

STUDIES ON A TECHNIQUE OF MICRO-TAPPING FOR THE ESTIMATION OF YIELDS IN NURSERY SEEDLINGS OF *HEVEA BRASILIENSIS*

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SUMMARY

A new method is described for estimating the rubber content of young Hevea brasiliensis seedlings by the collection of latex resulting from needle pricks. An evaluation is made of the repeatability of the results; a comparison is made of rubber yields from different systems of extraction for the purpose of estimating yielding capacity. The technique described is rapid and simple. It would be of value because selection for yield capacity could be made at an early stage.

INTRODUCTION

In view of the wide variability in the yield of *Hevea brasiliensis*, the estimation of the yield capacity of *Hevea* at the nursery and young plantation stage has received much attention. Senanayake & Wijewantha (1968) have stressed the early indications of yield in the selection of *Hevea* cultivars. Cramer (1938) adopted a system of grading young rubber plants using a special type of knife to make incisions in 12-18 month old plants; Morris & Mann (1931-1938) developed another early test consisting of successive tappings of four-year old trees: this system of early tapping gave a very close correlation with the initial four years' yields. The latex content of petioles (Middleton & Westgarth, 1963; Fernando & Samaranayake, 1967) has also been measured for an early evaluation of yield. More recently the Rubber Research Institute of Malaya has utilized a count of the number of latex vessel rings in the cortex as an indicator of yield. Lustinec & Resing (1965) used a micro-tapping technique to delimit the flow areas in clones. But all these methods are rather difficult to adopt for the rapid selection of 'elite' plants from large populations, in the field. In the course of studies on the chemistry of latex synthesis at the Natural Rubber Producers' Research Association, Dickenson (1969) developed a method of pricking young plants with a sharp needle; the latex that oozed out was collected into glass capillaries by suction: Mendes (1971) has reported on an early tapping test with a specially designed knife, collecting the latex in small aluminium capsules.

METHOD

A strip of Whatman No. 1 filter paper 10 cm long and 2 cm wide, was wrapped round the stem at the level at which it was to be micro-tapped and held firmly in position with a spring clip (Fig. 1). The bark was then pricked at one or more points through the filter paper, close to the upper edge of the strip, with a sharp needle of the dissecting needle type. After a few minutes, the paper on which the latex had collected was removed, dried to constant weight, and weighed on a precision direct reading balance.

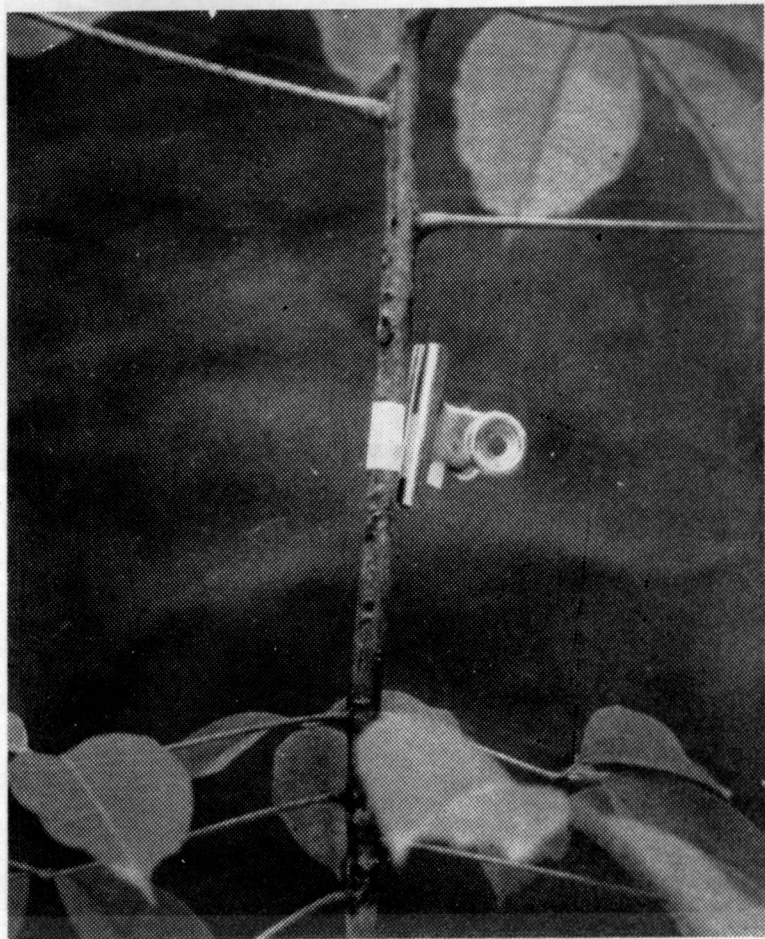


Fig. 1. Filter paper in position on the stem.

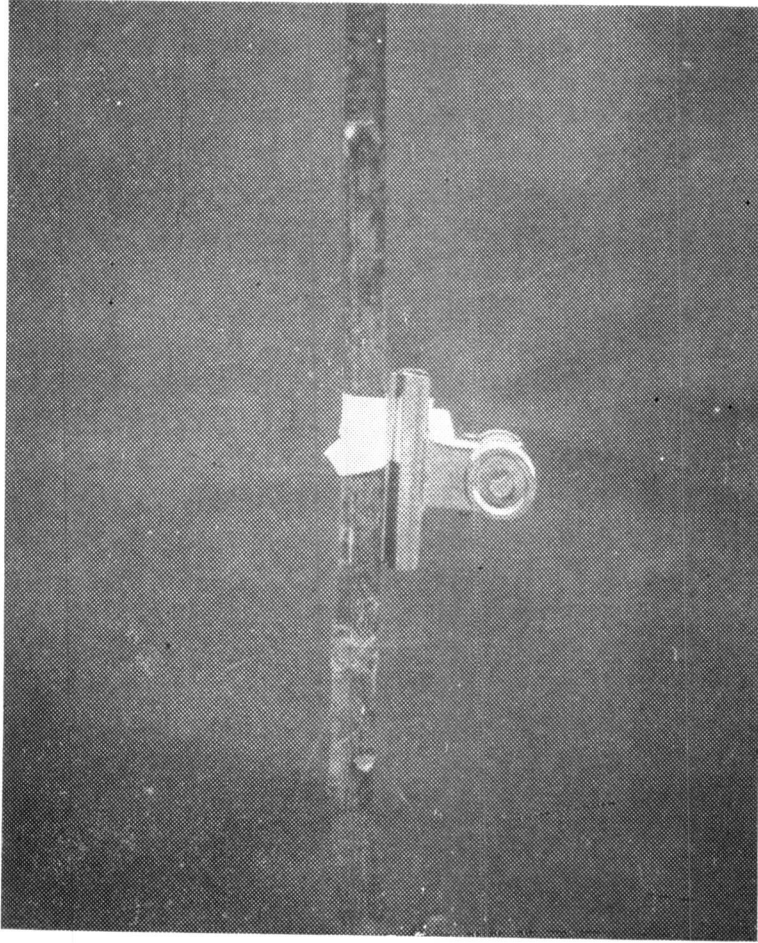


Fig. 2. Folded filter paper in position on older plant.

With older plants the latex tended to drip off the paper even though broad strips were used. In these cases the lower part of the paper was folded upwards along its length to form a flap (Fig. 2). Pricks were made along the inner flap of the paper and the latex collected into the fold of paper. In old seedlings, however, only half the circumferences of the stems (on the side of the fold) was available for pricking.

When a scalpel was used to make lateral incisions, instead of a needle, larger amounts of latex flowed from such incisions, but there was more of a tendency for the latex to collect between the paper and the bark. Therefore it was decided to use only a needle for further analysis of this method. Where the bark was ridged and corky, sand papering, using a fine grade of sand paper, was necessary in order to obtain sufficiently close contact between the paper and bark. Green bark was found to be the most suitable.

EXPERIMENTAL RESULTS

Repeatability

Nine RRIC 45 clonal seedlings, 13 months old, were pricked at the same height of 15 cm from ground level, on three successive days: the yields in mg of rubber were ranked and subjected to an intra-class correlation analysis (Table 1) after the values were transformed into normal scores (using Table 20 of Fisher & Yates, 1938). A very highly significant intra-class correlation coefficient of 0.6853 was obtained; this is indicative of the high degree of consistency or repeatability of this method (Abeywardena, 1963).

TABLE 1

YIELD AND RANKING OF MICRO-TAPPING OF RRIC 45 CLONAL SEEDLINGS PRICKED SEVEN TIMES PER DAY

Plant No.	1st day		2nd day		3rd day	
	Wt of rubber mg	Rank	Wt of rubber mg	Rank	Wt of rubber mg	Rank
1	8.2	7	10.0	2	10.0	8
2	13.8	4	6.0	5	11.9	6
3	8.9	6	5.6	7	10.7	7
4	22.1	1	15.7	1	31.6	1
5	17.0	3	7.6	3	25.0	3
6	21.0	2	7.2	4	26.2	2
7	13.3	5	5.9	6	13.4	4
8	6.9	8	2.2	9	12.9	5
9	5.1	9	4.1	8	5.2	9

Intra-class correlation coefficient (repeatability index) = 0.6853

Number of pricks

The same nine RRIC 45 seedlings were also used to compare yields from one, two and four pricks (Table 2).

TABLE 2
VARIATION IN MEAN YIELDS FOR DIFFERENT NUMBER
OF PRICKS

No. of pricks	Mean wt (mg) of rubber per	
	Treatment (pricking)	Prick
1	2.13	2.13
2	3.80	1.90
4	6.21	1.55
S.E.	0.64	0.22
L.S.D. (P = 0.05)	1.91	0.65

An analysis of variance showed a very highly significant difference in the effect of the number of pricks ($F_2, 16-10.2$). On separating the treatment effects to linear and quadratic components the linear effect was found to be highly significant ($F=74.83$) and the quadratic effect non-significant. The difference between treatments was not significant with regard to the mean yield per prick, but there is an indication of a decrease in yield per prick with increase in the number of pricks per plant.

Comparison with petiolar rubber content

Nine Tjir 1 clonal seedlings, 18 months old, were micro-tapped at a height of 23 cm (9 in.) from the ground level with four pricks per plant. After the flow of latex ceased, 10 to 12 petioles per plant were removed, dried, macerated, and the rubber content in mg/g dry weight was estimated using a Goldfish extraction apparatus as described by Fernando & Samaranyake (1967). As shown in Table 3 a very close correlation was obtained between the two types of estimation.

A second experiment of the same nature was carried out on 11 seedlings of hand-pollinated origin and parentage, PB 5/51 \times IAN 45-873. Two sets of micro-tappings were carried out at heights of 15 cm (6 in.) and 23 cm (9 in.) and the petiolar rubber content measured as before. A correlation coefficient (r) of 0.78, significant at $P = 0.001$ level, was obtained between petiolar rubber and micro-tapping at 15 cm height; r was 0.804, significant at $P = 0.001$ level for micro-tapping at 23 cm height; r was 0.78; significant at $P = 0.01$ level, for mean micro-tapping of the two heights.

TABLE 3
COMPARISON OF RUBBER CONTENT OF PETIOLES
AND MICRO-TAPPING YIELD

Tjir 1 seedling	Petiole rubber mg/g dry weight	Micro-tapped (four pricks)
1	0.21	0.21
2	0.24	0.20
3	0.36	0.36
4	0.45	0.41
5	0.48	0.39
6	0.73	0.64
7	0.80	0.71
8	0.82	0.49
9	0.99	0.53

Correlation coefficient $r = 0.85$ (highly significant)

Coefficient of determination $r^2 = 72.3\%$

Micro-tapping and yields of mature trees

In order to detect a correlation with the yields of mature trees the branches of ten-year old budded trees of clone RRIC 97 were micro-tapped on ten successive tapping days. The tapped latex was coagulated in the collection vessel, dried, rolled and weighed to give the yields per tree as dry rubber per tapping. The micro-tapped filter paper was dried and weighed. No significant correlation could be detected between tapping and micro-tapping in this experiment.

In order to investigate whether this lack of correlation could be due to the stock/scion effect, 12 seedling trees, five years old, were next selected. The seedlings were of hand-pollinated origin and had been cut back repeatedly at 1 m for budwood, leaving a trunk approximately 30 cm in girth. The leader branches, micro-tapped 2 m from ground level, were approximately one year old. In this case a correlation significant at 5% level ($r = 0.6180$) was obtained in the case of one tree. On two of the ten tapping days, correlations ($r = 0.6241$ and 0.6727), significant at 5% level, were obtained between tapping and micro-tapping across the 12 seedling trees.

DISCUSSION

In the bark the latex is contained within an anastomosing system of vessels arranged in concentric cylinders with lateral inter-connections between vessels of adjacent cylinders. Pricking would not only affect the turgor of these vessels punctured but also that of the neighbouring vessels, so that with successive pricks the yield per prick can be expected to decrease. This decrease in yield per prick, with increase in the number of pricks, was observed when a large number of pricks were made at the same height around the stem. In order to improve reliability of the method it may be advisable to prick at many heights on the same plant and at the same time. Practically, however, four pricks per paper was found most convenient.

Theoretically, a lateral cut should cause a greater flow than from a few pricks as a result of rapid loss of turgor and subsequent slower vessel plugging (Southern, 1968). In the present studies however the increase in yield obtained from lateral cuts over four needle pricks was not significant. Needle pricks are also preferable as an experimental method as they cause less injury to the tissues than lateral cuts.

For most physiological investigations adequate replication could circumvent errors, if any, caused by this method of micro-tapping. It may be that the lack of significant correlation with actual tapping yields in the case of seedlings may be due to sufficient replication of micro-tappings. The significant correlation in the case of two seedlings and the absence of correlation in the case of budded trees points to possible appreciable stock/scion effect in the case of yields of budded trees.

The high repeatability (consistency) index and the close correlation with petiolar rubber content indicate that this method will be very useful in genetic selection for yield. This method has also been used (Fernando *et al.* 1971) to assess the relationships between yield, growth, and oil content in a population of young seedlings of *Hevea*. Cramer's "Testatex" grader, which has been in use extensively, provides only a semi-quantitative assessment based on visual observation. The method of micro-tapping outlined here should prove superior to most methods on the basis of ease and rapidity of manipulation and even permit selection for the commercial planting of 'elite' seedlings.

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