Effects of Mineral Status and Light on Rubber Formation in Hevea

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The concentration of rubber and phosphorus in the stems of very young rubber seedlings increased with increased supply of phosphate. Later, when phosphate status markedly affected the dry weight of each seedling, this relationship disappeared and the rubber concentration decreased with increased seedling dry weight. More rubber per seedling was formed in the light than in the dark. The effect of light on rubber formation was more important than that of mineral status, the influence of which was indirect. The amount of rubber found in each seedling was proportional to the amounts of dry matter, carbohydrates and proteins present.

THE APPLICATION OF FERTILISERS to *Hevea brasiliensis* in the field may increase the yield of rubber produced per tree (HAINES 1946, OWEN, WESTGARTH & IYER 1957); this increase is believed due to the enhanced growth and vigour of the tree and the consequent greater output of latex. The concentrations and balance of the nutrient elements in leaves and latex may influence the yield of rubber per tree (BEAUFILS 1956) but information, reviewed below, on the effect of nutrient elements on the concentration of rubber in plant tissues is scant.

The concentration of rubber in the stems of soil grown guayule seedlings, *Parthenium argentatum*, was smaller at the higher level of available nitrogen supply than at the lower level (BENEDICT 1950). The concentration of rubber in the leaves of the same plant decreased as the proportion of ammonium ions, supplied in nutrient solution, increased; a high nitrate type of nutrient produced the highest concentration of rubber in the leaves (BONNER 1944). Reduction in the concentrations of rubber in the stems and roots of guayule and kok saghyz plants, due to boron deficiency, have also been noted (MITCHELL, BENEDICT & WHITING 1944, MEYER 1946).

It has been shown that a deficiency of any one of the nutrient elements decreased the percentage concentration of rubber in the stems and petioles of very young (up to four months old) *Hevea brasiliensis* seedlings grown in sand culture (BOLLE-JONES 1954a); but with continued growth, a reversal occurred and the healthy plants contained a smaller percentage concentration of rubber but grew much larger than the deficient plants. In a subsequent experiment (BOLLE-JONES 1954b) the effects of different levels of magnesium, potassium and phosphorus on the concentration of rubber in the stems and petioles of *Hevea brasiliensis* were found to be inconsistent from month to month. Significant concentration increases were obtained but they were never large (not more than 25%); their small magnitude suggested that the concentration of rubber within the plant was not directly affected by the nutrient concentration, but only the total amount produced per plant. The dry weight of the shoot appeared to be inversely related to the concentration of rubber found in the stems and petioles.

In another investigation, concerned with field grown *Hevea* plants, it was observed that the petiolar rubber concentration varied according to the concentration of nitrogen found in the laminae (BOLLE-JONES & RATNASINGAM 1954). Later it was found (BOLLE-JONES 1955), for *Hevea brasiliensis* seedlings grown in sand, that as the nitrogen concentration of the laminae increased the rubber concentration decreased. Increased nitrate level significantly decreased the concentration of rubber found in bulked stems and petioles.

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There was, therefore, evidence which indicated that the mineral nutrient status of the plant might affect the concentration of rubber found in the tissues. These effects were not always explicable and sometimes appeared to be contradictory but they did allow the inference that in the earlier stages of growth, before the dry weight effects due to differential mineral nutrient treatments became marked, increases in the concentration of rubber in the tissues might be obtained with improved mineral status. They also indicated that the total dry weight of the shoot of *Hevea brasiliensis* might be inversely related to the concentration of rubber in the stems and petioles and that different effects might be obtained according to the tissue analysed.

Most of the foregoing observations involved a comparison of foliar composition with the concentrations of rubber in the laminae or stems and petioles. However, it chanced in 1955 that analysis of hevea stem tissue for both minerals and rubber was carried out by the senior author and that an approximately linear relationship between the concentration of phosphorus and rubber was revealed. The first part of this paper describes an experiment designed to confirm and amplify this chance finding. It was found that a rough proportionality existed between the concentrations of phosphorus and rubber in the stems of plants, up to the four months old stage; but this relationship disappeared as the plants grew older and their dry weights varied widely among treatments. The effect of phosphorus was not specific as the same type of relationship appeared to hold in respect of the stem concentrations of other nutrient elements also.

It was suspected, even before these results became available, that as far as the investigation of the effects of nutrient elements on the concentration of rubber in tissues of pot grown plants was concerned valid comparisons could only be made in the absence of any marked plant dry weight changes and that any future investigations must involve very short term experiments. It was decided to adopt the alternative procedure of assaying the total rubber and certain other organic fractions within the plant and to attempt to relate them to nutrient treatment.

The objective of this latter approach was to determine the means by which mineral nutrient treatment affected the total rubber content per plant. While it was reasonably certain that none of the nutrient elements exercised a specific effect on the concentration of rubber in the tissues (so that in the absence of such an element the concentration of rubber would be greatly reduced and that this effect could not be brought about by any other element) it was not established with certainty that the formation of rubber was a mere consequence of growth and could only be influenced by any factor which, in the first instance, affected growth itself and the production of plant dry matter.

It was desirable to establish some indication of how rubber formation could be influenced by mineral nutrient status. With this objective an experiment was initiated in which the effects of mineral nutrient elements and also of light intensity on the production of rubber, carbohydrate and protein fractions were examined. The results, which are reported below, showed that:

a the effect of mineral status on rubber formation was an indirect one;

b the effect of light was more pronounced than that of mineral treatments and that more rubber per plant was formed in the light than in the dark;

c the rubber content per plant, whether in light or darkness, bore a linear relationship to the dry weight of plant, total carbohydrates per plant and total proteins per plant.

This preliminary study (Bolle-Jones & Mallikarjuneswara 1957) will serve as the basis for later studies on the elucidation of the effect of mineral nutrient elements on the formation of rubber in Hevea brasiliensis.

RELATIONSHIP BETWEEN CONCENTRATIONS OF PHOSPHORUS AND RUBBER IN THE STEM

Experimental

Seedlings of clone Tjirandji 1 were grown in sand contained in five gallon bitumen painted clay pots. The sand (particle size, 0.5 to 1.2 mm) was purified by washing with cold dilute hydrochloric acid (2% w/v) and subsequently restored to neutrality by leaching with nutrient salt solutions, as used for each respective treatment.

Phosphate was applied at five different levels which were 0.6 (P_1), 1.2 (P_2), 2.4 (P_3), 4.8 (P_4) and 9.6 (P_5) milligram equivalents per litre. In addition to phosphate, and an adequate supply of the micronutrient elements, each nutrient solution contained the following concentrations of the other essential elements (as m.eq./l): SO_4^{--} 6.3 to 9.3, NO_3^- 5, NH_4^+ 3, Mg^{++} 2.5, K^+ 3, Ca^{++} 4, Na^+ 2, Fe^{+++} 0.66. Each of the five phosphate treatments was duplicated and each replicate contained four pots. Eighteen seeds, each weighing more than 5 grams, were sown in each pot on 31 August 1956. Complete plants were successively removed from each pot at 60, 95, 131, 192 and 235 days after sowing. For the earlier samplings a minimum of three plants was removed from each pot until only one remained to be taken at the last sampling.

All the stems removed at the first two samplings were analysed, but for subsequent samplings the green portion only of each stem was used and the brown lower portion discarded. The brown portion was discarded in order to avoid the inclusion of an increasing proportion of non rubber bearing tissue in the material to be analysed and the consequent lower values which would be obtained for the rubber concentration values. The stem material was analysed for rubber (MEEKS, CROOK, PARDO & CLARK 1953), phosphorus, nitrogen, magnesium, potassium and calcium (BOLLE-JONES, MALLIKARJUNESWARA & RATNASINGAM 1957); the total dry weight per plant per replicate was also recorded.

Results

Plants which received the P_1 nutrient showed typical visual symptoms of phosphorus deficiency (BOLLE-JONES, 1956) in March; their growth became thin and retarded. Plants grown at the highest phosphate level (P_5 treatment) appeared paler and smaller than those of the P_3 treatment, which roughly corresponded to the phosphate level required for normal growth; but towards the end of the experiment they appeared to gain in relative vigour. Increased phosphate level caused an increased proportion of the lower leaves to develop a yellow mottling, especially near the tips and edges. In general, the heaviest dry matter production was obtained for plants grown at the P_3 level. Plants grown at the higher phosphate level (P_3 and P_4) usually produced a smaller dry weight during the period covered by this experiment.

The concentration of phosphorus in the stem increased with applied phosphate level. The concentration of rubber in the stems increased with increases in the stem phosphorus concentration up to and including samples taken 131 days after sowing (*Figure 1*). Later samplings showed that the concentration of rubber in the stems of the P_2 and P_3 plants were smaller than for the P_1 plants. Examination of the relevant graphs shows that a decrease in stem rubber concentration was usually, but not always, accompanied by an increase in the dry weight of the plant. A retarded growth, as was apparent in the phosphorus deficient plants, produced an increase in the stem rubber concentration. However, the beneficial effect of phosphate level on rubber concentration during the earlier samplings, cannot be solely attributed to the increased concentration of phosphorus found in the stems as concomitant increases in nitrogen, magnesium, potassium and calcium concentrations were also recorded (Table 1).

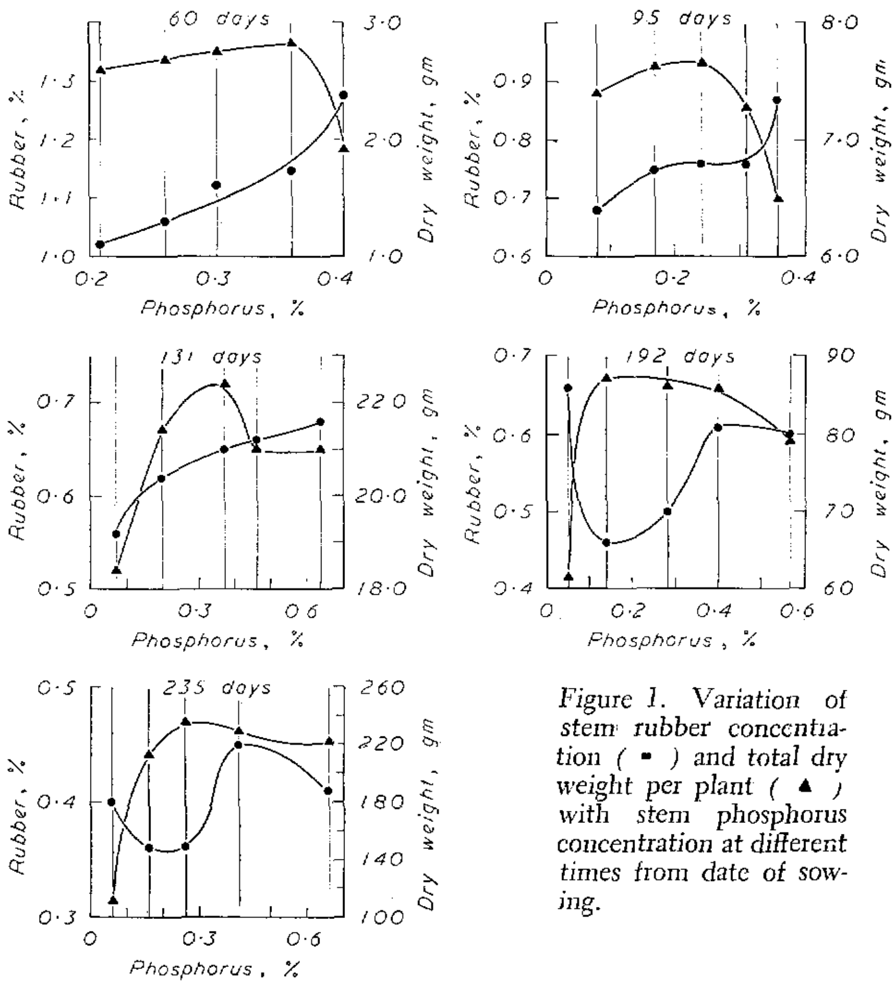

Figure 1. Variation of stem rubber concentration (•) and total dry weight per plant (▲) with stem phosphorus concentration at different times from date of sowing.

The general relationship found between stem rubber concentration and plant dry weight is represented in *Figure* 2. An increase in plant dry weight markedly reduced the concentration of rubber found in the green stems. This form of presentation requires qualification as all the values represented were not obtained at any one sampling but they were taken at different sampling times. However, the graph does serve to show that increased dry weight per plant was accompanied by a diminution of the concentration of rubber in the stems.

Conclusion

The results fully confirmed the tentative inferences which could be deduced from the literature discussed in the introduction to this paper. They proved that in the earlier stages of growth, during which the mineral nutrient treatments had not promoted any marked differences in terms of dry weight per plant, improved nutrient status increased the concentration of the rubber found in the tissue. This increased rubber

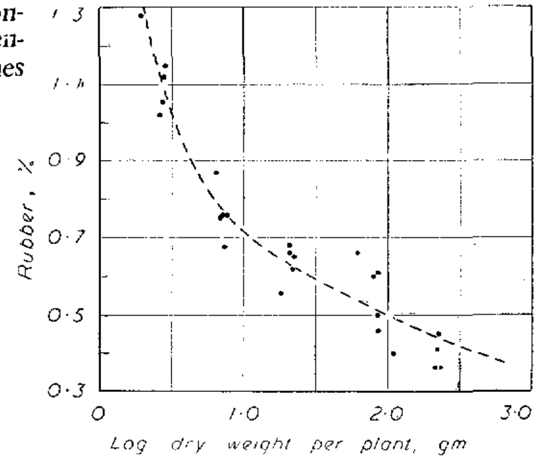

Figure 2. Illustration of general relationship observed between stem rubber concentration and total dry weight of plant; values derived from seedlings of different ages.

concentration could not be specifically related to an increased concentration of any one nutrient element. Once large plant dry weight differences became obvious the stem rubber concentration did not increase with increased phosphate level but rather the reverse. Any factor which tended to limit or retard growth, such as deficient or excessive phosphate supply, increased the concentration of rubber found in the stem.

TABLE 1. MEAN CONCENTRATIONS OF NITROGEN, MAGNESIUM, POTASSIUM AND CALCIUM IN GREEN PORTION OF STEMS OF DIFFERENT AGES

Age	60 days				95 days				131 days			
Treatment	N	Mg	K	Ca	N	Mg	K	Ca	N	Mg	K	Ca
P_1	1.38	0.14	0.82	0.37	0.99	0.16	0.70	0.48	0.91	0.20	0.92	0.62
P_2	1.34	0.14	0.88	0.42	1.12	0.17	0.88	0.51	1.24	0.27	1.14	0.67
P_3	1.29	0.14	0.90	0.40	1.12	0.19	0.92	0.54	1.20	0.28	1.35	0.65
P_4	1.32	0.14	0.87	0.48	1.18	0.20	0.96	0.56	1.18	0.28	1.34	0.66
P_5	1.49	0.16	0.93	0.51	1.20	0.19	0.91	0.58	1.32	0.31	1.56	0.73

Age	192 days				235 days			
Treatments	N	Mg	K	Ca	N	Mg	K	Ca
P_1	0.85	0.22	0.58	0.52	0.85	0.23	0.66	0.62
P_2	1.08	0.34	0.94	0.60	1.16	0.32	0.90	0.66
P_3	1.14	0.36	1.13	0.58	1.12	0.32	0.78	0.66
P_4	1.25	0.28	1.27	0.58	0.94	0.32	1.04	0.70
P_5	1.13	0.42	1.24	0.58	0.96	0.38	1.20	0.70

Values expressed as % of dry matter

Experimental

Tjirandji 1 rubber seedlings were grown in acid washed sand; four different nutrient treatments were applied:

1 Complete nutrient (Compt.);
2 Nutrient solution free of all the micronutrient elements (– mn);
3 Phosphorus free nutrient (– P);
4 Water only (H_2O).

All treatments were duplicated and each replicate contained three pots in each of which twenty seeds were sown. One such set of replicated treatments was placed in the light and another in the dark. The sand (particle size 0.5 to 1.2 mm) was purified by boiling with a mixture of hydrochloric acid (4% w/v) and oxalic acid (1% w/v) for seven hours. After leaching free of residual acid, the sand was contained in five gallon bitumen painted clay pots. The complete nutrient solution, on which treatments 2 and 3 were also based, possessed the following composition (in milligram equivalents per litre): NO_3^- 5, NH_4^+ 3, SO_4^{--} 7.5, PO_4^{---} 3, K^+ 3, Mg^{++} 2.5, Ca^{++} 4, Na^+ 1, Fe^{+++} 0.66, Mn^{++} 0.02, BO_3^{---} 0.067, Cu^{++} 0.002, Zn^{++} 0.002, Mo(hexavalent) 0.001, Al^{+++} 0.001, Ni^{++} 0.0002, Co^{++} 0.0002, Ga^{+++} 0.0002.

The solutions containing the macronutrient elements were purified of micronutrient elements by complexing them with alpha-nitroso beta-naphthol and adsorption of the complexes on carbon followed by extraction with dithizone in carbon tetrachloride. Iron, as acidified ferric chloride, was repeatedly extracted with ether, prior to conversion to ferric citrate. Demineralised water was used to supply the plants grown in the – mn and H_2O treatments and tapwater for the remainder. Seedlings grown in the dark received a daily eight hour illumination of two footcandle intensity from an artificial source, whereas those grown in the light, actually light shade, received about 800 foot candles. The seeds, each heavier than 5 grams, were sown in each pot on 31 August 1956. Seedlings, were removed from each pot at 31, 47, and 66 days after sowing. The seedlings, each of which was removed whole, were killed, dried at 55 to 60°C, milled and then analysed for rubber, hydrolysable carbohydrate, crude fibre, and protein (including free amino acids).

At the first sampling the seed residues were separated from the seedlings and both were analysed separately. The seed residues of the plants not sampled were also excised at the first sampling (and discarded) to facilitate the convenient interpretation of the results. Samples of the original seed supply as well as the seed residues were analysed for their fat content as well for the other fractions mentioned. All the results are expressed as contents of the constituent or fraction per seed, seed residue or seedling.

Rubber — This was determined by a method based on that of MEEKS, CROOK, PARDO & CLARK (1953).

Hydrolysable carbohydrates — These, including the 'hemicelluloses', were estimated as follows. One gram of dried plant material was hydrolysed with 50 ml of 1 N hydrochloric acid at 70 to 75°C for a period of 3½ hours. An aliquot of the filtrate was deproteinised and cleared with zinc sulphate and barium hydroxide solutions before its reducing sugar content was estimated in the usual manner with a copper sulphate and tartarate reagent. A set of invert sugar standards was always determined alongside every set of unknowns. The results are expressed as milligrams of invert sugar per seedling, seed or seed residue.

Crude fibre — The hydrochloric acid insoluble residue obtained during the estimation of the hydrolysable carbohydrates was boiled with 50 ml of 1.25% (w/v) sodium hydroxide for thirty minutes. The crude fibre value was obtained as the difference between the weights of the insoluble fraction before and after ignition; this value was divided by 0.9 so that the results could be expressed as milligrams of invert sugar.

Total carbohydrate — This value was obtained by summing the values for the hydrolysable carbohydrate and crude fibre fractions.

Protein content — An estimate which included any free alpha-amino acid originally present in the material was obtained after the hydrolysis of the plant material by estimation of the alpha-amino nitrogen with a ninhydrin reagent. Half a gram of the plant material sample was hydrolysed with 10 ml of 8 N hydrochloric acid at 80 to 85°C for eight hours. The solution was cooled and made up to 100 ml with water and then filtered. A 1 ml aliquot was taken, ninhydrin and other reagents were added, and the resultant colour was measured absorptiometrically. A description and preparation of the respective reagents and the mode of colour development were described by WIGGINS & WILLIAMS (1955). A series of glycine standards was run with each batch of determinations. Glycine was chosen for the standard as it was found in our preliminary trials that six out of the nine acids tested gave ninhydrin complexes whose absorption was close to that of glycine. To obtain a protein value for the plant material sample the concentration of alpha-amino nitrogen was multiplied by 6.25 and the results were expressed as milligrams of proteins (including free amino acids) per seedling, seed or seed residue.

Fatty oil — An estimate of the content of seeds and seed residue was obtained by soxhlet extraction of the powdered sample with dry ether for eight hours. The ether solution was removed, the ether evaporated and the residual fatty oil weighed after heating to a constant weight at 100°C. Carbohydrates were later estimated on the ether extracted seed and seed residue material.

Results

The plants grown in the dark became drawn and etiolated while those deprived of all nutrients, but grown in the light, also appeared pale and nitrogen deficient. Although no symptoms which could be attributed to a mineral deficiency were noted, the Compt and – P plants were, at the six weeks stage, 10 to 20% taller than those of the other treatments; that is, a lack of the micronutrient elements produced shorter plants.

Dry weight and analytical data for the third sampling were incomplete, owing to the lack of plant material for the H_2O (light), – mn (dark) and H_2O (dark) treatments. The dry weights of the dark grown plants varied little over the duration of the experiment but those of the light grown plants increased with time, according to mineral nutrient treatment. The effect of light on plant dry weight was greater than that of the nutrient treatment and was significant even at the earliest sampling whereas the nutrient treatments did not show significance until the second sampling (Table 2). The dry weights of the Compt. and – P plants were greater than those of plants grown in the – mn and H_2O treatments. Similar trends were observed for the rubber, hydrolysable carbohydrate, crude fibre, total carbohydrate, and protein contents, per plant. More of each constituent was found in plants of the same mineral nutrient treatment grown in the light than in the dark.

The rubber content became reduced in plants grown in the – mn and H_2O treatments (Table 2); this reduction was significant in the – mn (light) plants at the third sampling. The hydrolysable carbohydrate, but not the crude fibre content, was affected by nutrient treatment at the first sampling (Table 3). Plants which did not receive phosphate (– P and H_2O treatments) contained more hydrolysable carbohydrates than *those which did. A similar effect was noted for the third sampling.* Much less crude fibre was found in the plants of the – mn and H_2O, than in other treatments at the second and later samplings. The total carbohydrate content was, for the second sampling, least in plants grown in the – mn treatment; values for plants given the H_2O treatment were also much reduced. Plant protein content was not significantly influenced by mineral treatment at the first sampling (Table 2). Later for the second

TABLE 2. DRY WEIGHT, RUBBER AND PROTEIN CONTENTS PER PLANT

Dry weight, grams

Sampling time	Light or dark	Nutrient treatment					Min. 5% sig. diff.
		Compt.		– P		– mn	H_2O	
31 days	L	1.14		1.25	(±0.050)	1.25	1.21	0.169
	D	0.82		0.98		0.76	0.81	
47 days	L	1.63		1.50	(±0.065)	1.14	1.26	0.218
	D	0.90		0.90		0.58	0.70	
66 days	L	2.80		3.30	(±0.147)	2.17	—	0.893
	L	2.80	(±0.055)	3.30		—	—	0.248
	D	1.04		1.04		—	—	

Rubber, milligrams

Sampling time	Light or dark	Nutrient treatment						Min. 5% sig. diff.
		Compt.		– P		– mn	H_2O	
31 days	L	10.3		10.3	(±0.35)	11.0	10.5	1.16
	D	7.5		8.0		7.7	7.6	
47 days	L	13.4		13.1	(±1.04)	10.9	11.4	3.48
	D	9.1		8.9		6.7	7.5	
66 days	L	22.4		24.2	(±0.28)	19.5	—	1.72
	L	22.4	(±1.17)	24.2		—	—	5.27
	D	11.8		10.7		—	—	

Protein, milligrams

Sampling time	Light or dark	Nutrient treatment						Min. 5% sig. diff.
		Compt.		– P		– mn	H_2O	
31 days	L	81		84	(±3.7)	87	77	12.3
	D	57		64		50	53	
47 days	L	112		110	(±5.5)	85	73	18.4
	D	75		74		46	53	
66 days	L	201		193	(±16.9)	149	—	103.0
	L	201	(±3.1)	193		—	—	13.8
	D	84		83		—	—	

sampling, it was found that the absence of the micronutrient elements from the nutrient significantly decreased the plant protein content.

For the first and second samplings, there was no evidence of any interaction between light and mineral nutrient treatments. For the third, and incomplete, sampling there

TABLE 3. HYDROLYSABLE CARBOHYDRATE, CRUDE FIBRE AND TOTAL CARBOHYDRATE CONTENTS PER PLANT

Hydrolysable carbohydrate, miligrams

Sampling time	Light or dark	Nutrient treatment: Compt.		– P		– mn	H_2O	Min. 5% sig. diff.
31 days	L	214		259	(±9.2)	237	300	30
	D	148		201		150	197	
47 days	L	335		289	(±17.4)	203	304	58
	D	137		126		88	111	
66 days	L	484		661	(±8.2)	419	—	50
	L	484	(±10.3)	661		—	—	46
	D	108		127		—	—	

Crude fibre, milligrams

Sampling time	Light or dark	Nutrient treatment: Compt.		– P		– mn	H_2O	Min. 5% sig. diff.
31 days	L	275		289	(±12.4)	309	277	41
	D	184		190		157	169	
47 days	L	429		403	(±20.0)	325	330	66
	D	248		246		160	180	
66 days	L	837		880	(±36.3)	534	—	221
		837	(±25.0)	880		—	—	112
	D	322		314		—	—	

Total carbohydrate, milligrams

Sampling time	Light or dark	Nutrient treatment: Compt.		– P		– mn	H_2O	Min. 5% sig. diff.
31 days	L	490		548	(±20.0)	546	577	67
	D	333		391		307	366	
47 days	L	764		693	(±31.7)	528	633	106
	D	386		372		248	281	
66 days	L	1322		1541	(±44.5)	954	—	271
	L	1322	(±33.5)	1541		—	—	151
	D	430		441		—	—	

was some evidence that a beneficial effect due to the – P treatment on hydrolysable carbohydrate content and plant dry weight was obtained in the light but not in the dark.

From the standpoint of interest and record, rather than for the direct purpose of this paper, the significant effects of light and mineral treatments on the dry matter, rubber and other fractions within the seed are shown in Table 4. Neither light nor nutrient treatments influenced the amount of rubber and total carbohydrates removed from the seed by the seedling. There occurred a greater loss of dry matter per seed, and a greater translocation of protein and fatty oils from the seed to the seedling in the light than in the dark. For each of these variables the main effects of nutrient application were observed in the dark treatments. In general, dry weight losses and removal of protein and fatty oils from the seed were much lower in the – mn and H_2O, than other, treatments.

Correlationships between different components and rubber

When all the available values, regardless of whether they were obtained from light or dark grown plants, were considered, correlationship coefficients significant at the 0.1% level were obtained between rubber and each of the following: dry weight of

TABLE 4. AMOUNTS OF DRY MATTER, PROTEIN AND FATTY OIL LOST OR TRANSLOCATED FROM SEED TO SEEDLING

Seed residues excised 31 days after sowing

Fraction	Light or dark	Nutrient treatment					Min. 5% sig. diff.
		Compt.	– P		– mn	H_2O	
Dry matter (gm)	L	1.19	1.26	(±0.034)	1.24	1.12	0.114
	D	1.04	1.05		0.86	0.82	
Protein (mg)	L	82	95	(±3.2)	87	77	10.9
	D	75	71		59	53	
Fatty oil (gm)	L	0.57	0.61	(±0.026)	0.56	0.55	0.087
	D	0.45	0.48		0.27	0.28	

TABLE 5. CORRELATION COEFFICIENTS BETWEEN RUBBER CONTENT AND DRY WEIGHT OF PLANT, HYDROLYSABLE CARBOHYDRATE, CRUDE FIBRE, TOTAL CARBOHYDRATE AND PROTEIN CONTENTS PER PLANT

r = correlation coefficient *y = rubber (mg)*

Rubber (as y) correlated with:	r values		Light and dark	
	Light	Dark	r values	Regression equation
x, plant dry weight (gm)	0.946 ***	0.847 ***	0.960 ***	y = 2.46 + 7.12x
x, hydrolysable carbohydrate (mg)	0.917 ***	–0.184 NS	0.900 ***	y = 3.85 + 0.032x
x, crude fibre (mg)	0.932 ***	0.956 ***	0.956 ***	y = 3.45 + 0.025x
x, total carbohydrate (mg)	0.943 ***	0.849 ***	0.956 ***	y = 3.21 + 0.015x
x, protein (mg)	0.845 ***	0.926 ***	0.901 ***	y = 2.09 + 0.108x

*** significant at the 0.1% level NS not significant plant, hydrolysable carbohydrate, crude fibre, total carbohydrate and protein contents (Table 5). If, however, the values were segregated into light and dark treatments all but the hydrolysable carbohydrates maintained this high positive correlation. The rubber and hydrolysable carbohydrate values obtained for the dark grown plants yielded a negative correlationship which was not significant, but corresponding values for the light grown plants allowed of a significant positive correlationship to be obtained.

GENERAL DISCUSSION

The results showed that under certain conditions mineral nutrient status could influence the concentration of rubber found in the tissues but this response was in all probability an indirect one and mediated through an effect on factors which influenced growth and rubber production. Even where increased stem rubber concentration could be related to improved phosphate supply (*Figure 1*) the effect of this mineral nutrient could hardly be considered specific as its application caused an increase in the stem concentrations of other nutrient elements besides phosphorus (Table 1). Any factor which limited growth, such as deficient or excessive phosphate supply, increased the concentration of rubber found in the stems. Part of the reason why an increased dry weight per plant was accompanied by diminished stem rubber production may have been an increased proportion of non rubber bearing tissues. However, attempts were made to minimise this factor by the selection of the green portion of the stems for the purpose of analysis and the avoidance of the cork thickened brown stem portion. It was also known that the inverse relationship between dry weight and rubber concentration applied not only to stems but also to laminae; thus when the nitrogen concentration of the laminae and dry weight of the plant were found to increase, the rubber concentration of the laminae decreased (BOLLE-JONES 1955). It seemed that the proportion of rubber to plant dry matter might decline if growth was vigorous and that a constant bulk proportionality did not necessarily exist. This statement does not, however, preclude the existence of a rigid proportionality in the ratio of rubber to dry matter formed during the initial stages of growth of any one tissue. It may indicate that under conditions of restricted growth, due for example to a nutrient deficiency, the metabolism of the dry matter (perhaps carbohydrate) may be greater and this, not being replenished, makes the concentration of rubber in the tissue appear higher.

The second experiment described clearly revealed the paramount influence of light not only on the dry weight of the plant but on all other fractions examined (Table 2 and 3). The beneficial effects of light on rubber, crude fibre and protein contents were highly significant at the first sampling and the coresponding effects of mineral nutrient treatments were insignificant. These results might suggest that the formation or carbohydrates preceded the formation of rubber or that light was necessary for rubber to be formed. Our present findings indicated tentatively that light, per se, was not required for rubber formation as the rubber content per plant continued to show a slight increase under the 'dark' conditions of our experiment. However this point can be fully resolved by estimating the rubber contents of plants which are kept in the dark and fed with sugars; there is already some evidence to show that the rubber content would increase as a result of such treatment (J. S. LOWE, unpublished data).

If rubber is assumed to be formed directly from carbohydrates the correlationship coefficients (Table 5) demonstrate that it is more likely to be associated with the crude fibre than with the hydrolysable carbohydrate fraction. Other data (Table 3) indicate that although the mineral nutrient treatments significantly affected the hydrolysable carbohydrate fraction at the first sampling they did not cause a parallel variation in rubber content. On a priori grounds, if rubber was formed from carbohydrates it would be expected that the relationship would be closest to the hydrolysable carbohydrate rather than to the crude fibre fraction. If rubber is to be regarded as a plant food reserve, as has been suggested (SPENCE & McCALLUM 1935, TEAS 1956), it might be expected not only to show a close relationship to carbohydrate content but also to diminish under conditions of carbohydrate depletion. No such marked diminution was observed by DE HAAN & VAN AGGELEN-BOT (1949) or under the mild starvation conditions of the experiment described here.

We favour the theory that rubber is formed as a by-product of growth, perhaps as a result of anaerobic respiration (RITTER 1954); this allows us to reconcile the close relationships between rubber and protein and rubber and plant dry weight as well as the relationship of rubber to carbohydrate content (Table 5). As a waste by-product, it would be expected that rubber would be formed irreversibly from its precursor and therefore, under conditions of starvation, would not be metabolised within the plant. To determine whether rubber is derived from protein like. or carbohydrate like, fractions may involve future experiments in which the effects of nitrogen deficient nutrient solutions (with and without sucrose) on plant rubber content are studied.

The practical conclusion to be drawn from these present observations is that the formation of rubber in *Hevea brasiliensis* is intimately connected with growth. Any nutritional deficiency which impedes the optimal formation of carbohydrates and proteins within the plant will diminish the amount of rubber formed per plant. Of great interest, was the marked effect of micro-nutrient element supply on the plant's wellbeing. The absence of the essential traces of these elements from the nutrient solution decreased the dry weight of the plant and its carbohydrate and protein contents. As these are closely related to the amounts of rubber formed in the plant, it seems that the fulfilment of the requirement of *Hevea brasiliensis* for micronutrient elements may be an important factor in the realisation of optimal rubber yields under field conditions and they are worthy of further attention. It was also noted that seedlings grown in the dark and supplied with micronutrient elements were able to withdraw significantly higher amounts of proteins and fatty oils from the seed than those which were not so supplied.

SUMMARY

Seedlings of *Hevea brasiliensis* were grown in sand and supplied with nutrient solutions in experiments to determine the effect of light and mineral nutrient treatments on the formation of rubber in young plants. During the first four months of growth the concentration of rubber in the green portion of the stems increased with improved phosphate supply. This increased concentration was not specifically related to an increased concentration of phosphorus in the tissues as concentrations of other elements also increased.

Once large plant dry weight differences between treatments occurred, the direct relationship between the concentration of rubber and phosphorus in the stem disappeared and any factor which retarded growth increased the concentration of rubber in the stem.

The rubber content of the plant was closely correlated with its dry weight, carbohydrate and protein contents. As light intensity exerted a greater effect on these values than did nutrient status, variation in the latter was of lesser importance than light as a factor in the formation of rubber.

The effect of mineral nutrient status on rubber formation was an indirect one and was concerned with the role of the nutrient elements in the formation of either carbo-

hydrates, proteins or of plant dry matter. Of great significance was the apparent importance of traces of micronutrient elements in this formative role.

The interpretation of the results was facilitated if rubber was regarded as a by-product of growth rather than as a food reserve.

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