*Leaf Analysis as a Guide to the Nutrition of Hevea brasiliensis. V. A Leaf Sampling Technique For Mature Trees**

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The effects of ammonium sulphate, rock phosphate, potassium chloride, manganese sulphate and copper sulphate applications on the nutrient composition of leaves exposed to full sunlight and of two types of leaf in the shade of the canopy were investigated.

Shaded leaves collected from low branches were found to be more sensitive to changes in nitrogen and phosphorus status due to fertiliser applications to the soil and less sensitive to changes in the magnesium and calcium status than were leaves exposed to full sunlight. Little difference in the sensitivity to changes in potassium, manganese and copper status was found between shaded and exposed leaves.

The relationship between the number of trees sampled in a visually uniform field and the precision attained in estimating the content of each nutrient in the three leaf types is discussed.

It is concluded that the collection of shaded leaves from low branches forms a suitable and convenient sampling technique for studies on the mineral nutrition of mature Hevea brasiliensis.

Investigations on leaf sampling procedures for mature rubber (SHORROCKS, 1961a and 1962a) have shown that leaves exposed to full sunlight ('light leaves') and leaves in the shade ('shade leaves') differ markedly in their nutrient composition, and that in general shade leaves are more variable in their nutrient composition than light leaves.

While there are indications (SHORROCKS, 1961a) that shade leaves reflect some of the effects of fertilisers more clearly than do light leaves, this preliminary work did not demonstrate that shade leaves could be used to provide a sensitive and accurate index of the tree nutrient status.

The present study was designed mainly to investigate in detail the effects of fertilisers on light leaves and the two types of shade leaves, in order to determine whether one leaf type showed the effects of fertilisers on leaf nutrient composition more clearly and consistently than another and to determine whether the most convenient method, sampling low leaves in the shade, would prove to be a sufficiently sensitive index of nutrient status. The suitability of the methods was decided on the basis that the leaf type which was the most sensitive to changes in nutrient composition resulting from fertiliser treatments was likely to be the one which reflected most clearly the nutrient status of the tree. The investigations were carried out on six areas used for fertiliser experiments, including plantings of different clones, situated on a variety of inland soils.

In other areas a detailed investigation of the tree-to-tree variation in nutrient composition of each leaf type was also carried out, in

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order to determine the relationship between the number of trees sampled to provide leaves for a bulk leaf sample, and the precision attained in estimating the population mean for each nutrient: these investigations were carried out on four fields each of which was homogeneous in respect of planting material and fairly uniform in respect of tree growth.

METHODS

PART I. EFFECT OF FERTILISER APPLICATIONS ON THE NUTRIENT COMPOSITION OF SHADED AND EXPOSED LEAVES

Details of the six fertiliser experiments are summarised in *Table 1*. Three types of leaf were collected:

1. *Light leaves:* from terminal whorls exposed to full sunlight at the top of the canopy.
2. *Low leaves—shade:* from terminal whorls on any low branch in the shade, excluding spurs of limited growth.
3. *Spur leaves—shade:* from the single terminal whorl on spurs of limited growth in the shade of the canopy, according to the method of BEAUFILS (1955).

Figure 1. A terminal whorl of leaves from which the basal four leaves are taken for analysis.

Two whorls were collected from each of six trees in each plot and the basal four leaves were taken (*Figure 1*). A plot sample for each leaf type was formed by bulking the leaves taken from the six trees. In Experiments 1 and 2, where two samplings were carried out, the samples were taken from the same six trees on both occasions.

The petioles but not the midribs were discarded. The preparation of the leaf samples and the analytical procedures employed have been described by BOLLE-JONES (1954), BOLLE-JONES, MALLIKARJUNESWARA AND RATNASINGAM (1957) and SHORROCKS (1962a). All analytical results are quoted in the Tables as percentages or p.p.m. on a dry weight basis.

An analysis of variance was carried out for each nutrient determined, and the appropriate standard errors of the leaf type means were calculated and used to test for significant differences between means.

PART II. TREE-TO-TREE VARIATION IN THE NUTRIENT COMPOSITION OF SHADED AND EXPOSED LEAVES

The three types of leaf described above were collected from a large number of trees in four fields, the details of which are shown in *Table 2*. One whorl of each leaf type was taken from each tree and the basal four leaves removed to form a discrete sample for analysis. The nitrogen, phosphorus, potassium, magnesium, calcium and manganese concentrations in the leaves were determined.

The sampled trees were chosen at random and were evenly distributed over the entire field. The even distribution was achieved by sampling at the uniform rate of five trees from each tapping task in the whole field. The trees sampled from within each task were selected along a diagonal across the task, and were evenly distributed along this diagonal.

The variances of each nutrient element, for each leaf position, were calculated for each field, as an estimate of the tree-to-tree varia-

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tion for the individual nutrient content and position.

The following formula was employed to calculate the error (here called the permissible error) involved in the estimation of the population mean value for each nutrient content of each leaf type for different numbers (n) of trees sampled:

$$(\text{Permissible error})^2 = \frac{(t_{n-1})^2 \times \text{variance}}{n}$$

RESULTS

PART I. EFFECT OF FERTILISER APPLICATIONS ON THE NUTRIENT COMPOSITION OF SHADED AND EXPOSED LEAVES

A. Effects of Ammonium Sulphate

Nitrogen content. There were significant increases in the leaf nitrogen content (*Table 3*): the magnitude of this effect was greater in the shade leaves than in the light leaves, and it was slightly greater in low leaves—shade than in spur leaves—shade, except in Experiment 4 where only the spur leaves—shade showed the significant positive effect of N on nitrogen content.

Potassium content. There was a significant reduction in the leaf potassium content of all leaf types except low leaves—shade in Experiments 2 (1961 sampling) and 4 (*Table 4*). The effect of N on the potassium content was not consistently more marked in one leaf type than in another: in Experiment 1, low leaves—shade and spur leaves—shade showed the effect more clearly than light leaves, whereas in Experiment 2, the light leaves showed the effect more clearly; in the other experiment there was little difference between the magnitude of this effect in shade and light leaves. The spur leaves—shade appeared to show the negative effect of N on potassium content slightly more clearly than the low leaves—shade.

Magnesium content. Magnesium content was significantly reduced in Experiments 1 (1961 sampling), 2 and 4 only, but this effect was not shown by all leaf types in all experiments (*Table 5*) and there was no indication that one leaf type consistently showed the effect more clearly than another.

Calcium content. Calcium content was significantly reduced in Experiments 1, 2 and 4 and an indication of a similar effect was observed in Experiment 3 (*Table 5*). This negative effect of N was only observed in the shaded leaves in the 1960 samplings of both Experiments 1 and 2, whereas in the 1961 samplings of these two experiments the effect was observed in both the light leaves and the low leaves—shade. In Experiment 4 all leaf types showed the effect equally clearly.

Manganese content. There were significant increases in the manganese content of all leaf types in Experiment 1, and whilst there was no indication that the magnitude of this effect was greater in one leaf type than in another (*Table 6*) the effect reached a greater level of significance in the shade leaves than in the light leaves.

B. Effects of Potassium Chloride

Potassium content. There were significant increases in potassium content in all experiments (*Table 7*). The magnitude of this effect on shade leaves was found to be very similar to that in light leaves in Experiments 1 (1961 sampling), 2 and 4, whilst in Experiments 1 (1960 sampling) and 3 low leaves—shade showed the effect more clearly than did light leaves, and in Experiment 5 the light leaves showed the effect more clearly than did the shade leaves. Little difference in the magnitude of the positive effect of K on the potassium content of low leaves-shade and spur leaves—shade was observed.

Magnesium content. Leaf magnesium content was significantly reduced only in Experiments 1 (1960 sampling), 2 (1960 sampling) and 4 (*Table 8*), and in the light leaves the effect was either of greater magnitude or achieved a greater degree of significance than in the shade leaves.

Calcium content. There was significant reduction in the leaf calcium content in Experiments 1 and 4 only (*Table 8*). This negative effect of potassium chloride was observed only in the light leaves in Experiments 1 (1961

sampling) and 4, whereas in Experiment 1 (1960 sampling) all three leaf types showed the effect, in approximately equal magnitude.

C. *Effects of Rock Phosphate*

Phosphorus content. There was a significant increase in the leaf phosphorus content in all experiments except Experiment 2 (1961 sampling) *Table 9*. The magnitude of this effect was either greater in low leaves—shade than in the light leaves or approximately the same in both leaf types. Little difference in the magnitude of the positive effect of rock phosphate on the phosphorus content of low leaves—shade and spur leaves—shade was observed.

Calcium content. There was a significant increase in the leaf calcium content in Experiments 1, 2 and 4 (*Table 10*). This positive effect of rock phosphate was in general more clearly shown in the light leaves than in the low leaves—shade, and only in Experiment 2 (1961 sampling) was the effect evident in the low leaves—shade alone. Except in Experiment 4 the low leaves—shade showed this positive effect of rock phosphate on the calcium content more clearly than spur leaves—shade.

Magnesium content. Magnesium content was significantly reduced in Experiments 1, 2 and 4 (*Table 11*); there was no real indication that this effect was more marked in one leaf type than in another.

D. *Effects of Manganese Sulphate and Copper Sulphate*

Manganese content. Application of manganese sulphate resulted in significant increases in the leaf manganese content (*Table 12*) and there was slight indication that this effect was of greater magnitude in spur leaves —shade and low leaves—shade than in light leaves—shade.

Copper content. Application of copper sulphate caused very slight but significant increases in the leaf copper content of all leaf types and there was no difference between the three leaf types in the magnitude of this effect (*Table 13*).

PART II. TREE-TO-TREE VARIATION IN THE NUTRIENT COMPOSITION OF SHADED AND EXPOSED LEAVES

The variances of each nutrient element for each leaf position (*Table 14*) have been compared by calculating, for each nutrient, the ratio of variances between (a) low leaves—shade: light leaves, (b) spur leaves—shade: light leaves and (c) spur leaves—shade: low leaves—shade (*Table 15*). When the ratio of the variances was greater than unity the usual F variance-ratio test was used. When the ratio of the variances was less than unity its reciprocal was tested for significance in the same way.

Light: Shade

The variance of the potassium concentration of both types of shade leaves was significantly greater than that of the light leaves in all fields and a similar situation was found with respect to the phosphorus concentration with the exception of Field 1, where the variance for the phosphorus concentration of light leaves was significantly greater than that for low leaves—shade. The variances for both the calcium and manganese concentrations of leaves, and in the majority of the fields the variances differed significantly. No significant difference between the variance for the nitrogen concentration of low leaves—shade and light leaves was found, whilst in Fields 1 and 2 the light leaves had a greater variance than spur leaves—shade. The variances of the magnesium concentration of light and shade leaves did not exhibit any consistent pattern.

Spur Leaves—Shade: Low Leaves—Shade

The variances for the nitrogen and potassium concentrations of the two types of shade leaves did not differ significantly in any field. Whilst there were a few instances where a significant difference was observed between the variances of spur leaves—shade and low leaves—shade for the phosphorus, magnesium, manganese and calcium concentration, it appears that in general little difference occurs between the variances of the two types of shade leaf.

Relationship Between Number of Trees Sampled to Provide One Bulk Sample and the Precision in Estimating the Nutrient Composition

The variances for a given nutrient in low leaves—shade were found to differ between fields: Field 1 showed the minimal variance for nitrogen, phosphorus, potassium and calcium whilst Field 4 showed the minimal variance for magnesium and manganese (*Table 14*). In order to cover the maximum variability that is likely to be found in the nutrient composition of low leaves—shade the maximum variances for each nutrient in low leaves (underlined values in *Table 14*) have been taken for calculation in the formula:

$$(\text{Permissible error})^2 = \frac{(t_{n-1})^2 \times \text{variance}}{n}$$

The permissible error is the allowable difference between the population mean and the sample mean, and is not the minimal difference between the two means of a similar series that could be detected as significant at the 5% level (using t at $P < 0.05$). To calculate such a minimal difference the formula would be:

$$(\text{Minimal difference})^2 = \frac{(t_{2n-1})^2 \times \text{variance} \times 2}{n}$$

The permissible errors have been calculated from the maximum variances for each nutrient in low leaves—shade, for different numbers (n) of trees sampled (*Table 16*).

On the basis that a minimum precision of 10 – 15% of the mean value is sufficient for adequate representation of the population mean value for any nutrient it would appear from *Table 16* that under the most variable conditions, the sampling of forty trees from 65 acres enables a precision of under 10% of the mean value to be obtained in the estimation of the nitrogen, phosphorus, potassium and magnesium concentrations: the sampling of fifty trees would enable a precision of 10 – 15% of the mean value to be obtained in the estimation of the calcium and manganese concentration of low leaves-shade.

The increased precision obtained in the estimation of the manganese and the calcium concentration of low leaves by sampling fifty rather than forty trees is not considered sufficiently large to warrant the increased rate of sampling, particularly when it is considered that for the other four nutrient elements, which are of greater importance in rubber cultivation, a precision of under 10% is being obtained by sampling forty trees.

The precision obtained in the estimation of the nutrient content of all three types of leaves by sampling forty trees is shown in *Table 17* and the mean nutrient concentrations found in the three leaf types are given in *Table 18*. While the permissible error of a given nutrient for the three leaf types varies according to the magnitude of the variance it can be seen that there are no marked differences between the three leaf types in the permissible errors as a percentage of the mean: the phosphorus and potassium concentrations of shade leaves, which exhibit a higher variance for these nutrients than the light leaves, are estimated with a percentage precision of approximately the same order as the light leaves which have a lower phosphorus and potassium content.

It is apparent from *Table 17* that as with low leaves—shade, forty trees need to be sampled for both light leaves and spur leaves —shade in order to obtain a precision of under 10% of the mean value in estimating the potassium, nitrogen, phosphorus and magnesium concentrations, when the maximum variances of each nutrient for each leaf type are considered. To obtain a precision of 15% of the mean value in estimating the manganese and calcium concentration of light leaves it is necessary to sample more than forty trees.

DISCUSSION

The effects of fertilisers on leaf composition previously described (SHORROCKS, 1961b; RUBBER RESEARCH INSTITUTE OF MALAYA, 1962) have been confirmed in the present study. The results recorded in Part I made it possible to examine the relative sensitivity of light leaves and shade leaves as indicators of the nutrient status of the tree; in addition, the study of tree-to-tree variation in the re-

sults described in Part II permits an evaluation of the use of light and shade leaves on the basis of the precision obtainable and the size of sample required to give a desirable degree of precision.

RELATIVE SENSITIVITY OF LIGHT AND SHADE LEAVES TO NUTRIENT STATUS

Nitrogen and Phosphorus

The positive effects of ammonium sulphate on the leaf nitrogen content and the positive effect of rock phosphate on the leaf phosphorus content were in general more clearly shown in low leaves—shade than in light leaves.

Magnesium and Calcium

The negative effect of potassium chloride on the leaf magnesium content was in general more clearly shown in light leaves than in low leaves—shade, whereas the negative effect of rock phosphate and ammonium sulphate on leaf magnesium content were shown in both light leaves and low leaves—shade.

The positive effect of rock phosphate and the negative effect of potassium chloride on the leaf calcium content were in general both more clearly shown in light leaves than in low leaves—shade, whilst the negative effect of ammonium sulphate on the leaf calcium content was shown equally clearly in both low leaves—shade and light leaves. Thus light leaves are in general more sensitive to changes in magnesium and calcium content than are low leaves—shade.

Potassium, Manganese and Copper

Both low leaves—shade and light leaves showed the positive effects of potassium chloride and the negative effects of ammonium sulphate on the leaf potassium content and there was no indication that one leaf type showed the effect more clearly than another.

The positive effect of ammonium sulphate on the leaf manganese content was equally marked in both low leaves—shade and light leaves, whilst there was some indication that spur leaves—shade and low leaves—shade showed the positive effect of manganese sulphate on leaf manganese content more clearly than light leaves. Shade and light leaves showed the positive effect of copper sulphate on the leaf copper content equally clearly.

Choice of Leaf Type for Sampling

It is concluded that shade leaves collected from low branches are more sensitive to changes in the nitrogen and phosphorus status and less sensitive to changes in the magnesium and calcium status than leaves exposed to full sunlight, and that there is little difference in the sensitivity to changes in the potassium, manganese and copper status between these two types of leaf. Even where one leaf type was more sensitive than another in reflecting changes in the leaf nutrient composition imposed by treatment, the other leaf types generally indicated the same effect, but to a less marked degree. Only a few instances were recorded where one leaf type showed an effect without a similar effect being apparent on another leaf type.

On the assumption that the leaf type which is more sensitive to changes in the nutrient composition imposed by the treatment is likely to be the one which reflects more clearly the nutrient status of the tree (particularly in respect of the major nutrients), it is concluded that low leaves—shade are to be preferred to light leaves in all studies on the nutrient status of *Hevea brasiliensis*. Light leaves may be preferred to low leaves—shade only when studying the magnesium and calcium status of the tree, although, as has already been indicated, low leaves—shade are a suitable leaf type to indicate changes in these nutrient elements.

TREE-TO-TREE VARIATION IN THE NUTRIENT COMPOSITION OF LIGHT AND SHADE LEAVES

Study of the tree-to-tree variation in the nutrient composition of the different leaf types showed that shade leaves exhibited a greater variance for the potassium and phosphorus concentrations and a smaller variance for the manganese and calcium concentrations than

did the light leaves: these results provide confirmation and amplification of those of preliminary investigations (SHORROCKS, 1962a). No differences were found between the two types of shade leaf with respect to variances exhibited by either the nitrogen or the potassium concentration, and for other nutrients it appeared that little difference is to be expected between the variances for a given nutrient.

In order to obtain a precision of under 10% of the mean when estimating the population mean values for the concentration of nitrogen, phosphorus, potassium and magnesium in low leaves—shade, it was found necessary to sample forty trees, while fifty trees would be required to obtain a precision of 10 – 15% in the estimation of the manganese and calcium concentrations. Such errors were calculated on the basis of the maximum observed variance for any given nutrient.

For a given number of trees sampled, approximately the same percentage precision was obtained in estimating the nitrogen, phosphorus, potassium and magnesium concentrations in all three leaf types: there were indications that for manganese and calcium a greater number of trees would be required to be sampled for light leaves than for low leaves—shade in order to obtain the same percentage precision. This situation is to be contrasted with the finding that the shade leaves were less sensitive to changes in the calcium and manganese status than light leaves.

CONCLUSIONS

It is concluded that the collection of low leaves—shade provides a more satisfactory sampling technique than the collection of light leaves for studies of the mineral nutrition of mature *Hevea;* furthermore, this technique also has the advantage of being much simpler and safer than the collection of light leaves.

It is considered that the precision of estimating the nutrient concentrations in low leaves—shade obtained by sampling leaves from forty trees in an area no greater than 65 acres is sufficient to give an adequate representation of the population mean. Further consideration of the required precision is likely to be dependent upon the estimation of optimal values for the individual leaf nutrient contents and the errors involved in their estimation.

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TABLE 1. DETAILS OF EXPERIMENTS DISCUSSED IN PART I

	Experiment Number					
	1	2	3	4	5	6
Design	3^3 NPK	3^3 NPK	3^3 NPK	2^3 NPK	3^2 K, Cu	3 Mn
Number of plots	81	27	27	64	54	18
Clone	RRIM 501	PB 86	PB 86	Tjir 1	PR 107, Tjir 1 RRIM 501	Tjir 1, BR 2 PB 49, AVROS 157 RRIM 509
Planting distance	60′ × 4′	22′ × 11′	22′ × 11′	22′ × 11′	66′ × 4′	60′ × 4′
Year budded	1953	1951	1949	1952	1951	1950
Soil type	shale-derived	shale-derived	shale-derived	alluvial sand	granite-derived	granite-derived
Fertiliser applications:						
Ammonium sulphate (lb)	0, 8½, 17†	0, 2, 4*	0, 2, 4*	0, 7¾†	—	—
Potassium chloride (lb)	0, 2½, 5†	0, ⅓, ⅔*	0, ½, 1*	0, 2¾†	0, ¼, ½*	—
Rock phosphate (lb)	0, 8½, 17†	0, 1, 2*	0, 1½, 3*	0, 5¾†	—	—
Copper sulphate (oz)	—	—	—	—	0, 1½, 3*	—
Manganese sulphate (oz)	—	—	—	—	—	0, 4, 8*
Number of fertiliser applications	8	4	3	7	4	2**
Duration of experiment	1954 to 1961	1958 to 1961	1959 to 1961	1952 to 1958	1958 to 1961	1957, 1958

† Total fertiliser applications over experimental period, * Annual rate of fertiliser application, ** Only half the plots received the second application.

TABLE 2. DETAILS OF FIELDS DISCUSSED IN PART II

	Field Number			
	1	2	3	4
Size (acres)	65	65	65	115
Clone	RRIM 501	G1 1	PB 86	PB 86
Year budded	1952	1949	1949	1950
Soil type	granite-derived	alluvial-clay	granite-derived	shale-derived
Number of trees sampled	105	105	100	115
Number of tasks sampled	21	21	20	23

TABLE 3. THE EFFECT OF AMMONIUM SULPHATE ON THE NITROGEN CONCENTRATION OF LIGHT LEAVES, LOW LEAVES—SHADE AND SPUR LEAVES—SHADE

% N in dry lamina

	Experiment 1					Experiment 2		
Sampling dates	2.8.60			17.7.61		5.9.60		
	Light	Low, shade	Spur, shade	Light	Low, shade	Light	Low, shade	Spur, shade
Control (N_0)	3.33	3.33	3.35	3.66	3.74	3.07	3.29	3.34
N_1	3.44	3.64	3.54	3.74	3.80	3.42	3.70	3.66
N_2	3.52	3.73	3.66	3.76	3.90	3.46	3.73	3.75
s.e.	±0.030	±0.034	±0.030	±0.024	±0.031	±0.053	±0.099	±0.063
Linear effect ($N_2 - N_0$)	+0.19***	+0.40***	+0.31***	+0.10**	+0.16***	+0.39***	+0.44**	+0.41***

	Experiment 2			Experiment 3		Experiment 4		
Sampling dates	12.7.61			26.6.61		25.10.60		
	Light	Low, shade	Spur, shade	Light	Low, shade	Light	Low, shade	Spur, shade
Control (N_0)	3.52	3.57	3.67	3.39	3.43	3.38	3.38	3.33
N_1	3.66	3.95	3.90	3.67	3.67	3.40	3.39	3.43
N_2	3.61	4.15	4.10	3.65	3.90			
s.e.	±0.050	±0.128	±0.080	±0.076	±0.105	±0.022	±0.021	±0.026
Linear effect ($N_2 - N_0$)	+0.09	+0.58**	+0.43**	+0.26*	+0.47**	+0.02	+0.01	+0.10*

* $P<0.05$, ** $P<0.01$ and *** $P<0.001$

TABLE 4. THE EFFECT OF AMMONIUM SULPHATE ON THE POTASSIUM CONCENTRATION OF LIGHT LEAVES, LOW LEAVES—SHADE AND SPUR LEAVES—SHADE

% K in dry lamina

	Experiment 1					Experiment 2		
Sampling dates	2.8.60			17.7.61		5.9.60		
	Light	Low, shade	Spur, shade	Light	Low, shade	Light	Low, shade	Spur, shade
Control (N_0)	0.96	1.16	1.15	1.00	1.33	1.13	1.50	1.57
N_1	0.86	0.99	0.99	0.96	1.16	0.94	1.53	1.45
N_2	0.84	0.97	0.94	0.94	1.11	0.85	1.34	1.35
s.e.	±0.015	±0.024	±0.024	±0.016	±0.024	±0.047	±0.042	±0.059
Linear effect ($N_2 - N_0$)	-0.12***	-0.19***	-0.21***	-0.06**	-0.22***	-0.28***	-0.16*	-0.22*

	Experiment 2			Experiment 3		Experiment 4		
Sampling dates	12.7.61			26.6.61		25.10.60		
	Light	Low, shade	Spur, shade	Light	Low, shade	Light	Low, shade	Spur, shade
Control (N_0)	1.18	1.83	1.96	1.63	2.34	0.77	0.88	0.86
N_1	1.08	1.76	1.80	1.49	2.09	0.72	0.84	0.79
N_2	0.95	1.77	1.74	1·30	1.99			
s.e.	±0.038	±0.078	±0.075	±0.090	±0.065	±0.012	±0.021	±0.015
Linear effect ($N_2 - N_0$)	-0.23***	-0.06	-0.22†	-0.33*	-0.35*	-0.05**	-0.04	-0.07**

† $P<0.10$, * $P<0.05$, ** $P<0.01$ and *** $P<0.001$

TABLE 5. THE EFFECT OF AMMONIUM SULPHATE ON THE MAGNESIUM AND CALCIUM CONCENTRATIONS OF LIGHT LEAVES, LOW LEAVES — SHADE AND SPUR LEAVES—SHADE

% Mg in dry lamina

	Experiment 1		Experiment 2			Experiment 2			Experiment 4		
Sampling dates	17.7.61		5.9.60			12.7.61			25.10.60		
	Light	Low, shade	Light	Low, shade	Spur, shade	Light	Low, shade	Spur, shade	Light	Low, shade	Spur, shade
Control (N_0)	0.21	0.26	0.20	0.26	0.24	0.22	0.29	0.30	0.22	0.32	0.31
N_1	0.19	0.23	0.21	0.25	0.25	0.18	0.28	0.28	0.20	0.30	0.29
N_2	0.18	0.23	0.19	0.23	0.23	0.18	0.29	0.29			
s.e.	±0.004	±0.004	±0.010	±0.006	±0.007	±0.007	±0.008	±0.007	±0.003	±0.005	±0.005
Linear effect (N_2—N_0)	-0.03***	-0.03***	-0.01	-0.03*	-0.01	-0.04***	0.00	-0.01	-0.02*	-0.02*	-0.02*

% Ca in dry lamina

	Experiment 1					Experiment 2		
Sampling dates	2.8.60			17.7.61		5.9.60		
	Light	Low, shade	Spur, shade	Light	Low, shade	Light	Low, shade	Spur, shade
Control (N_0)	0.39	0.69	0.62	0.42	0.47	1.13	0.76	0.71
N_1	0.40	0.71	0.66	0.37	0.46	1.20	0.53	0.55
N_2	0.36	0.65	0.57	0.37	0.41	1.00	0.61	0.54
s.e.	±0.022	±0.016	±0.020	±0.016	±0.014	±0.061	±0.042	±0.053
Linear effect (N_2—N_0)	-0.03	-0.04†	-0.05†	-0.05*	-0.06**	-0.13	-0.15*	-0.17*

	Experiment 2			Experiment 3		Experiment 4		
Sampling dates	12.7.61			26.6.61		25.10.60		
	Light	Low, shade	Spur, shade	Light	Low, shade	Light	Low, shade	Spur, shade
Control (N_0)	0.83	0.61	0.54	0.72	0.55	0.45	0.64	0.56
N_1	0.66	0.52	0.50	0.74	0.55	0.41	0.59	0.52
N_2	0.56	0.47	0.46	0.68	0.52			
s.e.	±0.039	±0.042	±0.042	±0.037	±0.050	±0.011	±0.012	±0.013
Linear effect (N_2—N_0)	-0.27**	-0.14*	-0.08	-0.04	-0.03	-0.04*	-0.05*	-0.04*

† $P<0.10$, * $P<0.05$, ** $P<0.01$ and *** $P<0.001$

TABLE 6. THE EFFECT OF AMMONIUM SULPHATE ON THE MANGANESE CONCENTRATION OF LIGHT LEAVES, LOW LEAVES — SHADE AND SPUR LEAVES — SHADE (EXPERIMENT 1)

p.p.m. Mn in dry lamina

Sampling dates	2.8.60			17.7.61	
	Light	Low, shade	Spur, shade	Light	Low, shade
Control (N_0)	34	48	45	41	39
N_1	39	54	50	45	45
N_2	43	61	57	49	52
s. e.	±3.5	±4.4	±4.0	±2.8	±2.6
Linear effect (N_2 - N_0)	+9 †	+13*	+12*	+8 *	+13 ***

† $P < 0.10$, * $P < 0.05$ and *** $P < 0.001$

TABLE 7. THE EFFECT OF POTASSIUM CHLORIDE ON THE POTASSIUM CONCENTRATION OF LIGHT LEAVES, LOW LEAVES—SHADE AND SPUR LEAVES—SHADE

% K in dry lamina

	Experiment 1					Experiment 2		
Sampling dates	2.8.60			17.7.61		5.9.60		
	Light	Low, shade	Spur, shade	Light	Low, shade	Light	Low, shade	Spur, shade
Control (K_0)	0.74	0.80	0.80	0.78	0.99	0.84	1.37	1.35
K_1	0.92	1.10	1.08	1.01	1.24	0.99	1.47	1.43
K_2	1.00	1.21	1.20	1.12	1.37	1.09	1.53	1.59
s.e.	±0.015	±0.024	±0.024	±0.016	±0.024	±0.047	±0.042	±0.059
Linear effect (K_2—K_0)	+0.26***	+0.41***	+0.40***	+0.34***	+0.38***	+0.25**	+0.16*	+0.24**

	Experiment 2			Experiment 3		Experiment 4			Experiment 5		
Sampling dates	12.7.61			26.6.61		25.10.60			4.7.61		
	Light	Low, shade	Spur, shade	Light	Low, shade	Light	Low, shade	Spur, shade	Light	Low, shade	Spur, shade
Control	0.93	1.60	1.68	1.46	2.04	0.64	0.75	0.73	0.92	1.08	1.09
K_1	1.09	1.83	1.88	1.45	2.13	0.84	0.97	0.91	1.03	1.19	1.21
K_2	1.20	1.93	1.94	1.50	2.24				1.09	1.17	1.20
s.e.	±0.038	±0.078	±0.075	±0.090	±0.065	±0.012	±0.021	±0.015	±0.024	±0.036	±0.037
Linear effect (K_2—K_0)	+0.27***	+0.33**	+0.26*	+0.04	+0.20*	+0.20***	+0.22***	+0.18***	+0.17***	+0.09	+0.11†

† $P<0.10$, * $P<0.05$, ** $P<0.01$ and *** $P<0.001$

TABLE 8. THE EFFECT OF POTASSIUM CHLORIDE ON THE MAGNESIUM AND CALCIUM CONCENTRATIONS OF LIGHT LEAVES, LOW LEAVES—SHADE AND SPUR LEAVES—SHADE

% Mg in dry lamina

	Experiment 1			Experiment 2			Experiment 4		
Sampling dates	2.8.60			5.9.60			25.10.60		
	Light	Low, shade	Spur, shade	Light	Low, shade	Spur, shade	Light	Low, shade	Spur, shade
Control (K_0)	0.24	0.33	0.32	0.21	0.24	0.24	0.22	0.31	0.31
K_1	0.22	0.32	0.32	0.20	0.26	0.24	0.20	0.30	0.29
K_2	0.22	0.31	0.32	0.18	0.24	0.24			
s.e.	±0.004	±0.006	±0.006	±0.010	±0.006	±0.007	±0.003	±0.005	±0.005
Linear effect (K_2—K_0)	-0.02**	-0.02*	-0.00	-0.03*	0.00	0.00	-0.02**	-0.01*	-0.02*

% Ca in dry lamina

	Experiment 1					Experiment 4		
Sampling dates	2.8.60			17.7.61		25.10.60		
	Light	Low, shade	Spur, shade	Light	Low, shade	Light	Low, shade	Spur, shade
Control (K_0)	0.43	0.72	0.66	0.41	0.45	0.46	0.60	0.54
K_1	0.37	0.68	0.62	0.39	0.44	0.40	0.62	0.54
K_2	0.34	0.65	0.57	0.36	0.44			
s.e.	±0.022	±0.016	±0.020	±0.016	±0.014	±0.011	±0.012	±0.013
Linear effect (K_2—K_0)	-0.09**	-0.07*	-0.09**	-0.05*	-0.01	-0.06**	+0.02	0.00

* $P<0.05$ and ** $P<0.01$

TABLE 9. THE EFFECT OF ROCK PHOSPHATE ON THE PHOSPHORUS CONCENTRATION OF LIGHT LEAVES, LOW LEAVES—SHADE AND SPUR LEAVES—SHADE

% P in dry lamina								
	Fxperiment 1					Experiment 2		
Sampling dates	2.8.60			17.7.61		5.9.60		
	Light	Low, shade	Spur, shade	Light	Low, shade	Light	Low, shade	Spur, shade
Control (P_0)	0.17	0.19	0.19	0.20	0.20	0.19	0.24	0.23
P_1	0.19	0.22	0.21	0.21	0.24	0.20	0.25	0.24
P_2	0.19	0.22	0.21	0.21	0.24	0.21	0.26	0.26
s.e.	±0.002	±0.003	±0.002	±0.002	±0.003	±0.006	±0.009	±0.009
Linear effect ($P_2 - P_0$)	+0.02***	+0.03***	+0.02***	+0.01***	+0.04***	+0.02*	+0.02†	+0.03*
	Experiment 2			Experiment 3		Experiment 4		
Sampling dates	12.7.61			26.6.61		25.10.60		
	Light	Low, shade	Spur, shade	Light	Low, shade	Light	Low, shade	Spur, shade
Control (P_0)	0.20	0.32	0.31	0.21	0.26	0.17	0.22	0.22
P_1	0.21	0.29	0.31	0.23	0.28	0.19	0.26	0.26
P_2	0.21	0.31	0.31	0.23	0.29			
s.e.	±0.003	±0.015	±0.010	±0.007	±0.012	±0.001	±0.005	±0.004
Linear effect ($P_2 - P_0$)	+0.01	—0.01	0.00	+0.02*	+0.03*	+0.02***	+0.04***	+0.04***

† $P<0.10$, * $P<0.05$ and *** $P<0.001$

TABLE 10. THE EFFECT OF ROCK PHOSPHATE ON THE CALCIUM CONCENTRATION OF LIGHT LEAVES, LOW LEAVES—SHADE AND SPUR LEAVES—SHADE

% Ca in dry lamina

	Experiment 1					Experiment 2		
Sampling dates	2.8.60			17.7.61		5.9.60		
	Light	Low, shade	Spur, shade	Light	Low, shade	Light	Low, shade	Spur, shade
Control (P_0)	0.33	0.63	0.58	0.34	0.42	1.02	0.59	0.64
P_1	0.39	0.68	0.64	0.40	0.44	1.10	0.68	0.60
P_2	0.43	0.74	0.63	0.42	0.46	1.21	0.63	0.56
s.e.	±0.022	±0.016	±0.020	±0.016	±0.014	±0.061	±0.042	±0.053
Linear effect (P_2—P_0)	+0.10**	+0.11***	+0.05	+0.08***	+0.04*	+0.19*	+0.04	-0.08

	Experiment 2			Experiment 3		Experiment 4		
Sampling dates	12.7.61			26.6.61		25.10.60		
	Light	Low, shade	Spur, shade	Light	Low, shade	Light	Low, shade	Spur, shade
Control (P_0)	0.63	0.45	0.46	0.68	0.59	0.38	0.59	0.50
P_1	0.69	0.58	0.55	0.71	0.50	0.48	0.63	0.58
P_2	0.73	0.56	0.50	0.75	0.53			
s.e.	±0.039	±0.042	±0.042	±0.037	±0.050	±0.011	±0.012	±0.013
Linear effect (P_2—P_0)	+0.10	+0.11†	+0.04	+0.07	-0.06	+0.10***	+0.04*	+0.08***

† $P<0.10$, * $P<0.05$, ** $P<0.01$ and *** $P<0.001$

TABLE 11. THE EFFECT OF ROCK PHOSPHATE ON THE MAGNESIUM CONCENTRATION OF LIGHT LEAVES, LOW LEAVES—SHADE AND SPUR LEAVES—SHADE

% Mg in dry lamina

	Experiment 1					Experiment 2		
Sampling dates	2.8.60			17.7.61		5.9.60		
	Light	Low, shade	Spur, shade	Light	Low, shade	Light	Low, shade	Spur, shade
Control (P_0)	0.23	0.34	0.33	0.21	0.25	0.20	0.26	0.26
P_1	0.23	0.32	0.32	0.19	0.24	0.20	0.25	0.24
P_2	0.22	0.32	0.31	0.18	0.24	0.19	0.24	0.23
s.e.	±0.004	±0.006	±0.006	±0.004	±0.004	±0.010	±0.006	±0.007
Linear effect (P_2—P_0)	-0.01†	-0.02*	-0.02†	-0.03***	-0.01*	-0.01	-0.02	-0.03**

	Experiment 2			Experiment 3		Experiment 4		
Sampling dates	12.7.61			26.6.61		25.10.60		
	Light	Low, shade	Spur, shade	Light	Low, shade	Light	Low, shade	Spur, shade
Control (P_0)	0.21	0.30	0.29	0.22	0.31	0.22	0.31	0.31
P_1	0.19	0.29	0.29	0.24	0.31	0.20	0.31	0.22
P_2	0.18	0.29	0.29	0.22	0.30			
s.e.	±0.007	±0.008	±0.007	±0.016	±0.011	±0.003	±0.005	±0.005
Linear effect (P_2—P_0)	-0.03*	-0.01	0.00	0.00	-0.01	-0.02**	-0.01*	-0.02*

† $P<0.10$, * $P<0.05$, ** $P<0.01$ and *** $P<0.001$

TABLE 12. THE EFFECT OF MANGANESE SULPHATE ON THE MANGANESE CONCENTRATION OF LIGHT LEAVES, LOW LEAVES—SHADE AND SPUR LEAVES—SHADE

(Experiment 6, sampling date 4.11.60)

	p.p.m. Mn in dry lamina		
	Light	Low, shade	Spur, shade
Control (Mn_0)	32	38	32
Mn_1 1957 only	50	78	66
Mn_2 1957 only	44	53	50
Linear effect ($Mn_2 - Mn_0$)	+12	+15*	+18*
	s.e. of mean ± 4.7		
Control (Mn_0)	25	28	21
Mn_1 1957 and 1958	60	111	65
Mn_2 1957 and 1958	82	91	104
Linear effect ($Mn_2 - Mn_0$)	+57*	+63*	+83***
	s.e. of mean ± 16.4		

TABLE 13. THE EFFECT OF COPPER SULPHATE ON THE COPPER CONCENTRATION OF LIGHT LEAVES, LOW LEAVES—SHADE AND SPUR LEAVES—SHADE

(Experiment 5, Date of sampling 3.7.61)

	p.p.m. Cu in dry lamina		
	Light	Low, shade	Spur, shade
Control (Cu_0)	10	11	11
Cu_1	11	12	12
Cu_2	11	12	12
s.e	±0.2	±0.2	±0.3
Linear effect ($Cu_2 - Cu_0$)	+1***	+1***	+1***

TABLE 14. VARIANCES FOR THE NITROGEN, PHOSPHORUS, POTASSIUM, MAGNESIUM, CALCIUM AND MANGANESE CONCENTRATIONS IN LIGHT LEAVES, LOW LEAVES—SHADE AND SPUR LEAVES—SHADE

(The maximum variances shown by low leaves—shade for each nutrient are underlined. n denotes the number of observations used in calculating the variance)

Field	n	Light	Low, shade	Spur, shade	Light	Low, shade	Spur, shade
		Nitrogen			Phosphorus		
1	105	0.07430	0.05663	0.04901	0.000277	0.000156	0.000548
2	105	0.08927	0.07883	0.06041	0.001148	0.002425	0.002360
3	100	0.08797	0.09338	0.10652	0.000525	0.002249	0.001988
4	115	—	0.10318	0.10361	—	0.001691	0.002000
		Potassium			Magnesium		
1	105	0.03154	0.05126	0.05170	0.001277	0.002440	0.002767
2	105	0.04185	0.06289	0.06976	0.007968	0.003253	0.004284
3	100	0.07522	0.13711	0.13671	0.000926	0.001777	0.001133
4	115	—	0.13250	0.12986	—	0.001349	0.001632
		Calcium			Manganese		
1	105	0.06176	0.02760	0.03270	2287	1083	490
2	105	0.04711	0.02462	0.03142	7783	4414	3857
3	100	0.13545	0.11654	0.05254	8726	3792	4041
4	115	—	0.03971	0.07075	—	225	248

TABLE 15. RATIOS OF VARIANCES (a) LOW LEAVES—SHADE : LIGHT LEAVES, (b) SPUR LEAVES—SHADE : LIGHT LEAVES AND (c) SPUR LEAVES—SHADE : LOW LEAVES—SHADE FOR THE NITROGEN, PHOSPHORUS, POTASSIUM, MAGNESIUM, CALCIUM, AND MANGANESE CONCENTRATIONS IN THE LEAVES

(When the ratio of variances is less than one, and the reciprocal ratio is significant, the values are underlined)

Field	n	Low, shade / Light	Spur, shade / Light	Spur, shade / Low, shade	Low, shade / Light	Spur, shade / Light	Spur, shade / Low, shade
		Nitrogen			Phosphorus		
1	105	0.76	0.66*	0.86	0.56**	1.98***	3.51***
2	105	0.88	0.68*	0.77	2.11***	2.06***	0.97
3	100	1.06	1.21	1.14	4.28***	3.79***	0.88
4	115	—	—	1.00	—	—	1.18
		Potassium			Magnesium		
1	105	1.62**	1.64**	1.01	1.91**	2.17***	1.13
2	105	1.50*	1.67**	1.11	0.41***	0.54**	1.32
3	100	1.82**	1.82**	1.00	1.92**	1.22	0.62*
4	115	—	—	0.98	—	—	1.21
		Calcium			Manganese		
1	105	0.45***	0.53**	1.18	0.47***	0.21***	0.45***
2	105	0.52**	0.67*	1.28	0.57**	0.49***	0.87
3	100	0.86	0.39***	0.45***	0.43***	0.46***	1.07
4	115	—	—	1.78**	—	—	1 10

* $P<0.05$, ** $P<0.01$ and *** $P<0.001$

TABLE 16. PERMISSIBLE ERRORS FOR EACH NUTRIENT IN LOW LEAVES — SHADE CALCULATED FOR DIFFERENT NUMBERS OF TREES SAMPLED (n)

(The maximum observed variance has been used. Figures in parenthesis show the permissible error as a percentage of the mean)

n	Nitrogen (mean 2.94%)		Phosphorus (mean 0.27%)		Potassium (mean 1.20%)		Magnesium (mean 0.41%		Calcium (mean 0.84%)		Manganese (mean 129 p.p.m.)	
20	0.150	(5.1)	0.023	(8.5)	0.173	(14.4)	0.027	(6.6)	0.160	(19.0)	31.1	(24.1)
30	0.120	(4.1)	0.018	(6.7)	0.138	(11.5)	0.021	(5.1)	0.127	(15.1)	24.8	(19.2)
40	0.104	(3.5)	0.016	(5.9)	0.120	(10.0)	0.019	(4.6)	0.111	(13.2)	21.6	(16.7)
50	0.093	(3.2)	0.014	(5.2)	0.107	(8.9)	0.017	(4.1)	0.099	(11.8)	19.2	(14.9)
60	0.083	(2.8)	0.013	(4.8)	0.096	(8.0)	0.015	(3.7)	0.088	(10.5)	17.2	(13.3)
70	0.077	(2.6)	0.012	(4.4)	0.088	(7.3)	0.014	(3.4)	0.081	(9.6)	15.8	(12.2)

TABLE 17. PERMISSIBLE ERRORS FOR THE NITROGEN, PHOSPHORUS, POTASSIUM, MAGNESIUM, CALCIUM AND MANGANESE CONCENTRATIONS OF LIGHT LEAVES, LOW LEAVES—SHADE AND SPUR LEAVES—SHADE CALCULATED ON THE BASIS OF 40 TREES SAMPLED

(Figures in parenthesis show the permissible errors as a percentage of the mean)

Field	Light		Low, shade		Spur, shade		Light		Low, shade		Spur, shade	
	Nitrogen						Phosphorus					
1	0.088	(3.0)	0.077	(2.7)	0.072	(2.5)	0.005	(3.0)	0.004	(2.2)	0.008	(3.8)
2	0.097	(2.5)	0.091	(2.3)	0.080	(2.0)	0.011	(5.0)	0.016	(5.9)	0.016	(5.9)
3	0.096	(3.3)	0.099	(3.1)	0.106	(3.4)	0.007	(3.5)	0.015	(5.8)	0.015	(5.6)
4	—		0.104	(3.5)	0.104	(3.6)	—		0.013	(5.2)	0.015	(5.8)
	Potassium						Magnesium					
1	0.058	(5.0)	0.073	(5.4)	0.074	(5.5)	0.012	(6.7)	0.016	(7.3)	0.017	(7.8)
2	0.066	(5.4)	0.081	(5.3)	0.086	(5.8)	0.029	(7.8)	0.019	(4.6)	0.021	(5.3)
3	0.089	(10.0)	0.120	(10.0)	0.120	(9.8)	0.010	(7.1)	0.014	(7.2)	0.011	(6.1)
4	—		0.120	(6.9)	0.117	(6.8)	—		0.012	(4.9)	0.013	(5.7)
	Calcium						Manganese					
1	0.081	(17.6)	0.054	(7.9)	0.059	(9.5)	15.5	(18.2)	10.7	(12.3)	7.2	(7.7)
2	0.071	(11.1)	0.051	(9.6)	0.058	(11.7)	28.6	(18.4)	21.6	(16.7)	20.2	(16.2)
3	0.120	(16.9)	0.111	(13.2)	0.074	(10 0)	30.3	(24.0)	20.0	(14.6)	20.6	(15.8)
4	—		0.065	(10.8)	0.086	(15.7)	—		4.6	(11.8)	5.1	(13.8)

TABLE 18. NUTRIENT COMPOSITION OF LIGHT LEAVES, LOW LEAVES—SHADE AND SPUR LEAVES—SHADE

Field	n	Light	Low, shade	Spur, shade	s.e.	Min 5% sig. diff	Light	Low, shade	Spur, shade	s.e.	Min 5% sig. diff
		% N					% P				
1	105	2.94	2.88	2.88	±0.021	0.060	0.18	0.19	0.20	±0.002	0.005
2	105	3.82	3.93	3.90	±0.024	0.068	0.22	0.27	0.27	±0.003	0.008
3	100	2.92	3.22	3.14	±0.017	0.049	0.21	0.26	0.26	±0.003	0.009
4	115	—	2.94	2.87	±0.023	0.063	—	0.25	0.25	±0.003	0.009
		% K					% Mg				
1	105	1.17	1.34	1.33	±0.020	0.056	0.18	0.22	0.22	±0.003	0.008
2	105	1.21	1.52	1.49	±0.017	0.047	0.37	0.41	0.40	±0.005	0.015
3	100	0.89	1.20	1.23	±0.013	0.035	0.14	0.19	0.18	±0.002	0.006
4	115	—	1.75	1.72	±0.019	0.053	—	0.24	0.23	±0.002	0.006
		% Ca					p.p.m. Mn				
1	105	0.46	0.68	0.62	±0.022	0.061	85	87	93	±2.5	7.1
2	105	0.64	0.53	0.49	±0.014	0.039	155	129	125	±3.7	10.3
3	100	0.71	0.84	0.74	±0.023	0.063	126	137	130	±4.8	13.5
4	115	—	0.60	0.55	±0.021	0.058	—	39	37	±1.0	2.8