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A Modified Procedure for Foliar Sampling of Hevea brasiliensis

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The current procedures of foliar sampling and laboratory preparation of samples for chemical analysis were examined. A modified approach in which the foliar samples were collected and analysed without further sub-sampling in the laboratory was studied. Statistical analysis of results obtained shows that the modified procedure is comparable to the existing procedure and can be adopted for determining the nutritional status of Hevea *leaves.*

The reliability of foliar data to assess the fertiliser requirement of *Hevea* depends on the choice of foliar sampling technique. The current sampling procedure[1,2] consists of taking one composite leaf sample from thirty trees randomly selected over an area of 15–20 ha. For mature trees, four basal leaves (triplets) of a terminal whorl which constitute the low shade leaves are sampled. The total number of leaflets (three leaflets per leaf) for each composite sample will thus be 360 (30 trees × 4 leaves per tree × 3 leaflets per leaf). To reduce the bulk of a sample, sub-sampling before drying and grinding for chemical analysis is necessary.

The factors affecting accuracy and precision of routine field sampling and analysis had been extensively studied[3-6]. In general, the variability of foliar data is attributed to two major sources of errors, *viz.* laboratory errors and field sampling errors. While field sampling errors can be reduced by increasing the number of leaves per unit area, laboratory errors can be minimised by further simplifying the procedure for plant analysis.

In recent years, the total area under rubber on which soil and foliar nutrient surveys for discriminatory fertiliser recommendations were carried out had increased to more than 115 000 ha a year and the number of foliar samples collected for analysis had exceeded 13 000 samples[7]. As a result of this increase, the workload in leaf sampling has increased. To cope with the increase, there is a need to re-examine the current procedure of foliar sampling and laboratory analysis.

This paper attempts to determine whether modifications to the existing procedure can be made without affecting the precision and accuracy of results, especially the nutrient composition of the major elements. The benefits derived from the modification are also discussed.

EXPERIMENTAL

Field Sampling

Leaf samples from mature rubber were collected according to the established procedure reported by Chan[1]. The established procedure *(Procedure I)* and the modified procedure *(Procedure II)* are described below.

Procedure I. In each composite leaf sample, low shade leaves were sampled from thirty trees randomly selected over an area of 15–20 ha. From each tree, the four basal leaves from the terminal whorls on any low branch in the shade, excluding spurs of limited growth, were taken. The number of leaflets (three leaflets per leaf) from each tree was twelve and the total number of leaflets for each composite sample amounted to 360. The leaves, packed in paper bags, were sent immediately for analysis.

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Procedure II. The procedure for collecting leaf samples was similar to that described in *Procedure I.* Instead of taking all the basal leaves, only the centre leaflet of each of the four basal leaves was sampled. When the centre leaflet was not suitable, one of the other leaflets within the same whorl was taken. At four leaflets per tree, the total number of leaflets in each composite sample was 120.

Preparation of Samples for Analysis

After removing the petioles, the leaves were cleaned with a moist linen, dried at 75°C–80°C and ground to pass through a 0.55 mm screen. To reduce the bulk of leaf samples collected by *Procedure I,* sub-sampling for drying and grinding was required. Precautions were taken to ensure that the composite sample was thoroughly mixed before it was sub-sampled.

To estimate the errors due to sub-sampling and chemical analysis, each of ten composite samples obtained by *Procedure I* was divided into four sub-samples of ninety leaflets each. The sub-samples were dried, ground and analysed in triplicates for major elements. Estimates of errors were obtained from analysis of variance according to the scheme given in *Table 1.*

Chemical Analysis

The leaf samples were analysed according to the standard procedure of the Rubber Research Institute of Malaysia[8]. About 2 g of milled leaves were charred on a hot plate and then ignited in a muffle furnace for 2 h at about 580°C. The ashed material was treated with concentrated hydrochloric acid and then digested with 20% volume/volume nitric acid.The leaf extract was analysed for phosphorus, potassium, calcium, magnesium and manganese. Nitrogen in the leaf sample was determined by the Kjeldahl digestion method followed by semi-micro distillation in a Markham apparatus. In all cases, the nutrient composition was adjusted to those at optimum leaf age[9].

The relationship between nutrient composition in leaves obtained by *Procedures I* and *II* was assessed by chemical analysis of 330 samples simultaneously collected by the two procedures. The samples were from trees of different clones, age and exploitation and grown on different soils. In addition, to test the precision and reproducibility of results, twenty-nine composite samples collected by the two procedures were analysed in triplicates. All the results were statistically analysed and examined.

RESULTS

Sub-sampling and Analytical Errors

Procedures I and *II* for sampling leaves for chemical analysis are technically similar. The only difference is that *Procedure II* does not require sub-sampling before drying and grinding. Assuming that errors attributed to operator, day-to-day operation and chemical analysis are identical for the two procedures, the major source of error in *Procedure I* is sub-

TABLE 1. ANALYSIS OF VARIANCE TO ESTIMATE THE VARIOUS SOURCES OF ERRORS

Source of variation	Degrees of freedom	Mean squares expressed as components of variance
Bulk samples	1	
Between different bulk samples	9	$\sigma_r^2 + 3\sigma_{ss}^2 + 12\sigma_s^2$
Between sub-samples within samples	30	$\sigma_r^2 + 3\sigma_{ss}^2$
Between triplicate chemical analysis	80	σ_r^2
Total	120	

sampling error. The scheme for determining sub-sampling error as well as error in chemical analyses in *Procedure I* is given in *Table 1* in which mean squares, are expressed as components of variance. From the mean squares, the errors arising from sub-sampling and chemical analyses can be computed and expressed as coefficients of variation(%) through the following equations:

$$\text{Analytical error} = \frac{100}{\text{Mean}}\sqrt{\sigma_r^2}$$

$$\text{Sub-sampling error} = \frac{100}{\text{Mean}}\sqrt{\frac{(\sigma_r^2 + 3\sigma_{ss}^2) - \sigma_r^2}{3}}$$

where σ_r^2 and σ_{ss}^2 are variance associated with chemical analyses, and sub-sampling, respectively.

The estimates of sub-sampling and analytical errors expressed as coefficients of variation (CV) are shown in *Table 2*. The sub-sampling errors for nitrogen, phosphorus, potassium, calcium, magnesium and manganese as determined by the CV ranged from 3.2% to 8.6% whereas the analytical errors were within the range of 3.9% to 7.2%. Except for nitrogen and potassium, errors for sub-sampling and chemical analyses were much larger and exceeded the CV value of 5%. These relatively large errors were consistent with the CV values of 5%–10% in plant analyses cross-checks among Malaysian laboratories held in the 1983–85 period[10]. Additionally, the inter-laboratory cross-checks also showed that the number of 'rogue' values (analytical results that had deviated by more than twice the standard deviation from the overall mean) had increased tremendously in the participating laboratories. In this study, 'rogue' values as defined were not omitted when statistical computations were made. All analyses were carried out in a routine manner with no special emphasis given or precaution taken. Further to this, the sampling and analysis were done to coincide with the peak season of soil and foliar survey.

Relationship between Procedures I and II

In an attempt to determine whether there were significant differences in foliar data when the leaves were sampled by the two procedures, the analytical results of 330 composite samples were studied. Significant correlations between the results of samples collected by *Procedures I* and *II* were obtained. These correlations as given in *Table 3* were significant at $P < 0.001$. The regression equations as obtained for each element were also shown in *Table 3*. For all the major elements, the slopes of the regression lines were significantly different and so were the regression coefficients. The regression lines in all cases did not originate from the origin.

Comparing the nutrient composition of twenty-nine foliar samples collected by the two procedures, it was observed that samples collected by *Procedure II* had higher values. In *Table 4*, pairwise t-test showed that there were significant differences in the nitrogen, phosphorus, potassium, calcium and magnesium concentrations of the leaves. Nitrogen and phosphorus registered the most significant diference at the 0.1% level whereas there was no significant difference in the manganese values. The difference in leaf values is anticipated since it is not possible to sample leaves of the same age and position for this study. Although there are significant differences in leaf values, a study of the mean of each composite sample showed that the differences seldom exceed by more than 5% of the overall mean values. Furthermore, these differences were reduced when provisions were made for the leaf age.

The precision and accuracy of *Procedures I* and *II* in determining the leaf nutrient status of rubber were assessed. In *Table 5*, *F*-values to determine whether there was any significant difference in precision between the two procedures were given. With the exception of phosphorus, no significant differences in nitrogen, potassium, calcium, magnesium and manganese determinations were noted.

DISCUSSION

Apart from some minor changes, the two foliar sampling procedures are generally similar. In *Procedure I*, the bulk samples require sub-

TABLE 2. COMPONENTS OF ERRORS IN PROCEDURE I: STANDARD DEVIATION AND COEFFICIENTS OF VARIATION

Sources of variation	Overall mean C.V.(%)	Nitrogen		Phosphorus		Potassium		Calcium		Magnesium		Manganese	
		Mean = 3.15%		Mean = 0.230%		Mean = 1.21%		Mean = 1.121%		Mean = 0.291%		Mean = 130 p.p.m.	
		s.d.	CV(%)	s.d.	CV(%)	s.d.	CV(%)	s.d.	CV(%)	s.d.	CV(%)	s.d.	CV(%)
Analytical error	5.83	0.123	3.90	0.0160	6.96	0.0605	4.88	0.0652	5.82	0.0181	6.22	9.3980	7.22
Sub-sampling error	5.77	0.094	3.16	0.0121	5.26	0.0494	4.08	0.0757	6.75	0.0196	6.74	11.1800	8.60
Total error	8.28	0.160	5.09	0.0201	8.74	0.0782	6.46	0.1000	8.92	0.0267	9.18	14.66	11.28

Total error is made up of analytical and sub-sampling errors.

TABLE 3. CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS BETWEEN THE TWO SAMPLING PROCEDURES

Nutrient	Overall mean (%)	Regression equation	Correlation coefficient (r)
Nitrogen	Y = 3.38 X = 3.25	Y = 0.506 (±0.026) + 0.811X (±0.043)	0.72***
Phosphorus	Y = 0.234 X = 0.244	Y = 0.046 (±0.002) + 0.843X (±0.035)	0.96***
Potassium	Y = 1.55 X = 1.59	Y = 0.115 (±0.007) + 0.955X (±0.026)	0.89***
Calcium	Y = 0.943 X = 1.001	Y = 0.182 (±0.010) + 0.870X (±0.086)	0.80***
Magnesium	Y = 0.385 X = 0.389	Y = 0.102 (±0.006) + 0.748X (±0.057)	0.58***

Y = Procedure I; X = Procedure II; all values are adjusted for leaf age.
Number of composite samples is 330.
Figures within brackets are standard errors.
*** $P<0.001$

TABLE 4. PAIRWISE T-TESTS FOR DIFFERENCES BETWEEN THE MEANS OF THE TWO PROCEDURES

Item	Nutrient composition					
	N(%)	P(%)	K(%)	Ca(%)	Mg(%)	Mn(p.p.m.)
Mean value Procedure I	3.36 (3.63)	0.266 (0.223)	1.35 (1.37)	1.050	0.378	119
Mean value Procedure II	3.23 (3.58)	0.239 (0.241)	1.38 (1.39)	1.106	0.394	120
t-value	-6.55***	6.25***	2.52*	3.56**	2.09*	0.54NS
n	29	29	29	29	29	29

***$P<0.001$; **$P<0.01$; *$P<0.05$
NS - Not significant
Figures within brackets are values after adjustment for optimum leaf age.

sampling for drying and grinding. Unless steps are taken to ensure that all the sampling trees are adequately represented, the sub-sampling errors could be large as shown in *Table 2*. It is interesting to note that the sub-sampling errors for all the nutrient elements contributed as much as the analytical errors to the total error. Particularly during the peak sampling period between May and October, the need to prepare and analyse the large number of samples daily can affect the quality of results. Under these circumstances, the day-to-day operators' errors as studied by Middleton *et al.*[4] would tend to become significant.

TABLE 5. F-TESTS FOR SIGNIFICANCE OF DIFFERENCES IN PRECISION BETWEEN THE TWO PROCEDURES

Item	Nutrient element					
	N	P	K	Ca	Mg	Mn
Within group error variance						
Procedure I	0.0151	0.0897×10^{-3}	0.0030	0.0054	0.0004	125.59
Procedure II	0.0102	0.0483×10^{-3}	0.0028	0.0034	0.0003	124.06
F-value	1.48NS	1.86*	1.07NS	1.57NS	1.33NS	1.01NS

*$P < 0.05$

NS: Not significant

In *Procedure II*, the sub-sampling step in the laboratory is omitted and all the materials that are required for analysis have already been carefully selected and bulked together in the field. The question that all the sampling trees are not represented does not arise. The samples are immediately dried and ground. The problem of storage in a refrigerator is also minimised when the samples can not be dried and ground immediately upon arrival. Furthermore, it is more convenient to transport samples from the field to the laboratory as the number of cardboard boxes required to contain the same number of samples would be reduced. A normal cardboard box would then contain about thrice the number of samples compared to *Procedure I*.

Foliar results of samples collected by the two procedures were highly related to each other. There was no significant difference in the level of precision and accuracy in the two procedures. In view of this and coupled with the high correlation of the results, the current interpretation of foliar data for manurial purposes is unlikely to be affected if the foliar samples were collected by *Procedure II*.

CONCLUSION

Considering that the number of foliar samples for laboratory analysis had increased significantly over the years, it is pertinent that the current field sampling procedures be modified to reduce the various sources of errors. One of these sources of errors is sub-sampling in the laboratory for drying and grinding. The modified procedure does not require further sub-sampling and the samples thus collected truly represent the area. In addition to this, the procedure also minimises the cost of transporting samples to the laboratory, storage problems and space in the oven for drying. In view of the reduced volume, the samples collected would be easier to handle in smaller cardboard boxes both in the field and subsequent collection from the transport station. Additionally, the size of leaf bags can be smaller for convenience in handling and storage in the field as well as in the laboratory.

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