Variation in Leaf Nutrient Content of Hevea with Clone and Age of Leaf

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Changes with age in nitrogen, phosphorus, potassium, magnesium, calcium and manganese contents in leaves were studied using five clones of Hevea brasiliensis. Top-light leaves of six-year-old mature trees were examined. Nitrogen, phosphorus, potassium and magnesium contents in leaf showed sharp fall in concentration during the first three weeks after leaf emergence, probably due to the dilution caused by the rapid increase in dry weight of leaves at this stage. During the following eight months, a more gradual change in concentration of all elements was observed. Nitrogen, phosphorus and potassium contents showed negative linear relationships with leaf age, while calcium and manganese contents showed positive linear relationships. Magnesium content however did not show any such relationship with leaf age.

Clonal differences with regard to leaf nutrient contents are discussed. Further studies under widely varying conditions are considered necessary before firm conclusions with regard to clonal differences can be established.

Interpretation of leaf analytical data for determining nutritional status of trees has been shown to require correction of leaf nutrient contents for variation in leaf age. The relationships between leaf nutrient contents and age of leaves are used for such corrections. Where leaf age is not known, calcium content in leaf is used to indicate leaf age, and for correcting the contents of other nutrients in leaves. An increase or decrease by 0.065% N and 0.034% K for every 0.1% Ca in excess of or below 0.8% Ca in leaf respectively was considered necessary for correcting leaf nitrogen and potassium contents.

Tissue analysis has been used for determining nutritional status of various plants. Preliminary work at the Rubber Research Institute of Malaya (BOLLE-JONES, 1957) compared bark and leaf analysis for diagnosing nutritional status of rubber trees, but leaf was subsequently chosen (SHORROCKS, 1961, 1962a, b and c and SHORROCKS AND RATNASINGAM, 1962). Success of using leaf analysis as a diagnostic tool depends, among other things, on the sampling procedure and the accuracy of the critical leaf nutrient contents against which the results from the advisory samples are compared.

At present, a standard method of sampling leaves of Hevea, as recommended by SHORROCKS (1964), is followed. This method gives the mean value of the leaf nutrient content at the time of sampling. However, the leaf nutrient content changes with leaf age, and thus with the date of sampling. The result obtained is not, therefore, directly comparable with the critical values unless the effect of leaf age variation is taken into account. Age of leaves depends on the time of refoliation of trees. The situation is complicated as the time and degree of wintering and refoliation for Hevea in Malaya vary not only in different parts of the country, but also according to the clones and even from year to year.

To overcome this difficulty in using leaf analysis for diagnosing nutritional status of trees, variation in leaf nutrient content with age of leaf and with clone was studied during the season 1965 – 1966 (from April/May 1965 to February 1966). The results form the subject of discussion in this paper.
EXPERIMENTAL

The experiment was carried out using mature trees of clones RRIM 513, 605, 623, 701 and PB 86, all planted in 1959, on yellow latosol derived from granitic parent material, i.e., Rengam series (Owen, 1951). The trees received normal amounts of fertiliser during immaturity and later (Rubber Research Institute of Malaya, 1958 and 1963). At the beginning of refoliation (April–May 1965), the terminal whorls of a number of shoots of a tree were labelled with numbered tags. The shoots chosen were positioned within the top 5 feet of the canopy and received full sunlight. The leaves were therefore top-light leaves (Shorrocks, 1964). About 300 shoots from an average of thirty trees were labelled in each plot of about 0.5 acre in size. Leaf samples were collected and analysed separately from three such plots for each of the clones, except for RRIM 623 for which samples were collected from only one plot. At the time of labelling, the leaves of the whorls tagged were only one to three days old. Sampling of labelled leaves was carried out at weekly or fortnightly intervals. Each time a composite leaf sample was obtained from ten shoots selected at random from the labelled whorls within each plot. The samples were dried and analysed in the usual manner (Middleton et al., 1964).

RESULTS AND DISCUSSION

The changes in leaf contents of nitrogen, phosphorus, potassium, calcium, magnesium and manganese with leaf age for the five clones, are presented in Figure 1(a). The leaf age covered is from about 7 to 300 days. The values plotted are the averages from three plots for each of the clones, except for RRIM 623 for which the values are from only one plot. After the initial forty days, the changes in nutrient content of leaves were more gradual up to about 250 days of leaf age, although a rather marked recovery of concentration for nitrogen only was observed subsequent to the initial fall. The changes however, were different for different nutrients. For example, nitrogen, phosphorus and potassium showed a gradual fall over this period. Nitrogen concentration fell from about 3.3 to about 2.7% N; phosphorus from about 0.20 to about 0.15% P; potassium from about 1.1 to about 0.8% K over this period of about 230 days. For magnesium, only a slight decrease in content was observed for RRIM 605 and 623. For the other three clones, magnesium content remained

RRIM 605. In RRIM 623 and 701, the extent of the fall is considerably less. For phosphorus, the concentration fell in all clones from about 0.45 to about 0.21% P during the first six weeks after leaf emergence. For both potassium and magnesium, the fall in concentration during this initial period is also marked; the concentration of potassium decreased in all clones from about 1.7 to about 1.1% K during first six weeks after leaf emergence. For magnesium, the concentration fell from about 0.26 to about 0.20% Mg for the same period. For calcium and manganese however, there was no such fall at the beginning of the season; the concentration increased at every subsequent sampling.

The leaves rapidly increased in size during this period and marked increase in dry weight per leaf was also observed. For example, dry weight of one leaflet at the first sampling at about three days after leaf emergence was found to be about 0.4 g. At about forty days after emergence, the weight of one leaflet increased to about 1.6 g. Therefore, the fall in concentration of nitrogen, phosphorus, potassium and magnesium could, at least partly, be a dilution effect during this period. In other words, the dry weight of the leaves increased without corresponding transportation of these elements to the leaves. For calcium and manganese, the transportation of the elements to the leaves was even faster than the rapid increase in dry weight, resulting thus in increasing concentration of these two elements.

After the initial forty days, the changes in nutrient content of leaves were more gradual up to about 250 days of leaf age, although a rather marked recovery of concentration for nitrogen only was observed subsequent to the initial fall. The changes however, were different for different nutrients. For example, nitrogen, phosphorus and potassium showed a gradual fall over this period. Nitrogen concentration fell from about 3.3 to about 2.7% N; phosphorus from about 0.20 to about 0.15% P; potassium from about 1.1 to about 0.8% K over this period of about 230 days. For magnesium, only a slight decrease in content was observed for RRIM 605 and 623. For the other three clones, magnesium content remained
Figure 1(a). Relationship between leaf nutrient content and age of leaves: fitting of regression lines for the 50 – 250 days leaf age period (*** P<0.001, ** P<0.01, * P<0.05, N.S: not significant).
Figure 1(a). Relationship between leaf nutrient content and age of leaves: fitting of regression lines for the 50 – 250 days leaf age period (** P<0.001, ** P<0.01, * P<0.05, N.S: not significant).
Figure 1(a). Relationship between leaf nutrient content and age of leaves: fitting of regression lines for the 50 - 250 days leaf age period (*** P<0.001, ** P<0.01, * P<0.05, N.S: not significant).
TABLE 1. COMPARISON OF SLOPES OF THE LINEAR RELATIONSHIPS BETWEEN
LEAF NUTRIENT CONTENT AND AGE OF LEAVES FOR DIFFERENT CLONES

<table>
<thead>
<tr>
<th>Clone</th>
<th>N %</th>
<th>P %</th>
<th>K %</th>
<th>Ca %</th>
<th>Mg %</th>
<th>Mn p.p.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RRIM 513</td>
<td>-0.0031</td>
<td>-0.00008</td>
<td>-0.0015</td>
<td>0.0046</td>
<td>-0.00001</td>
<td>0.79</td>
</tr>
<tr>
<td>RRIM 605</td>
<td>-0.0033</td>
<td>-0.00021</td>
<td>-0.0017</td>
<td>0.0034</td>
<td>-0.00015</td>
<td>0.54</td>
</tr>
<tr>
<td>RRIM 623</td>
<td>-0.0031</td>
<td>-0.00017</td>
<td>-0.0011</td>
<td>0.0030</td>
<td>-0.00009</td>
<td>0.24</td>
</tr>
<tr>
<td>RRIM 701</td>
<td>-0.0021</td>
<td>-0.00012</td>
<td>-0.0017</td>
<td>0.0039</td>
<td>0.00005</td>
<td>0.93</td>
</tr>
<tr>
<td>PB 86</td>
<td>-0.0053</td>
<td>-0.00020</td>
<td>-0.0011</td>
<td>0.0064</td>
<td>-0.00009</td>
<td>1.01</td>
</tr>
<tr>
<td>S.E., average</td>
<td>0.00037</td>
<td>0.000033</td>
<td>0.00021</td>
<td>0.00036</td>
<td>0.000059</td>
<td>0.073</td>
</tr>
<tr>
<td>Min. sig. diff. (P=0.05)</td>
<td>0.0010</td>
<td>0.00009</td>
<td>0.0006</td>
<td>0.0010</td>
<td>0.00016</td>
<td>0.20</td>
</tr>
</tbody>
</table>

The standard errors of the slopes for the different clones with respect to each nutrient are somewhat different. For simplicity, an average error has been used for approximate comparison.

more or less constant throughout the period of 40 to about 270 days of leaf age. For calcium and manganese, the leaf content increased steadily throughout this period. Calcium concentration increased from about 0.4 to about 1.2% Ca, and manganese from about 30 to 150 p.p.m. Mn, during the same period.

There was no marked change in dry weight of leaves during this period. It would therefore appear that the fall in concentration of nitrogen, phosphorus and potassium in leaf is not due to dilution, but probably because of back translocation from leaf to the tree, leaching loss by rain or a combination of both.

Sampling of leaves for advisory purposes is usually carried out at a time when the fluctuations in nutrient contents are minimum. The initial period of about forty days after leaf emergence is not therefore suitable for such sampling. The present practice of sampling only leaves fully expanded but not senescent, or at a leaf age of between 40 and 250 days, is desirable. Even during this period, however, the concentration of nitrogen, phosphorus, potassium and manganese shows a gradual change — the pattern of it was therefore critically examined. Regression lines for changes in nutrient contents with age of leaves for this period are shown in Figure 1(a). Nitrogen is significantly correlated with leaf age, the correlation coefficients varying from -0.66 to -0.93 for the different clones. For phosphorus, the correlation coefficient for four clones varies between -0.59 and -0.91. For potassium, calcium and manganese, highly significant linear correlations were obtained, the correlation coefficients (negative for potassium) being of the order of 0.80 or above, except for potassium in clone PB 86. For magnesium, no consistent correlation with leaf age was found, the coefficient varying between +0.14 and -0.46.

Variation with Clones

Results of the study have been used for a comparison between clones, in respect of leaf nutrient content. Since the period after forty days of leaf age is important in practice, the comparison was made for this period only. Figure 1(b) shows the regression lines for the five clones for each nutrient. The comparison was made in respect of (a) the rate of change in nutrient concentration in relation to leaf age, as given by the slopes of the regression lines and (b) the levels of the nutrients. A comparison of the slopes of the regression lines, as given in Table 1, shows that the rate of change in concentration of nitrogen in relation
Figure 1(b). Relationship between leaf nutrient content and age of leaves: comparison of clones using the individual regression lines.
TABLE 2. LEAF NUTRIENT CONTENTS FOR DIFFERENT CLONES AT 50 DAYS LEAF AGE
(Calculated from regression lines with common slopes)

<table>
<thead>
<tr>
<th>Clone</th>
<th>(N) %</th>
<th>(P) %</th>
<th>(K) %</th>
<th>(Ca) %</th>
<th>(Mg) %</th>
<th>(Mn) p.p.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RRIM 513</td>
<td>3.32</td>
<td>N.D.</td>
<td>1.00</td>
<td>0.61</td>
<td>N.D.</td>
<td>23</td>
</tr>
<tr>
<td>RRIM 605</td>
<td>3.37</td>
<td>0.193</td>
<td>1.07</td>
<td>0.44</td>
<td>0.194</td>
<td>N.D.</td>
</tr>
<tr>
<td>RRIM 623</td>
<td>3.47</td>
<td>0.189</td>
<td>0.90</td>
<td>0.28</td>
<td>0.209</td>
<td>N.D.</td>
</tr>
<tr>
<td>RRIM 701</td>
<td>3.48</td>
<td>0.205</td>
<td>1.12</td>
<td>0.36</td>
<td>N.D.</td>
<td>41</td>
</tr>
<tr>
<td>PB 86</td>
<td>N.D.</td>
<td>0.199</td>
<td>1.09</td>
<td>N.D.</td>
<td>N.D.</td>
<td>44</td>
</tr>
<tr>
<td>S.E. of diff.†</td>
<td>0.025</td>
<td>0.0024</td>
<td>0.015</td>
<td>0.024</td>
<td>0.0044</td>
<td>4.8</td>
</tr>
<tr>
<td>Min.sig.diff. ((P=0.05))</td>
<td>0.05</td>
<td>0.005</td>
<td>0.03</td>
<td>0.05</td>
<td>0.009</td>
<td>10</td>
</tr>
</tbody>
</table>

N.D. = not determined since a common slope is not applicable
† Approximate, based on average number of observations in each case

To leaf age is similar for clones RRIM 513, 605, 623 and 701, but that of PB 86 is different. For phosphorus, the slopes are somewhat similar for RRIM 605, 623, 701 and PB 86, but that of RRIM 513 is different. For potassium, all clones have almost similar slopes, although the slopes for RRIM 623 and PB 86 are the most divergent ones. For calcium, PB 86 shows a significantly different slope. Although the slope for RRIM 623 is somewhat different from that for RRIM 513, this is not different from the slopes of the other two clones. The regression lines for magnesium concentration show similar but small significant slopes for RRIM 605 and 623. The regression lines of leaf magnesium content for the three other clones are almost horizontal. RRIM 513, 701 and PB 86 show similar slopes for manganese. The slopes for RRIM 605 and 623 differ significantly between themselves and from the other clones.

Thus it is possible to combine the slopes of four clones (excluding PB 86) for nitrogen; of four clones (excluding RRIM 513) for phosphorus; of all the five clones for potassium; and of four clones (excluding PB 86) for calcium. For magnesium, the slopes for the two clones (RRIM 605 and 623) are combined. For manganese, the slopes for three clones (RRIM 513, 701 and PB 86) are combined. The regression lines with these common slopes are shown in Figure 1(c). These regression lines with common slopes permit easy comparison between clones with respect to the levels of nutrient contents in leaves, at any age, as the effect of leaf age variation has been made similar for the clones. These levels of leaf nutrient contents for the clones with common slopes are shown in Table 2. It can be seen that significant differences exist between the clones. The maximum differences observed for the different nutrients are of the order of 0.16% N (for example, the values at fifty days leaf age were 3.32% N for RRIM 513 and 3.48% N for RRIM 701), 0.016% P, 0.23% K, 0.33% Ca, and of the order of about 20 p.p.m. Mn.

Although these differences are significant, the magnitudes are rather small. Further studies on other sites and for different years are required before this clonal differences can be fully substantiated. Until such differences are found under more widely varying conditions, common regression lines showing the changes in nutrient contents with leaf age may be used. These common regression lines
Figure 1(c). Relationship between leaf nutrient content and age of leaves: comparison of clones using regression lines with common slopes.
Figure 1(d). Relationship between leaf nutrient content and age of leaves: common regression lines for similar clones (***P<0.001, **P<0.01).
over the clones which gave similar slopes are drawn in Figure 1(d). These relationships however, should not be used for clones like PB clones, GT 1 and Gl 1 etc., which are suspected to have different leaf nutrient levels.

**Interpretation of Leaf Analytical Results**

The relationship between leaf nutrient content and leaf age can be used for better interpretation of leaf analytical data for advisory purposes. It can be seen from Figure 1(d) that while nitrogen, phosphorus and potassium contents show negative correlations, that for calcium shows a positive correlation with age of leaves. If the leaf age is known, the relationships can be used to determine the effect of age variation on leaf nutrient content. In practice, however, leaf age is not known unless the leaves are marked at the time of emergence and is only roughly known even if wintering or refoliation time is carefully observed.

Where leaf age is not known, calcium content can be used to estimate leaf age; and corrections to the other nutrients can be made accordingly. This is possible only if leaf calcium content is not affected by any factor other than leaf age. Calcium status of rubber trees is generally found to be satisfactory in Malaya. Except possibly on some very acid soils, mainly in coastal areas, application of calcium to change the calcium content in leaf is not generally necessary.

Figure 2(a) shows the N-Ca and K-Ca relationships for the five clones for the 50 to 250 days leaf age period. Linear regressions with correlation coefficients varying from -0.70 to -0.92 (significant at 0.1% level) are obtained in all cases, except for K-Ca relationship in PB 86. The regression lines for the different clones are compared in Figure 2(b). Table 3 gives the slopes of the regression lines with their standard errors. It is seen that for N-Ca relationship, the slopes for the five clones are somewhat similar. For the K-Ca relationship, the slopes are similar for four clones, with the exception of PB 86. For the N-Ca relationship, therefore, the slopes of the five clones are combined, while for K-Ca relationship only four clones are combined. These are shown in Figure 2(c). Irrespective of the clonal differences with respect to leaf nitrogen and potassium contents, the common N-Ca and K-Ca relationships, over the five and four clones respectively, are obtained. These two common regression lines, as shown in Figure 2(d), can therefore be taken to indicate general N-Ca and K-Ca relationships.

The critical values for *Hevea* leaf nutrient contents published earlier (RUBBER RESEARCH INSTITUTE OF MALAYA, 1963) represent the values for the leaves which are fully expanded but not senescent. If the leaf age is more rigidly specified in terms of leaf calcium content, and if the age chosen is that when the leaf calcium content is 0.8%, the nitrogen and potassium contents of leaves of varying age as obtained by advisory sampling can be corrected making use of the above relationships. The corrections required will be:

(a) if the calcium content is above 0.8%, add 0.065% N, or 0.034% K in the case of potassium, to the figure obtained by analysis for every 0.1% Ca by which the observed calcium value is in excess of 0.8%; and

<table>
<thead>
<tr>
<th>Clone</th>
<th>N-Ca</th>
<th>K-Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>RRIM 513</td>
<td>-0.58</td>
<td>-0.29</td>
</tr>
<tr>
<td>RRIM 605</td>
<td>-0.75</td>
<td>-0.40</td>
</tr>
<tr>
<td>RRIM 623</td>
<td>-0.91</td>
<td>-0.35</td>
</tr>
<tr>
<td>RRIM 701</td>
<td>-0.51</td>
<td>-0.40</td>
</tr>
<tr>
<td>PB 86</td>
<td>-0.67</td>
<td>-0.17</td>
</tr>
<tr>
<td>S.E., average</td>
<td>0.098</td>
<td>0.057</td>
</tr>
<tr>
<td>Min. sig. diff. (P = 0.05)</td>
<td>0.27</td>
<td>0.16</td>
</tr>
</tbody>
</table>

The standard errors of the slopes for the different clones in each case are somewhat different. For simplicity, an average error has been used for approximate comparison.
Figure 2(a). $N$--Ca and $K$--Ca relationships in leaf: fitting of regression lines (** P<0.001, * P<0.05).
Figure 2(b). $N$--$Ca$ and $K$--$Ca$ relationships in leaf: comparison of clones using the individual regression lines.

Figure 2(c). $N$--$Ca$ and $K$--$Ca$ relationships in leaf: comparison of clones using regression lines with common slopes.

Figure 2(d). $N$--$Ca$ and $K$--$Ca$ relationships in leaf: common regression lines for similar clones (***$P<0.001$).
(b) if the calcium content is below 0.8 %, subtract 0.065% N, or 0.034% K in the case of potassium, from the figure obtained by analysis for every 0.1 % Ca by which the observed calcium value is below 0.8 %.

CONCLUSIONS

Variation in leaf age, even after the leaves were fully expanded, affects the leaf nutrient contents significantly. Proper interpretation of leaf analytical data is therefore possible only if leaves of standard age are sampled or the nutrient contents in leaves of variable age are corrected for age variation. The linear relationships between the changes in leaf nutrient content and age of leaves reported above may be used for such correction, when the age of leaves sampled is known.

Where age of leaf is not known, it is suggested that calcium content in leaf may be used to estimate the leaf age and for correcting the leaf nutrient contents, provided that the leaf calcium content is not affected by any factor other than leaf age. It was found that an increase or decrease by 0.065 % N and 0.034 % K for every 0.1 % Ca in excess of or below 0.8 % Ca in leaf, respectively, was necessary for correcting leaf nitrogen and potassium contents.

ACKNOWLEDGEMENT

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REFERENCES


DISCUSSION

Chairman: Dr. S.K. Ng

Professor G.E. Blackman observed that three phases could be distinguished in the development of leaves. The first was cell multiplication, when the cells had not yet become vacuolated and the cell walls were very thin; during this phase, the proportion of cytoplasm and the contents of nitrogen and phosphorus were highest. In the next phase, the vacuoles, in which nutrients such as potassium accumulated, formed after cell division had almost completely ceased. In the final phase, expansion ceased, but there was a continued
thickening of the cell walls in which calcium continued to be deposited. Therefore, as the cell walls attained a progressively higher proportion of the total dry weight, an increase in the content of calcium could be anticipated. He queried the existence of a causative relationship between Ca and N and K.

Dr. J-P. Poliniere said that very similar variations in foliar contents of nutrients with clone and age had been found in Viet-Nam, except that comparisons were complicated by a range of six weeks in the date of wintering of different clones. Dr. Guha considered that the corrections based on the content of calcium would be valid in these circumstances. Mr. R. Shepherd said a survey of commercial estates in Malaya had given similar clonal differences in the foliar contents of nutrients, and had also shown that the size of mature leaves (a ninefold range in leaves from the same trees) had no significant effect on the analytical results. He asked whether a correction for variation in phosphorus content with age could be devised similar to those for nitrogen and potassium, based on the content of calcium. Dr. Guha agreed that such a correction for phosphorus could be calculated, but it was a less critical and urgent problem than either nitrogen or potassium, because the variation in content of phosphorus with age was relatively small. Moreover, application of phosphate to mature trees was seldom necessary in practice owing to the large reserves accumulated in the soil during immaturity.

Dr. P. Compagnon remarked that PB 86 seemed to exhibit symptoms of deficiencies of phosphate and magnesium on a given soil more readily than other clones. Dr. Guha agreed that different critical levels might prove necessary in the interpretation of foliar analysis for particular clones, especially with respect to potassium and magnesium. Mr. E.C. Paardekooper remarked that leaves were commonly collected for analysis in September or October when, allowing for differences between clones in the date of wintering, leaves were 200–250 days old. The data presented showed little variation over this period. In practice there might be little error using uncorrected figures for samplings in September and October. Dr. Guha agreed this was satisfactory for the limited acreage of experimental areas, but such a restricted sampling season did not permit economic deployment of labour for countrywide surveys of commercial areas. Mr. Paardekooper added that a threefold range in the clone means for the foliar content of calcium had been found when sampling clone trials at a particular date; this cast doubt on calcium as the universal basis to correct for age. Incidentally, significant differences between clones had never been detected for foliar manganese. Dr. Guha said that although the sampling throughout the clone trial was carried out at one time, there might yet be confounding of the true age of the leaves when sampled with clones according to their dates of wintering. Clonal differences could only be established with confidence by examining the foliar contents for the different clones at several ages.