Leaf Analysis as a Guide to the Nutrition of Hevea brasiliensis VI. Variations in Leaf Nutrient Composition with Age of Leaf and with Time

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The variations in leaf nutrient composition with age of leaf and time of sampling were studied on branched trees. Variations in the potassium, magnesium and phosphorus concentrations of low leaves—shade were relatively small during the period August to December; this period is recommended for sampling when leaf analysis is being used for the diagnosis of fertiliser requirements. Considerable month-by-month variation in the nitrogen, calcium and manganese concentrations occurred and there was no period in the year when the variations were small: the use of leaf analysis for the assessment of the nitrogen, calcium and manganese status of trees is discussed.

Information concerning the most suitable leaf and the best time in the year for sampling is essential for the successful use of leaf analysis in diagnosing tree nutrient status. Previous investigations of sampling procedure for mature rubber with a closed canopy (SHORROCKS 1962a and 1962b) have shown that taking leaves from branches low down in the canopy ('low leaves—shade') is a more sensitive and a more convenient sampling technique than taking leaves from the top of the canopy ('light leaves'): for young branched rubber where the canopy is not closed over, the sampling of light leaves from terminal whorls is advocated.

The seasonal variation in nutrient composition of leaves taken from top, second and third whorls on unbranched trees of clones PB 86, Tjir 1 and RRIM 501 was studied by BOLLE-JONES AND RATNASINGAM (1954), who found significant month-by-month variation in leaves at the same stage of development during one twelve-month period. It was not possible to select a period when variation in nutrient composition was relatively slight and when leaf sampling could be confidently recommended in the knowledge that little error would be introduced by time of sampling.

The current investigations, carried out on branched mature trees, were designed to determine whether the nutrient composition of light leaves and low leaves—shade varies markedly month by month or whether there are periods when variation in nutrient composition are slight, thereby enabling an optimal sampling period to be described.

METHODS

Several experiments were carried out in which leaves were sampled at different times in the year: in Experiments 1 and 2, low leaves—shade of known age were sampled and in Experiments 3, 4, 5, 6 and 7, light leaves and low leaves—shade of unknown age were sampled.

Low Leaves—Shade of Known Age

In Experiment 1 low leaves—shade were collected at monthly intervals during the period April 1963 to March 1964 from ten trees of clone PR 107 and ten Tjir 1 clonal seedling trees. The same trees, which were sampled throughout, were initially selected in March 1963 as trees of average vigour that had undergone complete defoliation during the wintering period in February 1963. The ten trees of each clone were distributed over

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an area of about half an acre, in the same field. Development of all low leaves—shade was completed by the end of March 1963 and there was no subsequent development of low leaves—shade during the year: all sampled leaves were formed at the same time and accordingly their age was known; for example leaves sampled in April were one month old and those in December nine months old.

Two whorls were sampled from each tree every month:

(i) a terminal whorl developed from an apical meristem (here called the leader whorl) and

(ii) a terminal whorl developed from an axillary meristem (here called the side shoot whorl).

The leader whorl and side-shoot whorls were taken from the same terminal section of a branch. The basal six leaves were taken from each whorl to form one discrete sample for analysis: the results presented are the mean values for the ten trees, for each type of whorl.

The Tjir 1 clonal seedling trees received 4 lb per tree of a mixed N, P, K, Mg fertiliser, R.R.I.M. Mixture M (RUBBER RESEARCH INSTITUTE OF MALAYA, 1963), and the PR 107 trees received 2 lb per tree of a mixed N, P, K, Mg fertilser, R.R.I.M. Mixture Magnesium C_2 (RUBBER RESEARCH INSTITUTE OF MALAYA, 1963), both applications being made in April 1963.

In Experiment 2 low leaves—shade (side-shoot whorl) were collected from Tjir 1 clonal seedling trees which had received no fertilisers since planting. Trees of average vigour which defoliated completely in February 1963 were selected for sampling: refoliation was nearly completed by the end of March and since there was no further development of low leaves—shade the age of the sampled leaves was known. Six groups of seven trees were sampled every month during the period July 1963 to February 1964. One whorl was sampled from each tree and the basal four leaves were taken to form a bulk sample for each group of seven trees.

Low Leaves—Shade and Light Leaves of Unknown Age Sampled at Different Times in the Year

The variation in leaf nutrient composition that can be expected month by month under more normal sampling circumstances where the age of the leaves sampled is not known, was studied by examination of data from fertiliser experiments where frequent sampling was undertaken.

Experiment 3. Low leaves—shade and light leaves were sampled, once before defoliation (in February) and eight times after refoliation, from thirty-two plots in an experiment designed to study the effect of time of fertiliser application on leaf nutrient status, the details and results of which have been reported (SHORROCKS, 1964). In each plot leaves were collected from the same twenty trees on each sampling occasion and bulked to form one plot sample for analysis: in the present paper the mean monthly values for the entire experiment calculated from the data of the thirty-two plots are considered.

Experiments 4, 5, 6 and 7. Data similar to those for Experiment 3 were obtained from four other fertiliser experiments (designed to study the effects of ammonium sulphate, potassium chloride and rock phosphate on growth and yield) where less intensive sampling of light leaves and low leaves—shade was carried out. All experiments were of a 3^3 NPK design with three replicates in Experiment 4 and single replication in Experiments 5, 6 and 7; details of the planting material, age and soil type are given in *Table 1*. In each plot of each experiment, leaves were taken from six trees and combined to form one plot sample for analysis. Light leaves and low leaves—shade were not sampled over the same periods as in Experiment 3 and accordingly these four experiments are considered as a separate group. The data presented here are the mean values for an experiment calculated from all plots at each sampling occasion.

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TABLE 1. DETAILS OF EXPERIMENTAL AREAS

Experiment	R.R.I.M. file no.	Planting material	Year budded	Soil series[b]
1	—	PR 107	1956	Sungei Buloh
1	SE.1/11	Tjir 1 seedlings	1955[a]	Sungei Buloh
2	SE.1/23	Tjir 1 seedlings	1957[a]	Rengam
3	SE.1/10(i)	RRIM 501	1953	Malacca
4	SE.1/10	RRIM 501	1953	Malacca
5	SE.29/2	PB 86	1951	Malacca
6	SE.28/3	PB 86	1949	Malacca
7	SE.1/21	Tjir 1	1950	Rengam

[a] Year planted. [b] (OWEN, 1951).

RESULTS

Low Leaves—Shade of Known Age, Experiments 1 and 2

Nitrogen. A marked, similar decline in nitrogen concentration with age was observed in both types of whorl of both planting materials (*Figure 1*). The rate of decline in nitrogen concentration was fairly regular, except for the ten- to twelve-month-old leaves (sampled January to March) and, for Tjir 1 only, the four- to seven-month-old leaves (sampled July to October), when relatively little variation was observed. It is probable, that, in Experiment 1, the relatively heavy fertiliser dressing given to the Tjir 1 clonal seedlings trees in April 1963 was responsible for the slight increase in leaf nitrogen concentration that occurred in the period July to September. The nitrogen concentrations of leader and side-shoot whorls were very similar.

Potassium. A reduction in potassium concentration occurred in both types of whorl of both planting materials between the period April to August (one- to five-month-old leaves): thereafter relatively little variation was found (*Figure 1*). For a given planting material, potassium concentration of side-shoot whorls was slightly greater than that in leader whorls.

Phosphorus. A slight decline in the phosphorus concentration with age was observed between the period April to July (one to four month-old leaves) in both types of whorl of both planting materials (*Figure 1*), thereafter relatively little fluctuation occurred. The phosphorus concentrations of leader and side shoot whorls were very similar.

Magnesium. An increase in the magnesium concentration with age was observed between April and August (one- to five-month-old leaves) in the case of PR 107 and between April and June (one- to three-month-old leaves) in the case of Tjir 1 clonal seedlings (*Figure 2*). Subsequent variations were slight but for both planting materials the magnesium concentration tended to fall after November. The magnesium concentrations of leader and side shoot whorls were very similar.

Calcium and manganese. Marked and fairly regular increases in the calcium and manganese concentrations with age occurred in both types of whorls throughout the entire year (*Figure 2*) in Experiment 1. In Experiment 2 there was little variation in the manganese concentration which is interesting since the concentration of manganese was low and the leaves showed slight deficiency symptoms, suggesting that insufficient manganese was available for absorption and accumulation in the leaves. For a given planting material, leader whorls contained higher calcium and manganese concentrations than side-shoot whorls.

Low Leaves—Shade and Light Leaves of Unknown Age Sampled at Different Times in the Year

Experiment 3. A marked decline in the nitrogen concentration of both light leaves

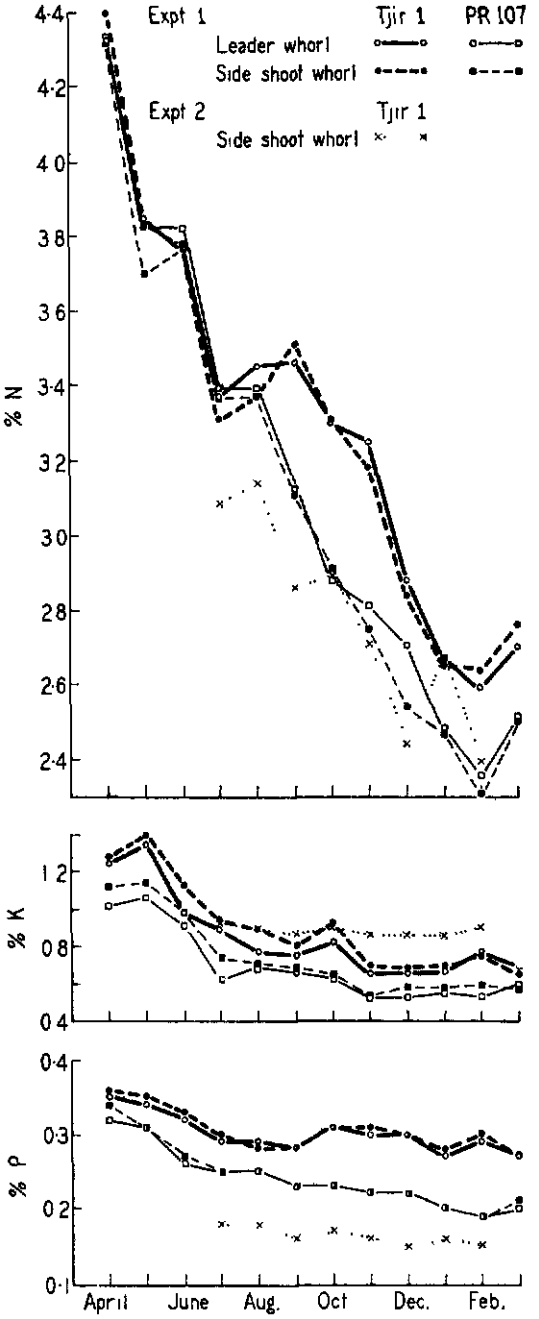

Figure 1. Experiments 1 and 2. Variations with age of leaf in the nitrogen, potassium and phosphorus concentrations of low leaves—shade (leaves sampled in April were one month old, those in May two months etc.)

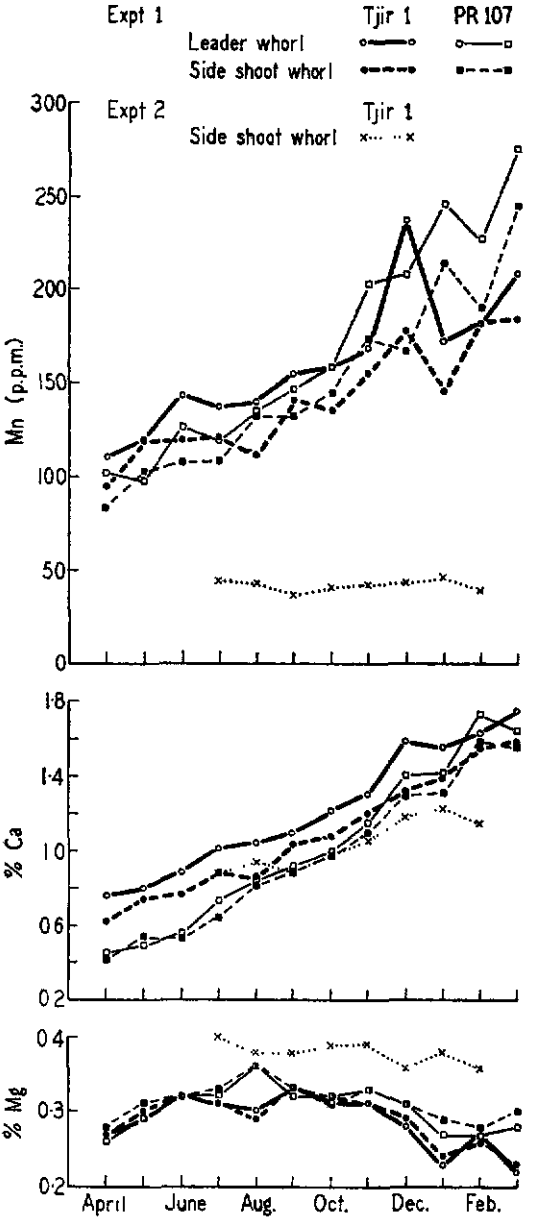

Figure 2. Experiments 1 and 2. Variations with age of leaf in the manganese, calcium and magnesium concentrations of low leaves—shade (leaves sampled in April were one month old, those in May two months etc.)

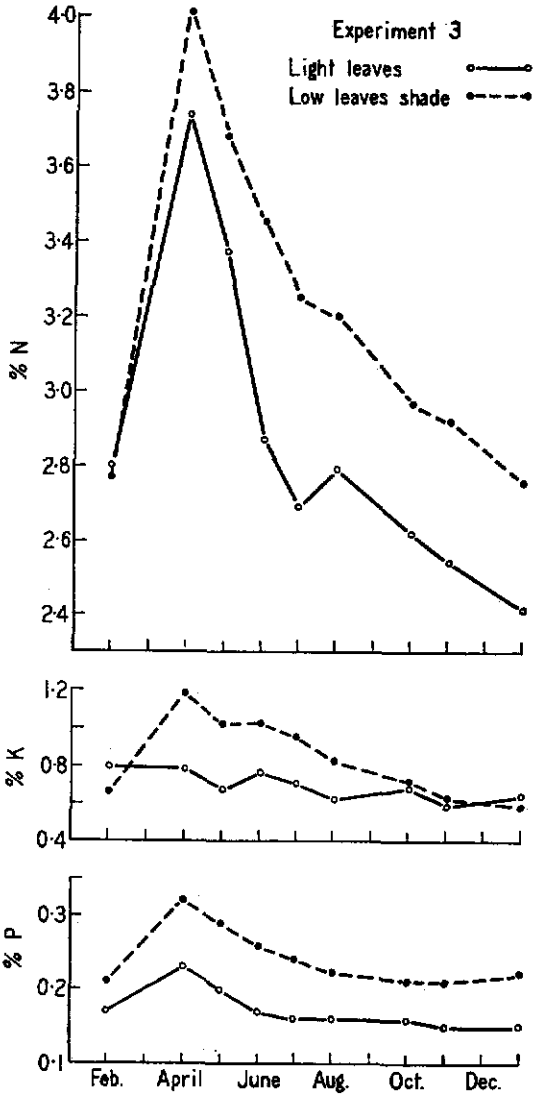

Figure 3. Experiment 3. Variations with time in the nitrogen, potassium and phosphorus concentrations of light leaves and low leaves—shade of unknown age.

and low leaves—shade occurred, commencing after the wintering period in March, and continuing throughout the whole period, April to January (*Figure 3*). A regular decline in potassium concentration of low leaves—shade with time was observed (*Figure 3*), and although there was a similar tendency in light leaves, the relative fluctuation month by month was slight. As in Experiments 1 and 2, a decline in the phosphorus concentration of low leaves—shade was observed during the period April to August and of light leaves in the period April to July (*Figure 3*); thereafter up to January there was very little variation for either type of leaf. There was very little evidence of any fluctuation in magnesium concentration in low leaves—shade during the period May to January, and in light leaves during the period July to January (*Figure 4*). An increase in the calcium concentration of low leaves—shade occurred with time after wintering, but in the light leaves the calcium concentration first declined up to July and then increased gradually in the following period up to January (*Figure 4*). A similar situation with regard to the manganese concentration of light leaves

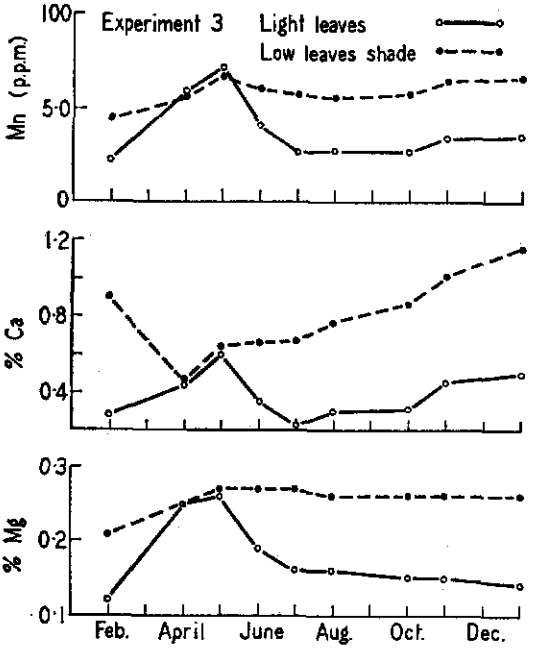

Figure 4. Experiment 3. Variations with time in the manganese, calcium and magnesium concentrations of light leaves and low leaves—shade of unknown age.

was observed, and in the case of low leaves—shade a very slight increase in manganese concentration with time was recorded. The levels of manganese and calcium were low, indicating that little manganese or calcium was available for absorption and accumulation in the leaves.

Experiments 4, 5, 6 and 7. The decline in nitrogen concentration of low leaves—shade with time after wintering was evident in all experiments, there being a tendency for the decline to be at a minimum in the period September to December (*Figure 6*).

For light leaves the situation was not as clear cut (*Figure 5*): in Experiments 5 and 6 the nitrogen concentration declined with time whereas in Experiment 4 much less overall fluctuation was found, and during the period October to March the nitrogen concentration was fairly constant. Divergent results were also obtained in Experiment 7 where the nitrogen concentration varied very little in 1959 but in 1960 declined regularly with time.

There was relatively little fluctuation in the potassium concentration of either light leaves or low leaves—shade with time after wintering, although the concentration in low leaves—shade tended to decline with time (*Figure 6*). A very slight gradual decline in phosphorus concentration of both light and low leaves—shade was generally observed with time after wintering (*Figures 5 and 6*); in the case of low leaves—shade there was very little variation during the period August to December. A slight gradual decline was observed in the magnesium concentration of low leaves—shade, with only small variations during the period September to October (*Figure 6*). The light leaves (*Figure 5*) showed slightly less month-to-month variability than low leaves—shade.

Inspection of the more detailed treatment data of Experiments 3, 4, 5, 6 and 7 showed that the basic patterns of variation in leaf nutrient concentrations with age were not affected by fertiliser treatment; although the concentration of a given nutrient could be increased or decreased by fertiliser treatment, the same variations with age were observed regardless of treatment.

DISCUSSION

It is clear from the results that considerable changes occur in the major nutrient composition of both light leaves and low leaves—shade as the leaves age and also with the time of sampling of leaves of unknown age.

Low Leaves—Shade of Known Age

Investigations on low leaves—shade of known ages showed that the nitrogen concentration declined and the calcium and the manganese concentrations increased with age of leaf throughout the entire year, except under conditions of nutrient deficiency in the case of manganese. The potassium concentration showed an initial decline followed, from about August, by a period when there was relatively little fluctuation. The magnesium and phosphorus concentrations, by comparison with the other nutrients, showed relatively little fluctuation; there was a tendency for the magnesium concentration to increase slightly in the first few months after refoliation followed by a very slight decline in concentration, and for the phosphorus concentration to decline in the first few months after refoliation. It appears from these results that the sampling of low leaves—shade during the period August to December would be satisfactory for estimating the potassium, phosphorus and magnesium status in leaves in the knowledge that little error is likely to be introduced by the actual time or month of sampling.

No period in the year can be described during which variations in leaf nitrogen concentration are at a minimum, apart possibly from the latter two or three months before defoliation when the nitrogen concentration is at a minimum. The diagnosis of the nitrogen status of the leaves, by comparison with standard values, might thus only be possible by comparing minimal nitrogen values for any given field with standards relating to the same period just before defoliation. Similarly difficulties are likely to be encountered in the accurate diagnosis of the calcium and manganese status of leaves owing to the fluctuations

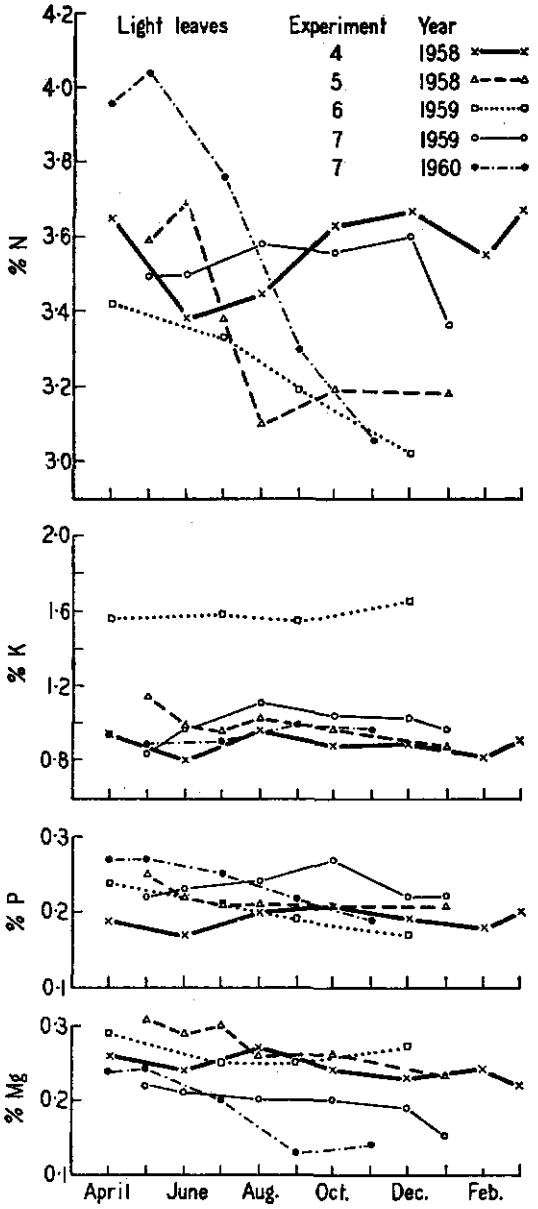

Figure 5. Experiments 4, 5, 6 and 7. Variations with time in the nitrogen, potassium, phosphorus and magnesium concentrations of light leaves of unknown age.

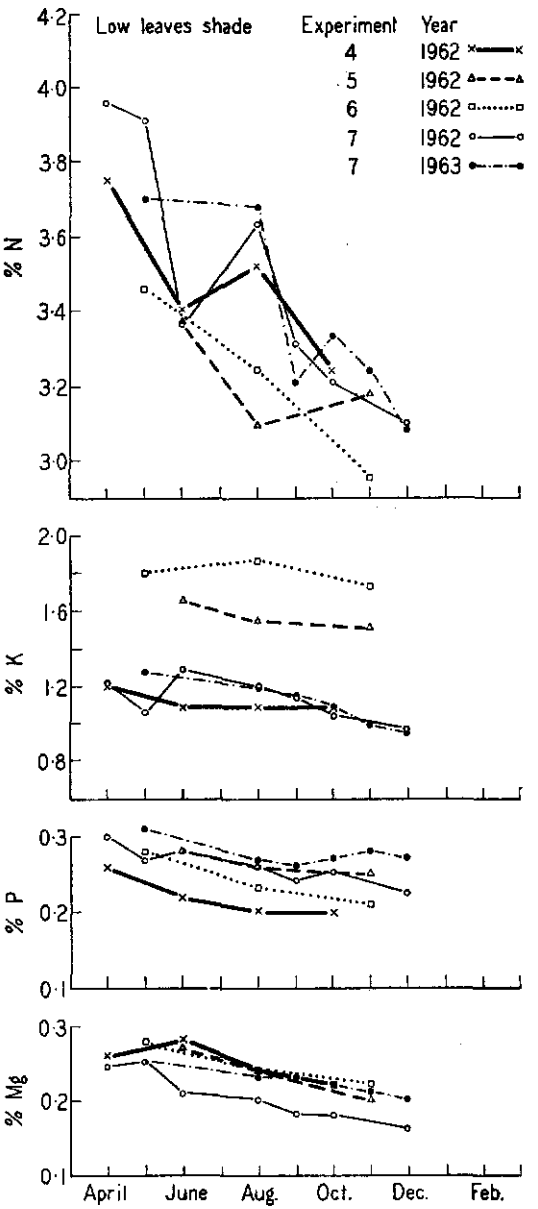

Figure 6. Experiments 4, 5, 6 and 7. Variations with time in the nitrogen, potassium, phosphorus and magnesium concentrations of low leaves—shade of unknown age.

which occur during the year. However, an assessment of the calcium and manganese status of leaves in the period of August to December (also recommended above for diagnosis of the potassium, magnesium and phosphorus status) could be safely made using standard values determined for the period between refoliation and July; the finding of a calcium or manganese concentration similar to or lower than such standard values would indicate a deficiency, as in healthy leaves the calcium and manganese concentrations would be expected to increase with time.

It is recommended that leaf sampling should be carried out during the period August to December when leaf analysis is being used for the diagnosis of fertiliser requirements: interpretation of the nutrient composition of leaves sampled outside this period can only be made in the light of expected variation in leaf nutrient composition with time and will accordingly be imprecise.

Slight differences were found between the nutrient composition of leader whorls and side-shoot whorls, there being a tendency for the side-shoot whorls of a given plant material to have higher concentrations of potassium and lower concentrations of manganese and calcium than leader whorls. It is necessary to standardise on one type of whorl and on the basis that the selection and sampling of side-shoot whorls is likely to be easier owing to their greater number, side-shoot whorls are preferred to leader whorls.

Light Leaves and Low Leaves—Shade of Unknown Age

The investigations carried out on light leaves and low leaves—shade of unknown age sampled at different months in the year confirm the basic picture shown by the study on low leaves—shade of known ages. However, in general, the variations in leaf nutrient composition with month of sampling were not as great as those observed in the leaves of known ages: this was particularly evident for the potassium concentration of light leaves and to a lesser extent for the nitrogen concentration of both low leaves—shade and light leaves. Owing to the recurrent development of light leaves during the year, a situation which is not found in low leaves—shade, it is possible that the sampling of light leaves which are recently mature frequently allows leaves of a more uniform age and similar nutrient composition to be sampled each month. However, this is not always so, for in some experiments the nitrogen concentration of light leaves declined during the period following wintering and in other experiments it remained fairly constant; these divergent results may be due to the sampling of leaves of increasing age from trees which were not actively flushing in one experiment and to the sampling of leaves of approximately the same age from trees which were actively flushing in another experiment. Thus the use of light leaves for investigations of tree nutrient status is complicated as the leaf age is unknown to the sampler: low leaves—shade would be preferred as their age at any time in the year can be assessed provided the date of refoliation is known.

ACKNOWLEDGEMENTS

The author wishes to acknowledge the work of Mr N. Samuel in the collection of the leaves, and the analytical work of Mr K. Ratnasingam and associated staff of the Soils Division.

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Kuala Lumpur *July 1964*

REFERENCES

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

OWEN, G. (1951) A provisional classification of Malayan soils. *J. Rubb. Inst. Malaya* **13**, Comm. 274, 20.

RUBBER RESEARCH INSTITUTE OF MALAYA (1963) Revised manuring programme for young replantings. *R.R.I. Plant. Bull.* No. 67, 79.

SHORROCKS, V. M. (1962a) Leaf analysis as a guide to the nutrition of *Hevea brasiliensis* II. Sampling technique with mature trees: Variations in nutrient composition of the leaves with position on the tree. *J. Rubb. Res. Inst. Malaya* **17**, 91.

SHORROCKS, V. M. (1962b) Leaf analysis as a guide to the nutrition of *Hevea brasiliensis* V. A leaf sampling technique for mature trees, *J. Rubb. Res. Inst. Malaya* **17**, 167.

SHORROCKS, V. M. (1964) Effect of time of fertiliser application on leaf nutrient composition. *J. Rubb. Res. Inst. Malaya* **18**, 276.