantas crecieron en maceta de porcelana conteniendo 5.5 Kg. de arena de cuarzo. Las soluciones se ponían todas las mañanas y se drenaban por la tarde.

Con excepción de P y S las plantas de todos los tratamientos en que había ausencia de un elemento mostraron reducción de tamaño y síntomas de deficiencia similares a los descritos por Curtis & Clarke (8). Las plantas de los tratamientos con ausencia de P y S casi no mostraron reducción en la parte aérea de la planta y las raíces en esos dos grupos de plantas fueron considerablemente más grandes que las raíces de las plantas del tratamiento de solución completa.

Los tratamientos más drásticos fueron aquellos en que hubo ausencia de N, de Ca y de Fe. A las plantas del tratamiento con ausencia de N fué necesario darles por algún tiempo 12.5% de la cantidad de N que se estaba administrando a las plantas de la solución completa. Con esta cantidad de N esas plantas reaccionaron muy favorablemente y

reanudaron su crecimiento normal. En las plantas de los tratamientos con ausencia de Ca y Fe la parte terminal de los tallos se secó y muchas de las plantas estaban a punto de morir cuando se cosecharon. La ausencia de K y de Mg también produjo una gran reducción en el tamaño de las plantas, pero en éstas no hubo secamiento o muerte de los tallos. Las plantas en los tratamientos con ausencia de B, Cu y Zn desarrollaron raíces más grandes que las de las plantas testigos, pero sus partes aéreas fueron bastante más pequeñas.

Se hicieron aplicacinnes foliares de soluciones minerales pero no se consiguió corrección de las deficiencias.

Se hizo análisis de raíces, tallos y hojas por hule y los tallos resultaron con el porcentaje de hule mayor por unidad de peso. Hubo algunas diferencias en cuanto al porcentaje de hule en algunos de los tratamientos pero no es posible establecer una correlación entre las deficiencias minerales y las concentraciones de hule en las plantas con tales deficiencias.

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