Foliar Diagnosis of Mineral Status of Hevea in Relation to Bark Analysis

E. W. BOLLE-JONES

Leaf sampling and diagnosis procedures are discussed and compared with results obtained by the analysis of bark for the assessment of the response of mature rubber trees to fertilisers. Significant correlationships were established between the bark and lamina concentrations of many of the nutrient elements investigated. Because of the difficulties of leaf sampling it is suggested that bark composition may become a means of evaluating the mineral nutrient status of mature trees. A diurnal variation in the concentration of magnesium is shown to occur in the laminae.

THE CHEMICAL COMPOSITION OF LEAVES, which readily show symptoms of nutritional disorders, may be employed as a method of diagnosis of mineral status; comparison is made with values obtained for leaves which either exhibit known deficiency symptoms or are healthy. To apply this approach towards the estimation of the nutrient status of a crop grown in the field requires a standardisation of sampling positions and samples which are representative of the area under examination. For young rubber plants, up to the unbranched stage, sampling is relatively easy as the leaves are found in well defined storeys, the uppermost three of which are separately sampled and combined with corresponding storeys from other plants which also contribute towards the 'bulk' sample used for analysis. This method of sampling, based on storey position, automatically takes into account the relative maturity of the leaves for plants similar in regard to age and growing conditions. Nevertheless, to make a diagnosis based on the analyses of such samples requires some measure of experience as storey or whorl position markedly influences the concentration of the foliar constituents, the effect of storey position on chlorophyll and certain minerals may vary according to the time of year, and for some mineral nutrients significant storey × treatment interactions are obtained (BOLLE-JONES 1954b, BOLLE-JONES & RATNASINGAM 1954).

Little is stated in the literature on the sampling of hevea leaves but a statement (CHAPMAN 1941) which indicated that a diurnal variation of foliar constituents occurred and that it was necessary to carry out leaf sampling at fixed times, between sunrise and 10.30 hours. This statement was not supported by experimental evidence and it was thought necessary to confirm its validity. The first section of this paper examines the variation, during the course of the day, of the mineral nutrients within the leaves of young hevea plants. It was found, for nitrogen, phosphorus, potassium, calcium and manganese, that there was no evidence of any significant variability associated with the time of day. However, the foliar magnesium concentration showed a significant diurnal variation; the values fell markedly after midday but increased towards evening. Diurnal changes also occurred in the iron concentration but they were not generally consistent from day to day. It seemed that the best time to sample leaves was before 10.00 hours. It was assumed that a similar variation occurs in mature trees.

After this stage in the investigations had been reached it was concluded that a reasonably satisfactory method of diagnosis of mineral status was available for young hevea trees up to the one year old stage. Thus it was preferable to take leaf samples in the

COMMUNICATION 310

forenoon and they should be taken according to storey position. The number of leaves which comprised any one sample was not less than forty and they were derived from a minimum of five trees which number could be increased according to the area being assessed and the number of replicates required for analyses. Samples were taken from healthy and affected areas and the diagnosis took into consideration any visual symptom apparent in the unhealthy leaves. A knowledge of these symptoms (BOLLE-JONES 1956) and the changes in foliar chemical composition which accompany their appearance, has proven a valuable aid in the assessment of mineral nutrient status of hevea plants.

However, the application of foliar diagnosis techniques to tall mature trees posed many more difficult problems, such factors as: the height of the sampling position above ground, the part of the crown to be sampled in regard to sun and shade positions, the ages of individual leaves and trees, and lastly the inaccessibility of the selected leaves. CHAPMAN (1941) sampled from spur shoots of limited growth on the tree; the largest leaf was taken from each of two well developed storeys 'high up in the light and on opposite sides of the tree'. Probably many trees were sampled in such fashion and all leaves bulked before analysis. BEAUFILS (1955) adopted a similar system and sampled the three most mature leaves of any one shoot of limited growth; these 'boughs are located inside the crown and grow generally on main or primary limbs.' About 40 leaves, taken from a minimum of 15 trees comprised one sample and all sampling was carried out between 07.00 and 10.30 hours. Apart from the manifest difficulty in choosing such few representative leaves out of the many hundreds present in any area to be examined, there is the hazard inherent in their collection* and the long time required to collect even one sample. For such reasons, this method of assaying the nutrient status of mature trees has never been favoured by the author but compulsorily adopted in the absence of any other well investigated alternative. Two possibilities presented themselves: the use of the bark composition as a method of diagnosis, and to relate mineral nutrient status to the composition of the latex. The former has been investigated and the results follow. Examination of the analyses of leaf and bark samples from field manurial trials showed that, in many respects, analysis of bark gave as good an indication of plant nutrient status as did that of leaves. The ease and rapidity of bark sampling and its amenity to standardisation may lead to its adaptation for the assessment of mineral status of mature trees.

DIURNAL VARIATION IN CONCENTRATION OF NUTRIENT ELEMENTS IN LAMINAE

Method of Investigation

It was planned to analyse leaves sampled at 2 hourly intervals, between 06.00 hours and 22.00 hours, from young nursery grown plants. It was anticipated that the amplitude of any diurnal variation would be small so that any attempt at its detection would require well standardised conditions. As all the samples could not be killed and oven dried immediately after being taken, the technique must allow for the leaves to be stored for some period of time, without deterioration, before being finally oven dried. If, for instance, the leaves were to be removed from the trees at 16.00 hours and left until the following morning before being killed, some loss in dry weight might occur, due to respiration, and the mineral concentration values obtained might appear higher than they actually were. One technique, which appeared promising and convenient to operate, was to kill the leaf tissue by dipping it in liquid air and then to store the killed tissue in a refrigerator until such time as it could be oven dried. To test the validity of such a procedure the following trial was carried out.

* CHAPMAN (1941) stated, in regard to leaf sample collection: 'This work was carried out by gangs of coolies who were directed from the ground when cutting spurs'.

Eight plants of *Hevea brasiliensis* clone RRIM 604, not more than a year old and as much alike as possible, were selected; they were grouped into 4 pairs. The terminal bud was quiescent and leaves of the top storey were mature and fully expanded. All the leaves of the top storey were removed, for analysis, from each pair, which was regarded as one unit or replicate; the first replicate was sampled at 08.15 hours and the subsequent ones at 08.45 hours, 09.15 hours and 09.45 hours. Immediately after all the top storey leaves were removed the midrib and petiole were cut out and discarded and the residual lamina, of each leaf, was quartered. All the quarter sections of lamina for one replicate were mixed and then placed at random in one of 4 beakers. The first beaker — treatment W — was immediately placed in the oven and its contents dried at 80°C in a current of air. The second beaker — treatment X — contained liquid air and the lamina sections were frozen almost immediately on being placed in the beaker, the beaker was then transferred to an oven and the lamina material dried as for treatment W. The third beaker — treatment Y — also contained liquid air; the lamina sections were frozen and the beaker subsequently placed in a refrigerator for 6 hours before its contents were oven dried. The fourth beaker — treatment Z — also contained liquid air in which the lamina sections were frozen; the beaker was kept in a refrigerator for a further 24 hours before its contents were oven dried.

TABLE 1. EFFECT OF FREEZING AND SUBSEQUENT DRYING ON CONCENTRATION OF NUTRIENT ELEMENTS IN LAMINAE OF HEVEA BRASILIENSIS

Clone RRIM 604 top storey laminae. Results expressed in terms of dry matter of laminae.
W *immediately oven dried*
X *frozen, then dried*
Y *frozen, stored in refrigerator for 6 hours, then dried*
Z *frozen, stored in refrigerator for 24 hours, then dried*

Treatment	N%	P%	Mg%	K%	Ca%	Fe p.p.m.	Mn p.p.m.
W	3.03	0.24	0.22	1.11	0.64	76	89
X	2.95	0.24	0.23	1.15	0.65	79	88
Y	2 94	0.24	0.23	1.16	0.65	75	90
Z	2.94	0.22	0.24	1.15	0.67	73	90
Min. 5% sig. diff.	0.08	0.01	0 01	0.05	0.02	6	7

When dried, each sample was ground and analysed according to methods previously described (BOLLE-JONES 1954a); the results are summarised in Table 1. It appeared that there was no significant difference between the effects of treatments X and Y on the concentration of all the nutrient elements examined. For nitrogen, magnesium, potassium, manganese and iron this statement could be extended to include treatment Z. However, the phosphorus and calcium concentrations of samples subjected to treatment Z differed significantly from those of treatments X and Y. Treatment W gave different results from X, Y and Z for nitrogen and potassium.

In view of these results, it was decided to freeze all laminae sampled during the investigation of diurnal variation and to oven dry as soon as convenient after freezing. This method would ensure that the results were not influenced by technique as far as

Table 2. Diurnal Variation in Concentration of Nutrient Elements in Laminae

Top storey laminae sampled at intervals of 2 hours on three separate days; Tjir 1 plants sampled on 22 October and Clone RRIM 604 on 18 and 19 December 1952. Triplicate samples taken at each sampling time. Results expressed in terms of dry matter of laminae.

ANALYSIS OF VARIANCE: MEAN SQUARES

Source	d.f.	Nitrogen	Phosphorus	Magnesium	Potassium	Calcium	Iron	Manganese
Days	2	0.8020	0.00108	0.00065	0.0525	0.6043*	13220***	7257.0
Times (of sampling)	8	0.0106	0.00025	0.00043***	0.0019	0.0051	859	76.6
Days x times	16	0.0069	0.00031	0.00008	0.0030	0.0058	734***	43.1
Replicates within days	6	0.1654***	0.01445***	0.00229***	0.3456***	0.0785***	229**	1473.6***
(Replicates x times) within days	48	0.0073	0.00042	0.00006	0.0029	0.0045	66	37.3
Pooled error	64	0.0072	0.00039	0.00006	0.0029	0.0048	—	38.8
Coefficient of variation (%)		2.6	9.1	4.2	4.5	8.3	8.6	6.4

* 5% sig. **1% sig. *** 0.1% sig.

MEAN VALUES

Time	N%	P%	Mg%	K%	Ca%	Fe p.p.m.	Mn p.p.m.
6.00 hours	3.28	0.22	0.20	1.23	0.83	114	102
8.00 hours	3.33	0.23	0.19	1.21	0.81	103	99
10.00 hours	3.29	0.22	0.19	1.20	0.86	101	101
12.00 hours	3.26	0.22	0.19	1.21	0.83	93	96
14.00 hours	3.27	0.22	0.18	1.20	0.83	85	94
16.00 hours	3.23	0.22	0.18	1.21	0.80	86	95
18.00 hours	3.33	0.23	0.18	1.22	0.88	85	100
20.00 hours	3.31	0.23	0.19	1.19	0.85	91	98
22.00 hours	3.32	0.23	0.19	1.23	0.83	93	94
Min. 5% sig. diff.	0.08	0.02	0.01	0.05	0.07	27	6

nitrogen, magnesium, potassium, manganese and iron were concerned but that for phosphorus and calcium the results should be interpreted with caution. It subsequently ensued that during the examination of diurnal variation effects none of the lamina samples was left more than 14 hours before being oven dried. This was a period of 8 hours more than allowed in treatment Y but 10 hours less than treatment Z.

The examination of diurnal variation effects was carried out in the following fashion. Leaf samples were taken at 2 hourly intervals on three separate days: 22 October 1952, 18 December 1952 and 19 December 1952. Tjirandji 1 seedling plants were sampled on 22 October and RRIM 604 plants on the remaining two days; all plants were grown in an uniform nursery area and were about one year old. Sampling was carried out in triplicate; each sample was derived from two trees which appeared to be almost of identical appearance. Each tree possessed fully expanded uppermost storey leaves and the terminal bud was quiescent. From each member of a pair of trees, one leaf (that is, three leaflets) was removed at each sampling time from the topmost storey and combined for analysis — the sequence of leaf removal being consistently basipetal for one member of the pair and acropetal for the other. This procedure was executed at the following hours: 06.00, 08.00, 10.00, 12.00, 14.00, 16.00, 18.00, 20.00 and 22.00, so that 9 leaves were removed from the uppermost storey of each selected plant.

After sampling the petioles and midribs were discarded, the laminae frozen in liquid air and stored in the refrigerator until taken later for oven drying.

Results

Significant variability, according to time of day, did not occur in the concentrations of nitrogen, phosphorus, potassium, calcium and manganese found in the lamina (Table 2). However, the magnesium concentration showed a significant diurnal variation; the values fell after midday and increased again towards evening. The absence of a significant days × times interaction demonstrated that the diurnal change in magnesium concentration was similar on all three sampling days. Diurnal changes also occurred in the iron concentration but the variation was not consistent for each of the three days. On the first day there was a general decrease in the iron concentration as the day progressed, with the most marked decrease occurring between noon and 14.00 hours. On the second day an initial decrease occurred but thereafter the changes were less pronounced; the lower values occurred at noon and 14.00 hours. On the third day the diurnal changes were not significant. These diurnal changes are shown in Table 3.

TABLE 3. DIURNAL VARIATION IN CONCENTRATION OF IRON IN THE LAMINAE

For details see Table 2. Day x times (hours). Iron concentration, means (p.p.m.)

Clone and day	6.00	8.00	10.00	12.00	14.00	16.00	18.00	20.00	22.00	Day means
Tjir 1 on 22 October	160	151	136	135	104	97	97	97	102	120
RRIM 604 on 18 December	101	82	81	66	74	84	86	91	91	84
RRIM 604 on 19 December	82	75	86	77	78	76	72	84	86	80
Min. 5% sig. diff.					13					17

Other observations, made during the present study, indicated (Table 2) that the concentration of calcium in the lamina may vary according to the day of sampling. The same could not be said of iron concentration, which also showed a significant day

effect, as the data for the first day, which gave substance to this significance, was derived from different clonal material to that of succeeding two days. It has been shown elsewhere (BOLLE-JONES & RATNASINGAM 1954) that different clones are characterised by different iron concentrations in the laminae.

Conclusion

If consideration is given to the significant diurnal variation of magnesium concentration then the best practical time of day for leaf sampling is before 10.00 hours. Maximum concentration values are obtained up to this time limit and the drop of values soon after noon is avoided. Similarly for iron an early sampling will tend to yield higher values and avoid the afternoon drop. Taking all the nutrient elements, as estimated here, into consideration it can be seen (Table 2) that a sampling period from 06.00 to 10.00 hours is usually satisfactory and can be recommended for general use. Whenever possible this recommendation was followed.

COMPARISON OF BARK ANALYSIS WITH LEAF ANALYSIS AS A DIAGNOSTIC PROCEDURE

Methods of Investigation

When dealing with tree crops, sampling of leaves for diagnostic purposes may often present a difficult problem because of variations in chemical composition according to age of leaf, diurnal and seasonal fluctuations and, above all, the practical difficulty of collecting representative samples from tall mature trees. The possibility of assessing nutrient status of trees by analysing bark samples is considered below. The requirement was the practical one of determining responses to fertiliser addition by trees grown in manurial trials; it is hoped that the initial findings reported here may be extended to become a means of diagnosis of mineral status.

It was necessary, as a first step, to take samples of bark at varying heights to determine whether position of sampling influenced composition. Two 14 years old trees, grown from basal cuttings made from unselected seedlings, were used for this test and bark was removed at heights of 5, 10, 15, 20 and 25 feet above ground level. The bark was removed in slivers down to the wood. A similar method to that employed for later sampling trials was used and is described below. The composition of the bark samples as affected by height is given in Table 4.

TABLE 4. VARIATION OF BARK COMPOSITION ACCORDING TO HEIGHT OF SAMPLING POINT

Concentration values given in terms of oven dried bark

Height above ground	Ash%	N%	P%	K%	Ca%	Mg%	Mn p.p.m.
5 ft	11.4	0.41	0.042	0.85	3.77	0.12	74
10 ft	10.4	0.42	0.044	0.78	3.48	0.16	75
15 ft	11.2	0.44	0.046	0.93	3.68	0.20	80
20 ft	10.4	0.46	0.046	0.93	3.32	0.18	69
25 ft	10.1	0.50	0.053	0.84	3.25	0.19	69
Min. 5% sig. diff.	1.4	0.10	0.016	0.21	0.57	0.05	19

Ash, nitrogen, phosphorus, potassium, calcium and manganese concentration did not vary according to height. However, the magnesium concentration was significantly influenced by height of sampling but there was little indication of a simple relationship between the magnesium concentration and height. It was concluded that varying height was a relatively unimportant source of variation in the composition of the bark and it was decided to fix on an arbitrary and convenient height for all future investigations. The analysis of variance, which is not presented, also showed that replicate samples taken at different heights on the same tree did not provide more information than was obtained from one sample taken per tree; duplicate determinations on a given sample were also superfluous.

Three manurial trials, which formed part of an extensive programme of field experiments (OWEN, WESTGARTH & IYER 1957), and which had shown yield and girth responses to fertiliser application, were selected for these investigations. The trees were more than 15 years old. A maximum of 100 (Seaport Estate trial) and a minimum of 20 (Field 31 trial) trees, at a density of about 100 trees per acre, occurred per plot. Of this number, 5 trees only, which were centrally located and chosen to avoid poaching effects, were selected for leaf and bark sampling purposes; this number may be considered small but for a well replicated experiment, of the type examined, it proved to be adequate.

Sections of the bark, four inches by one inch, taken down to the wood, were removed at a height of 90 inches (Field 31 and Wardieburn Estate trials) or 100 inches (Seaport Estate trial) above the union. This sliver of bark (the 4 inches being in the vertical plane) was taken from the side of the trunk away from the tapping cut. On excision, the section was sprayed with 15% v/v formic acid solution, to coagulate the rubber, and then placed in clean cotton bags. The samples were oven dried, powdered and then analysed for the nutrient elements, according to methods previously described (BOLLE-JONES 1954a).

From each tree selected for sampling, approximately 20 leaves were removed, they were neither very small nor shade leaves and they were borne on the lower region of the spur or lateral branches. These branches were borne as high up in the crown as could be reached by an Indian boy climber, who was equipped with a very long handled knife. As far as possible, 10 leaves were picked on one side of the crown and 10 leaves on the opposite side; a total of 100 leaves per plot was, therefore, collected. These leaves were returned to the laboratory as soon as possible, the midribs excised and discarded, the laminae oven dried and then ground to a powder before mineral analysis.

Each manurial trial contained at least six fertiliser treatments, sometimes as many as twelve, and a minimum of two replicates was sampled. This meant, in practice, that a leaf and bark sampling of any one trial took four days. On the first day leaf samples were taken in the early morning; bark was sampled from the same trees on the following day. On the third morning the remaining replicates were leaf sampled to be followed by the corresponding bark sampling on the fourth day.

To collect one leaf sample (that is, leaves from 5 trees) required the attention of one field assistant and two boy climbers and took about one hour. Bark sampling (from the same number of trees) was much more rapid and was easily completed by one field assistant within half an hour.

Results

Field 31 Trial (Rubber Research Institute Experiment Station) — This experiment consisted of 6 manurial treatments in a latin square design; control, n, nk, np, pk and npk; the trees were budded in 1937 and were first tapped in 1947. Prior to the present

TABLE 5. FIELD 31 TRIAL

Concentration of nutrient elements in dried laminae and girth of trees at sixty inches measured in December 1954

TREATMENT MEANS

Treatment	Ash%	N%	P%	Mg%	K%	Ca%	Fe p.p.m.	Mn p.p.m.	Girth cm
(1) Control	4.14	3.19	0.15	0.23	1.18	0.49	96	114	82.2
n	4.04	3.02	0.15	0.19	0.97	0.58	107	93	76.5
np	3.96	3.22	0.18	0.22	1.19	0.39	91	77	89.2
nk	3.96	3.27	0.15	0.18	1.20	0.38	96	56	81.4
pk	3.94	3.15	0.18	0.22	1.08	0.49	100	76	86.9
npk	4.07	3.11	0.18	0.20	1.15	0.46	99	104	87.0
Min. 5% sig. diff.	0.53	0.27	0.03	0.05	0.30	0.19	16	75	7.5

MEAN EFFECTS

Effect	Ash	Nitrogen	Phosphorus	Magnesium	Potassium	Calcium	Iron	Manganese	Girth
N	−0.0338	−0.0160	−0.00125	−0.0275	−0.00125	−0.0388	0.125	−12.375	−1.038
P	−0.0431	−0.0119	0.03125**	0.0200	0.01625	−0.0269	−3.812	3.750	7.721**
K	−0.0481	0.0406	0.00125	−0.0200	0.02375	−0.0319	1.438	−16.875	−0.119
95% conf. limit	0.3263	0.1648	0.01938	0.0313	0.18657	0.1184	9.482	46.166	4.560
99% conf. limit	—	—	0.02680	—	—	—	—	—	6.306

Table 6. Field 31 Trial

Concentration of nutrient elements in dried bark and yield of rubber in grams per tree per tapping during October 1954 to February 1955

TREATMENT MEANS

Treatment	Ash%	N%	P%	Mg%	K%	Ca%	Fe p.p.m.	Mn p.p.m.	Rubber gm
(1) Control	6.05	0.51	0.030	0.12	0.88	1.66	46	174	106
n	6.29	0.54	0.030	0.12	0.64	1.90	53	159	84
np	5.73	0.58	0.042	0.12	0.97	1.48	54	147	150
nk	5.00	0.53	0.029	0.10	0.82	1.32	44	124	110
pk	6.12	0.54	0.046	0.10	0.88	1.72	48	134	121
npk	6.88	0.53	0.044	0.13	0.88	1.93	53	195	117
Min. 5% sig. diff.	2.17	0.07	0.008	0.04	0.27	0.92	17	93	50

MEAN EFFECTS

Effect	Ash	Nitrogen	Phosphorus	Magnesium	Potassium	Calcium	Iron	Manganese	Rubber
N	–0.1088	0.0206	–0.00212	0.0094	–0.04875	–0.0350	3.750	2.562	1.819
P	0.5288	0.0294	0.01375***	0.0088	0.13188	0.1081	5.438	10.688	31.519*
K	0.2012	–0.0206	0.00150	–0.0112	–0.01562	–0.0619	–4.812	–12.562	8.031
95% conf. limit	1.3302	0.0438	0.00520	0.0255	0.16351	0.5638	10.648	57.201	30.375
99.9% conf. limit	—	—	0.00995		—	—	—	—	—

sampling the respective fertilisers had been applied to each plot in 1947, 1949, 1951 and 1953. Tjirandji 1 trees grown in replicates 1, 2, 5 and 6, were sampled for the purposes of this investigation. Sampling was carried out in early December 1954. The yield of rubber per tree per tapping recorded for the period October 1954 to February 1955 showed a significant beneficial effect due to the application of fertilisers containing phosphorus. A similar significant improvement of girth measurement due to phosphate addition was detected in the girth measured in December 1954.

Summaries of the effects of fertiliser treatments on girth measurement, yield of rubber and mineral compositions of laminae and bark are given in Tables 5 and 6. The chief feature was that the mean effect of the phosphate fertiliser on the concentration of phosphorus in the tissues was equally reflected in bark as well as in lamina composition. It was also evident that whenever phosphate fertiliser had been included in any treatment, the concentration of phosphorus in the bark tissue showed relatively greater increases than those found in the laminae.

Wardieburn Estate Trial — This trial was laid out as $2 \times 2 \times 2$ factorial experiment in which two levels (with and without) of nitrogen–, phosphorus–, and potassium–, containing fertilisers were applied in all combinations, and with five replications. The Tjirandji 1 trees grown in this experiment were budded in 1939 and first tapped in 1946; each treatment received its respective fertiliser application in 1948, 1950 and 1952. Leaf and bark samplings were carried out on trees grown in replicates B and C of this trial towards the end of August 1955. Girth measurements taken at a height of 72 inches above the union and recorded in July 1955 showed a significant positive effect of phosphate application. The average yield of rubber per tree per tapping, recorded during the period June to October 1955, was significantly depressed whenever potassium had been applied, except for the npk treated trees; a very definite negative mean effect due to potassium was recorded. However, yield records recorded during the period September 1954 to August 1955 inclusive showed that phosphate addition had a significant beneficial influence on yield although this effect did not achieve significance for the average based on the more limited June to October 1955 period. Summaries of most of these effects and the mineral compositions of laminae and bark are given in Tables 7 and 8.

The mineral analyses showed that phosphate, not only favourably influenced girth and yield production, but also produced significant mean effects on the concentrations of phosphorus and calcium in the laminae and bark. A significant mean effect of phosphate application was also noted on iron and manganese concentrations in the laminae but not for bark, and vice versa in regard to ash. Consequently the bark and laminae did not mirror the mean effects of phosphate addition in the same way or to the same extent.

Where nitrogen fertiliser only had been applied a negative effect on the manganese concentration of both bark and laminae was produced but it was only the laminae which showed a significant positive mean effect on nitrogen concentration when all treatments were taken into consideration. Thus the laminae registered more sensitively the addition of nitrogen fertilisers than did the bark.

Neither bark nor lamina composition indicated any significant effect attributable to the addition of fertilisers containing potassium.

Seaport Estate Trial — This was a factorial experiment in which three levels of potassium were applied, with two replications. Trees of clone PB 25, which were budded in 1938, were grown in this trial; each fertiliser treatment was applied in 1951, 1953 and 1955, and the trees had been tapped since 1947. Leaf and bark sampling were carried out on trees of both replicates (that is a total of 24 plots) in early

Table 7. Wardieburn Estate Trial

Concentration of nutrient elements in dried laminae and girth of trees at seventytwo inches measured in July 1955

TREATMENT MEANS

Treatment	Ash%	N%	P%	Mg%	K%	Ca%	Fe p.p.m.	Mn p.p.m.	Girth cm
(1) Control	4.31	3.15	0.18	0.18	1.22	0.52	78	118	67.0
n	3.89	3.32	0.16	0.12	1.29	0.38	74	56	63.8
p	4.74	3.13	0.20	0.13	1.44	0.70	76	164	71.0
np	4.15	3.35	0.25	0.12	1.04	0.72	88	144	69.3
k	4.37	3.19	0.17	0.13	1.38	0.50	78	92	63.6
nk	4.16	3.38	0.18	0.12	1.40	0.40	76	98	64.0
pk	4.64	3.09	0.18	0.12	1.44	0.64	71	130	67.5
npk	4.82	3.22	0.22	0.19	1.45	0.67	96	132	69.7
Min. 5% sig. diff.	1.05	0.29	0.04	0.12	0.39	0.08	10	41	8.4

MEAN EFFECTS

Effect	Ash	Nitrogen	Phosphorus	Magnesium	Potassium	Calcium	Iron	Manganese	Girth
N	–0.2600	0.1775*	0.0192	0.0000	–0.0725	–0.0475*	7.625**	–18.50	–0.608
P	0.4075	–0.0600	0.0435**	0.0025	0.0225	0.2325***	5.875*	51.75***	4.768*
K	0.2250	–0.0150	–0.0075	0.0050	0.1700	–0.0300	0.875	– 7.50	–1.565
95% conf. limit	0.5229	0.1448	0.0214	0.0589	0.1950	0.0399	5.026	20.27	4.193
99% conf. limit	—	—	0.0316	—	—	0.0590	7.435	30.00	—
99.9% conf. limit	—	—	—	—	—	0.0912	—	46.33	—

TABLE 8. WARDIEBURN ESTATE TRIAL

Concentration of nutrient elements in dried bark and yield of rubber in tahils per tree per tapping during September 1954 to August 1955

TREATMENT MEANS

Treatment	Ash%	N%	P%	Mg%	K%	Ca%	Fe p.p.m.	Mn p.p.m.	Rubber (tahils)
(1) Control	5.84	0.50	0.041	0.058	0.71	1.70	50	190	0.82
n	3.89	0.56	0.034	0.034	0.69	0.98	46	146	0.75
p	6.54	0.50	0.058	0.034	0.88	1.90	54	188	0.76
np	6.56	0.54	0.058	0.016	0.59	2.14	53	184	0.84
k	4.86	0.52	0.034	0.037	0.90	1.16	52	188	0.71
nk	4.60	0.56	0.028	0.026	0.80	1.15	46	184	0.70
pk	5.96	0.50	0.054	0.031	0.86	1.68	54	173	0.75
npk	6.32	0.52	0.056	0.070	0.76	1.86	52	156	0.86
Min. 5% sig. diff.	2.04	0.10	0.013	0.087	0.30	0.66	16	34	0.08

MEAN EFFECTS

Effect	Ash	Nitrogen	Phosphorus	Magnesium	Potassium	Calcium	Iron	Manganese	Rubber
N	–0.4625	0.0412	–0.00225	–0.0034	–0.12625	–0.0800	–3.25	–17.125*	0.0300
P	1.5475**	–0.0188	0.02200***	–0.0011	–0.00375	0.6500**	5.00	– 2.125	0.0600**
K	–0.2725	–0.0038	–0.00525	0.0056	0.11375	–0.2125	0	– 1.375	–0.0375
95% conf. limit	1.0205	0.0511	0.00636	0.0437	0.14883	0.3302	7.82	16.785	0.0384
99% conf. limit	1.5098	—	0.00942	—	—	0.4885	—	—	0.0568
99.9% conf. limit	—	—	0.01454	—	—	—	—	—	—

TABLE 9. SEAPORT ESTATE TRIAL

Concentration of nutrient elements in dried laminae and girth of trees at sixty inches measured in June 1956

TREATMENT MEANS

Treatment	Ash%	N%	P%	Mg%	K%	Ca%	Fe p.p.m.	Mn p.p.m.	Girth cm
$n_0p_0k_0$	3.68	3.34	0.16	0.17	0.65	0.72	99	66	77.8
$n_1p_0k_0$	3.54	3.58	0.18	0.17	0.62	0.68	83	92	78.6
$n_2p_0k_0$	3.12	3.74	0.18	0.18	0.68	0.52	94	44	77.6
$n_0p_1k_0$	4.72	3.22	0.26	0.14	0.80	1.06	107	93	78.6
$n_1p_1k_0$	4.28	3.40	0.22	0.13	0.76	0.92	98	64	80.7
$n_2p_1k_0$	3.82	3.40	0.19	0.12	0.58	0.86	98	104	74.8
$n_0p_0k_1$	4.35	3.18	0.16	0.16	0.96	0.71	98	85	77.4
$n_1p_0k_1$	3.74	3.56	0.16	0.12	1.02	0.53	94	51	77.6
$n_2p_0k_1$	3.46	3.68	0.17	0.15	0.92	0.50	92	50	81.4
$n_0p_1k_1$	4.56	3.42	0.23	0.16	0.95	0.83	88	78	81.0
$n_1p_1k_1$	4.17	3.52	0.21	0.13	0.86	0.82	92	99	78.7
$n_2p_1k_1$	4.14	3.64	0.20	0.12	0.87	0.79	94	86	79.8
Min. 5% sig. diff.	0.94	0.37	0.10	0.04	0.31	0.27	16	40	8.1

MEAN EFFECTS

Effect	Ash	Nitrogen	Phosphorus	Magnesium	Potassium	Calcium	Iron	Manganese	Girth
N (linear)	–0.692**	0.325**	–0.014	–0.014	–0.079	–0.159*	– 3.38	– 9.50	–0.250
95% conf. limit	0.470	0.182	0.049	0.018	0.158	0.135	7.86	19.95	4.061
99% conf. limit	0.663	0.257	—	—	—	—	—	—	—
N (quadratic)	0.096	–0.119	0.006	0.020	–0.039	0.019	8.62	– 1.74	–0.775
95% conf. limit	0.813	0.316	0.084	0.031	0.275	0.234	13.62	34.55	7.032
P	0.634**	–0.080	0.050*	–0.026**	–0.002	0.269***	2.84	23.00**	0.508
K	0.209	0.055	–0.010	–0.011	0.248**	–0.099	– 3.66	– 2.50	1.292
95% conf. limit	0.383	0.149	0.040	0.015	0.129	0.110	6.42	16.29	3.315
99% conf. limit	0.541	—	—	0.021	0.183	0.156	—	22.99	—
99.9% conf. limit	—	—	—	—	—	0.223	—	—	—

TABLE 10. SEAPORT ESTATE TRIAL

Concentration of nutrient elements in dried bark and yield of rubber in tahils per tree per tapping during September 1956 to January 1957

TREATMENT MEANS

Treatment	Ash%	N%	P%	Mg%	K%	Ca%	Fe p.p.m.	Mn p.p.m.	Rubber tahils
$n_0p_0k_0$	6.49	0.50	0.034	0.067	0.44	2.18	39	96	0.52
$n_1p_0k_0$	4.63	0.50	0.028	0.052	0.32	1.53	30	99	0.53
$n_2p_0k_0$	4.20	0.57	0.032	0.052	0.35	1.34	38	74	0.60
$n_0p_1k_0$	6.50	0.46	0.056	0.048	0.38	2.23	48	87	0.50
$n_1p_1k_0$	5.23	0.54	0.056	0.048	0.42	1.68	32	69	0.69
$n_2p_1k_0$	5.33	0.58	0.054	0.065	0.29	1.80	32	124	0.48
$n_0p_0k_1$	6.52	0.44	0.032	0.058	0.62	2.06	46	120	0.61
$n_1p_0k_1$	6.87	0.55	0.034	0.057	0.70	2.14	48	102	0.64
$n_2p_0k_1$	5.12	0.51	0.036	0.060	0.61	1.54	36	124	0.74
$n_0p_1k_1$	5.56	0.45	0.042	0.034	0.60	1.71	32	76	0.62
$n_1p_1k_1$	5.20	0.48	0.044	0.040	0.56	1.57	28	94	0.60
$n_2p_1k_1$	5.28	0.54	0.044	0.038	0.47	1.70	38	86	0.68
Min. 5% sig. diff.	2.74	0.10	0.014	0.024	0.28	0.90	30	58	0.21

MEAN EFFECTS

Effect	Ash	Nitrogen	Phosphorus	Magnesium	Potassium	Calcium	Iron	Manganese	Rubber
N (linear)	–1.285	0.090**	0.00037	0.002	–0.081	–0.450	– 5.00	– 5.25	0.065
95% conf. limit	1.372	0.051	0.00702	0.012	0.142	0.452	15.15	29.22	0.105
99% conf. limit	—	0.072	—	—	—	—	—	—	—
N (quadratic)	0.290	–0.023	0.00089	0.007	–0.056	0.182	8.00	2.25	–0.045
95% conf. limit	2.376	0.088	0.01215	0.021	0.246	0.782	26.24	50.60	0.182
P	–0.124	0	0.01650***	–0.012*	–0.055	–0.016	– 4.25	– 1.50	–0.013
K	0.359	–0.028	–0.00483	–0.008	0.227**	–0.004	1.75	0.17	0.097*
95% conf. limit	1.120	0.042	0.00573	0.010	0.116	0.369	12.37	23.86	0.086
99% conf. limit	—	—	0.00808	—	0.164	—	—	—	—
99.9% conf. limit	—	—	0.01154	—	—	—	—	—	—

November 1956. Girth, measured at 60 inches above the union in June 1956, did not show any significant response to fertiliser application. Yield of rubber per tree per tapping, averaged over the period September 1956 to January 1957, showed a significant mean effect attributable to fertilisers containing potassium. The mineral analyses of bark and laminae samples are given in Tables 9 and 10.

Both bark and lamina compositions reflected the effect of the addition of fertilisers containing potassium; the mean effect of this addition on potassium concentration in these tissues was significant. Thus the increase in yield of rubber on the addition of potassium fertilisers was accompanied by increases in the potassium contained in laminae and bark.

The inclusion of fertilisers containing phosphate in any treatment gave rise to a positive significant mean effect on the phosphorus concentration in both laminae and bark, but the effect was more strongly shown in terms of bark composition. Phosphate addition also significantly reduced the concentration of magnesium in both laminae and bark tissues. The application of phosphate fertilisers produced significant positive mean effects on the concentration of ash, calcium and manganese in the laminae but such effects were not recorded for the bark. Thus, as for the Wardieburn Estate trial, phosphate fertiliser addition affected the concentration of a greater number of elements in the laminae than in the bark; bark composition however, did show marked increases in phosphorus concentration wherever phosphate had been applied.

The addition of nitrogen fertilisers produced a significant increase in the nitrogen concentration of both bark and laminae; it also reduced the calcium concentration of the laminae but not of the bark.

Correlationships Between Compositions of Laminae and Bark

Significant correlationships were established between lamina and bark concentrations of phosphorus, potassium and manganese for each of the three manurial trials examined herein (Table 11); these correlationships were significant at the 1% level. Similar significant correlationships were also established in respect of nitrogen and calcium for samples taken from two out of the three trials. Significant correlationships were for ash and magnesium concentrations for samples taken from the Wardieburn Estate trial but none of the trials gave any indication of a significant correlation in respect of iron.

TABLE 11. CORRELATION COEFFICIENTS BETWEEN CONCENTRATIONS OF NUTRIENT ELEMENTS IN LAMINAE AND BARK

Trial	Ash	N	P	Mg	K	Ca	Fe	Mn
Field 31 (24 pairs of values)	0.204	0.125	0.538**	0.265	0.792***	0.723***	0.330	0.897***
Wardieburn Estate (16 pairs of values)	0.759***	0.689**	0.774***	0.923***	0.797***	0.868***	0.127	0.740**
Seaport Estate (24 pairs of values)	0.405	0.523**	0.660***	0.108	0.872***	0.198	–0.007	0.538**

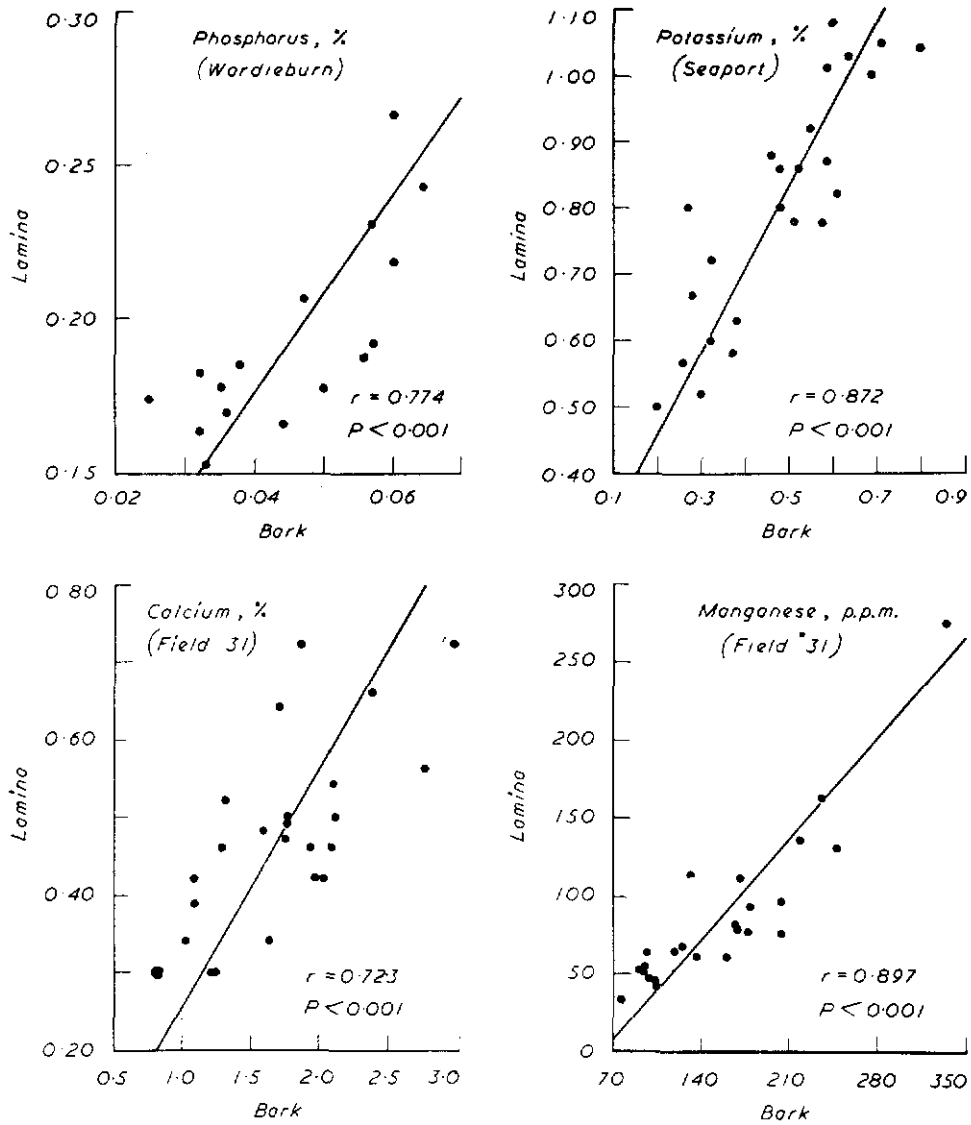

Figure 1. Relationships between concentrations of nutrient elements of bark and laminae. Concentrations expressed in terms of dried bark and lamina.

Some of these correlationships are demonstrated in *Figure 1* which also serves to illustrate that, for calcium and manganese the bark concentration values were usually of a higher order than those of laminae but for phosphorus the opposite was true. The concentrations of nitrogen, magnesium and potassium were smaller for the bark than for the laminae.

On the basis of the above findings it could be assumed that the concentrations of nitrogen, phosphorus, potassium, calcium and manganese in both bark and laminae could be regarded as capable of reflecting the same response to fertiliser addition but that the same could not, with confidence, be said in respect of magnesium.

GENERAL DISCUSSION

As indicated in the introduction to this paper, a satisfactory procedure is now available for the sampling of leaves of young hevea trees up to the one year old stage. The number of trees to be sampled per acre is a matter to be settled at the discretion and experience of the collector. A minimum unit of 5 trees has been mostly used and these have been usually located within half an acre; for large uniform soil areas the unit may be kept the same but the area increased. When leaves are removed according to storey position, about 40 leaves will be found to comprise one sample. For mature trees about 100 leaves are removed from a similar unit of trees, as described in the text. This larger number of leaves is taken in an attempt to balance the variability, according to crown position, found in mature trees. All recommendations regarding sampling are clearly arbitrary and made mainly to suit the convenience of the collector. It would undoubtedly be more efficient and accurate to double or treble the number of trees sampled and to reduce the number of leaves taken per tree. However, with a large number of samples to be taken this approach has been found to be impracticable in the field, owing to the long time required to take even one sample. Time may become an important limiting factor to be considered in any sampling as, based on the findings described in the early part of this paper, leaf samples should be taken between 06.00 to 10.00 hours. The collection of a large number of samples is preferred within this time limit by taking leaves from few trees, rather than to take a few samples derived from a large number of trees. Once a boy has climbed the tree to a selected spot it is hardly more trouble for the collector to take 20 leaves per tree from the cut down branches than it would be to collect about 3.

It was largely because of the unsatisfactory means of selection and collection of leaf samples from mature trees that the use of bark composition, as a means of determining the mineral nutrient status of trees, was considered. While it was never contemplated that this approach would excel the method of diagnosis based on leaf analysis it was nevertheless hoped that the analysis of easily collected bark samples would give a similar, but perhaps less sensitive index of nutritional status. This hope was partly realised and is discussed more fully below.

The concentrations of phosphorus, potassium and manganese in the laminae were closely correlated with the respective concentration found in the bark, for each of the manurial trials examined. Similar correlationships were also established for nitrogen and calcium for two out of the three trials considered. Therefore it seemed warranted to assume that when a response to fertiliser addition was obtained for laminae, in terms of concentration of any of the above elements, the bark also would show a similar response. This assumption was correct to a limited extent. Thus the addition of phosphate fertiliser in the Field 31 trial produced significant mean effects on the phosphorus concentrations of both laminae and bark. For the Wardieburn Estate trial, the same was true in regard to phosphorus and calcium concentration but not for effects on the manganese and iron concentrations, which were only given by the laminae. Similarly for the Seaport Estate trial, while both bark and lamina compositions showed responses to phosphate fertiliser in terms of phosphorus and magnesium concentrations the laminae also showed responses in terms of calcium and manganese concentrations.

Thus, for all trials, the phosphorus concentration of the bark as well as that of the laminae, reflected the addition of phosphate fertiliser but frequently the laminae composition showed effects which were not detectable in the bark. In Malaya a response, as measured in terms of girth and yield of rubber per tree, is frequently obtained as a result of the addition of phosphate fertiliser (OWEN, WESTGARTH & IYER 1957). See

Tables 5 to 8. Bark composition affords a ready means of assessing this response to phosphate fertiliser addition. For such a limited purpose, it seems credible that the bark may serve as a better index than the laminae; the bark composition may reflect the previous history and treatment given to the tree whereas the composition of the laminae can only mirror the outcome of these effects.

A response to potassium fertilisers, as measured by changes in the concentration of minerals in the laminae, was obtained for potassium, but only for one of the manurial trials examined (Seaport Estate). This same response was also reflected in the concentration of potassium found in the bark. As the potassium concentrations of laminae and bark were so closely correlated for all three trials and particularly in view of the effect obtained for the Seaport Estate trial it is considered that whenever laminae are likely to show responses, in terms of potassium concentration, to potassium fertilisers addition, bark will also respond similarly.

For nitrogen the evidence is less satisfactory, the correlationship coefficients, in respect of the nitrogen concentrations in bark and laminae, were inferior to those obtained for potassium and phosphorus. However, for the Seaport Estate trial, bark composition was as satisfactory an index (as measured by nitrogen concentration) as that of the laminae but for the Wardieburn Estate trial the mean effect of nitrogen fertiliser addition was shown only by the laminae.

These findings lead to the conclusion that bark composition may be used as a means of detecting responses to phosphate and potassium fertiliser additions but this may not always be so for nitrogen fertilisers. Further investigations in regard to the effect of the latter are required. By an extended application of bark sampling and analysis it should become possible to establish the normal level of potassium and phosphorus concentrations in the bark (and perhaps nitrogen also) and to relate them to yield performance. It is already clear from Tables 6, 8 and 10, that whenever a significant mean effect on yield is attributable to fertilisers containing phosphorus, or potassium, the bark composition mirrors this effect in terms of the concentrations of those elements. The ease of bark sampling is much in favour of its eventual adoption for routine evaluation of responses in manurial trials. The method of taking bark slivers can be altered to that of taking circular discs if this proves to be more amenable for routine purposes. The number of trees sampled must be varied according to the area being assessed. The advantages of the collection of bark samples as compared with the collection of leaf samples are obvious and need no further enumeration.

SUMMARY

An evaluation of the variation in the concentration of mineral nutrients in the laminae of young rubber plants during the day, showed that a significant diurnal variation occurred only in respect of magnesium; there were indications, but not proven, that a diurnal variation in iron concentration also occurred. Leaf sampling procedures are described and discussed in relation to diagnosis based on the composition of bark.

The effects of fertiliser addition on the mineral status of the tree were assessed, in three manurial trials, in terms of bark and lamina composition. It was concluded that bark composition offered a means of detecting responses to fertilisers containing phosphate, and potassium, almost as satisfactory as that of the lamina. Whenever a significant mean effect on yield of rubber produced per tree was attributable to the addition of phosphorus or potassium, the composition of the bark was found to mirror this effect in terms of the concentrations of those elements.

The concentrations of phosphorus, potassium and manganese in the lamina were closely correlated with the respective bark concentration; a similar relationship held for nitrogen and calcium in respect of samples taken from two out of the three trials examined. The concentration of iron in the lamina was not correlated with that found in the bark.

Soils Division
Rubber Research Institute of Malaya
Kuala Lumpur

May

REFERENCES

BEAUFILS, E. R. (1955) Mineral diagnosis of some "*Heveas brasiliensis*" *Arch. Rubber. Cult.* 32, 1.

BOLLE-JONES, E. W. (1954a) Nutrition of *Hevea brasiliensis*. I. Experimental methods. *J. Rubb. Res. Inst. Malaya 14*, 183.

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

BOLLE-JONES, E. W. (1956) Visual symptoms of mineral deficiencies of *Hevea brasiliensis*. *J. Rubb. Res. Inst. Malaya, 14*, 493.

BOLLE-JONES, E. W. & RATNASINGAM K. (1954) Nutrition of *Hevea brasiliensis*. III. Interclonal and seasonal variation in composition of leaves. *J. Rubb. Res. Inst. Malaya 14*, 257.

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

OWEN G., WESTGARTH D. R. & IYER, G. C. (1957) Manuring Hevea: Effects of fertilisers on growth and yield of mature rubber trees. *J. Rubb. Res. Inst. Malaya, 15*, 29.