

A NEW BASIS FOR THE SELECTION OF *HEVEA* SEEDLINGS

BY

D. M. FERNANDO AND M. S. C. DE SILVA

SUMMARY

Clonal seedlings from seven clones were germinated and periodically measured in height and diameter. The oil content of some seedlings was estimated by removing the cotyledons. The latex content was measured by a method of micro-tapping. A positive correlation was detected between latex content and growth. An inverse relationship was found between oil content and growth in some clones. Indications of an inverse correlation between latex and oil content were also detected. This assay of oil content therefore appears vital in the selection of seedlings and provides a link which considerably expands the possibilities of early selection of Hevea seedlings for growth and yield.

INTRODUCTION

Rubber was propagated exclusively from seed for the first few decades following introduction of the crop into the Far East. The seed for initial local distribution was collected from the original introductions from the Peradeniya and Heneratgoda Botanical Gardens. The variation between seedlings is however very great and Whitby (1919) showed that 9.6% of the trees in a seedling area yielded three to six times as much rubber per tree as the remaining trees.

After the perfection of the budgrafting technique by van Helten in 1917, propagation by this method was generally adopted. In view of the easier establishment of seedlings, attempts were made to develop sources of proven seed ; but the heterozygosity of *Hevea*, resulting in extensive variability in clonal populations, decided in favour of selection and subsequent budgrafting as the preferred method. Parallel with the adoption of budgrafting the relationship between stock and scion came into scrutiny and attempts were made to select the best possible clones for supply of stock seedlings on the basis of budgrafting success, resistance to wind damage, support of the high yields of the scion, and possibility of positive selection in the nursery.

Workers in the field of stock-scion effects on other crops have revealed that the transfer of substances such as minerals appears to be dominated more by the scion in some plant genera and more by the stock in others, but no general rule has emerged. In a recent review, Tukey (1969) has described the allelopathic influence of dwarfing in apples; also mentioned are rootstocks for disease resistance in watermelon, virus tolerance in pears, *Phylloxera* resistance in grapes, temperature and pest resistance in roses.

In rubber van der Hoop & Ostendorf (1932) showed that the nature of the latex is primarily determined by the scion. The search for suitable rootstocks has however resulted only in the selection of Tjir 1 as a seed parent : this early finding has had to be sustained for many years until now the danger of replacement of Tjir 1 as a clone may leave a serious and substantial gap in the field of suitable stocks. LCB 1320 was tried out for a few years in Indonesia but has now been discontinued.

The present study was commenced as a genetic inquiry into the behaviour of Tjir 1 seedlings and the possibility of finding a replacement clone for stocks from among more modern selections. In addition to the usual measurements of growth and latex, a new parameter, the oil content of the cotyledon of individual seedlings, was also estimated.

MATERIALS AND METHODS

Thirty six seeds each from seven different clones were germinated in polythene bags 2' x 9" filled with soil. The seven clones were Tjir 1, RRIC 37, RRIC 52, RRIM 513, RRIM 623, PB 86 and 506. Clone 506 is not released for general distribution but is a very heavy seed bearer of parentage RRIC 52 x Tjir 1. In order to compare differences in growth on a more extensive basis six replications of 36 plants of six clones were also planted as randomized blocks in the field: the clones used for this trial as female parents were RRIC 36, RRIC 37, RRIC 52, RRIM 623, Tjir 1 and PB 86. Measurements of diameter and height were taken every three weeks on all these plants.

Estimation of oil content

The oil content was estimated as mg per g dry weight of the excised cotyledons. Previous experience on excision of the cotyledons showed that seedlings are very susceptible to transplanting shock soon after excision of the cotyledons. Therefore in this experiment the seeds were germinated in the polythene bags. Some surface soil was removed when the first leaves were green and the cotyledons were removed: this stage was approximately four weeks after emergence of the radicle. Owing to adverse weather conditions it was possible to remove the RRIC 37 seedling cotyledons only six weeks after germination. 1% Fermate in vaseline was applied to the point of removal of the cotyledons in order to prevent invasion of fungi such as *Fusaria*.

The detached cotyledon was separated from the seed coat and dried for twelve hours at 80°C in a vacuum oven: it was then macerated in a high speed macerator and extracted for two hours in a Goldfish extractor using ether as the solvent. Unlike the Soxhlet extractor usually used, the Goldfish extractor works completely in the atmosphere of the solvent and under reduced pressure; this is possible owing to the incorporation of a stainless steel valve in the extractor. The ether was evaporated from the extracted oil and the percentage of oil per dry weight of the individual cotyledon was determined.

Micro-tapping

The method adopted was devised by Waidyanatha (personal communication) and consists of four punctures made on a strip of filter paper wrapped round the smoothed plant stem at about 15 cm height from ground level (Fig. 1). Drying and subsequent weighing would yield the weight of dry rubber extruding from each puncture. Owing to the small size of the droplet one hour's drying at 80°C in a vacuum oven was found sufficient.

RESULTS AND CONCLUSIONS

Growth

The diameter and height of the seeds from different clonal sources is shown in Fig. 2. In the case of the plants grown in polythene bags there are significant differences in growth between seedlings from different sources (Table 1). The differences in growth within seedlings from each clone were not however significant.

Differing behaviour was shown with reference to removal of the cotyledons; RRIC 52 and RRIC 37 seedlings showed significantly depressed growth with removal of the cotyledons. This expected depression with removal of the cotyledon was however not observed in the case of seedlings from Tjir 1, 506, PB 86 and RRIM 623; on the other hand seedlings from RRIM 513 showed a significant increase of growth with removal of the cotyledons.



Fig. 1. Micro-tapping in progress on a young rubber plant

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SEEDLING GROWTH

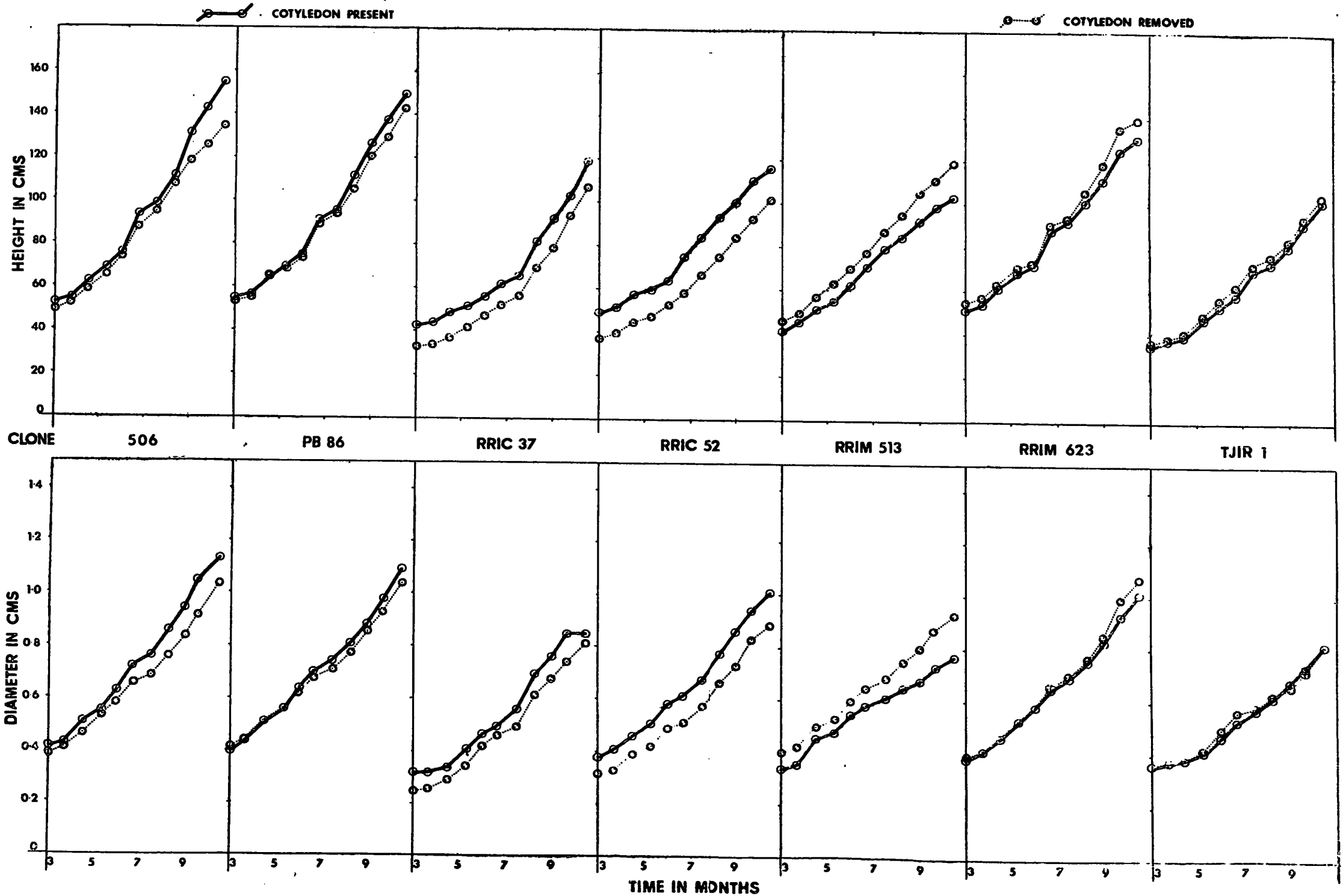


Fig. 2. Growth of seedlings from different clonal sources (all grown in polythene bags).

RELATIONSHIP BETWEEN OIL CONTENT AND SEEDLING GROWTH

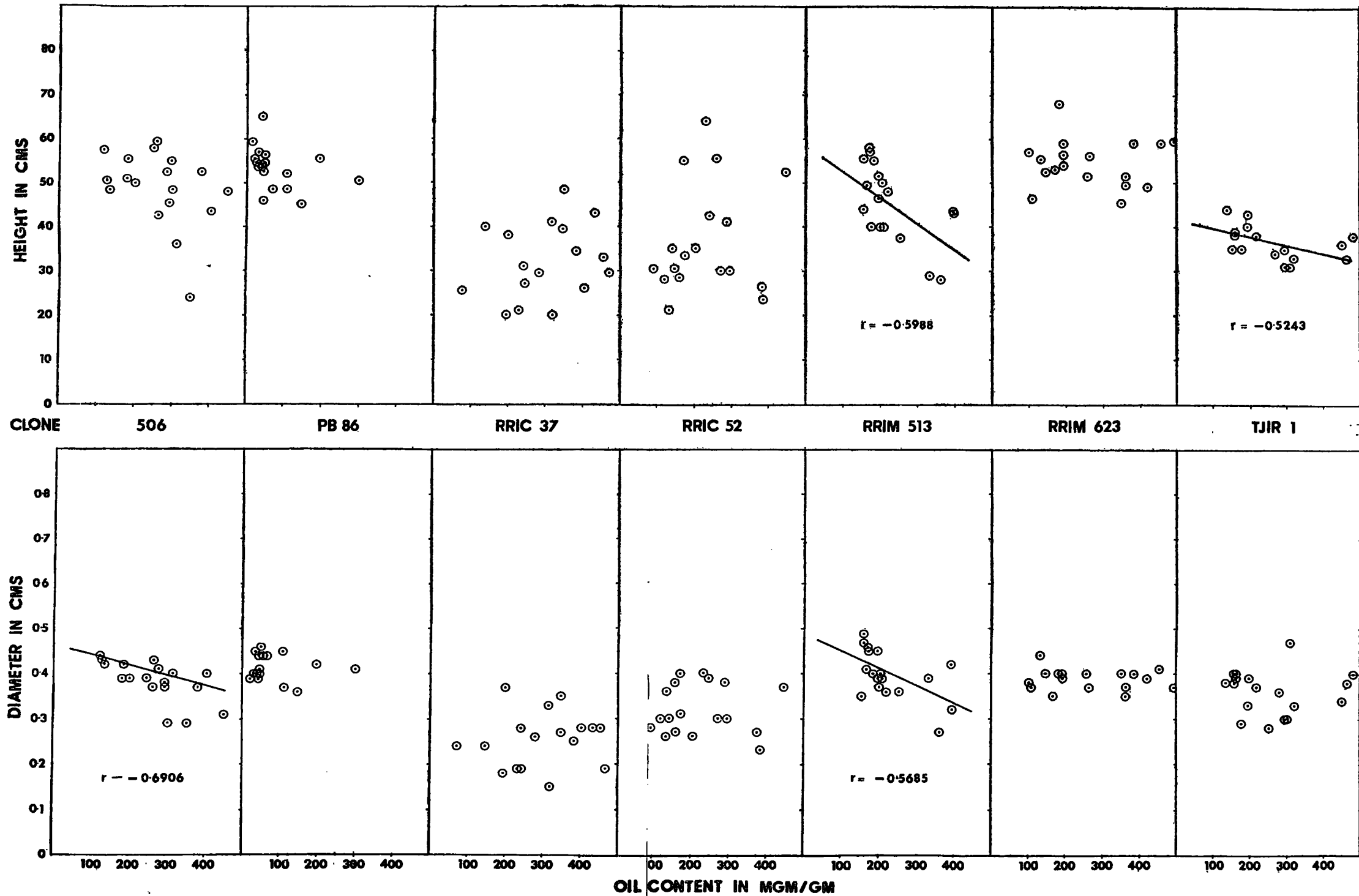


Fig. 3. Varying patterns of oil content and seedling growth in seedlings from different clones.

TABLE 1
SIGNIFICANCE OF VARIATIONS IN GROWTH, LATEX AND OIL CONTENT

Character	Age in months	Variance ratio		
		Between clones $n_1=6, n_2=235$	With and without cotyledons $n_1=1, n_2=235$	Interaction $n_1=6, n_2=235$
Height	3	27.38***	4.15*	5.74***
Diameter	3	23.09***	2.17	8.00***
Height	4 $\frac{1}{2}$	39.30***	4.16*	5.34***
Diameter	4 $\frac{1}{2}$	43.51***	2.86	4.78***
Diameter	6	26.81***	2.36	4.03***
Height	7 $\frac{1}{2}$	24.61***	2.90	2.84*
Diameter	7 $\frac{1}{2}$	18.64***	2.97	3.42**
Height	9	24.13***	1.27	2.44*
Diameter	9	8.83***	2.38	2.53
Diameter	10 $\frac{1}{2}$	9.08***	1.09	2.15*
Latex content	9	9.54***	—	—
Seed oil content	1	9.50***	—	—

* Significant at 5% level

** " " 1% "

*** " " .1% "

Seedling growth and oil content of cotyledons

The relationship between oil content of the seedling and growth is given in Fig. 3. It is seen that in seedlings of Tjir 1, RRIM 513 and 506 origin, there was a significant inverse relationship between oil content and growth. The coefficient of correlation in the case of all the other clones was below 0.5.

TABLE 2
RELATIONSHIP BETWEEN OIL CONTENT
AND GROWTH

Clone	Coeff. of correlation	
	Height	Diameter
Tjir 1	-0.5243*	-0.0255
RRIM 513	-0.5988*	-0.5685*
506	-0.3992	-0.6906*
PB 86	-0.3611	-0.1405
RRIM 623	-0.0313	-0.1669
RRIC 37	0.3335	0.1545
RRIC 52	0.1766	0.0581

* Significant.

Growth, latex content and oil content

Analysis of values of growth, latex content and oil content showed a direct correlation between latex content and growth and an inverse correlation between oil content and growth. The relationships are illustrated in Figs. 4 and 5.

TABLE 3

RELATIONSHIP BETWEEN GROWTH, LATEX AND OIL CONTENT

Age	Height				Diameter			
	Between clones		Within clones		Between clones		Within clones	
	Latex r	Oil r	Latex r	Oil r	Latex r	Oil r	Latex r	Oil r
3 months	0.5330	-0.5193	0.2715	-0.0937	0.5813	-0.6049	0.1921	-0.1532
4½ months	0.6326	-0.5727	0.2433	-0.0898	0.7296	-0.6367	0.2026	-0.1580
6 months	0.6814	-0.4579	0.4892	-0.0988	0.6953	-0.5984	0.2794	-0.2337
7½ months	0.6900	-0.4795	0.4607	-0.1007	0.6989	-0.5374	0.3940	-0.0149
9 months	0.6667	-0.4708	0.4458	-0.0630	0.6877	-0.5278	0.3550	-0.0645
10½ months	0.4983	-0.4875	0.4169	-0.0916	0.5946	-0.4483	0.3892	-0.0651

Significant value of r between clones > 0.7067

,, ,, ,, r within clones > 0.1275



Fig. 4

Tjir 1 seedlings

(Mean oil content — 260.70)

	<u>A</u>	<u>B</u>
Plant No.	771	762
Oil content (mg/g)	267	157.3
Latex (mg)	15	350
Height (cm)	63	114



Fig. 5

RRIM 513 seedlings

(Mean oil content — 229.80)

	<u>A</u>	<u>B</u>
Plant No.	808	813
Oil content (mg/g)	220	154
Latex (mg)	495	495
Height (cm)	109.5	158

Parallel field trial

The replicated plots planted in the field were measured at the same intervals as the polythene-bagged plants. Analysis of these measurements revealed no significant differences in growth between seedlings of the different clones : the coefficients of variation for diameter analyses were around 20%. This lack of significant differences in the field could be due to low fertility levels. The method of growth in polythene bags would therefore appear to be essential in order to equalise environment when experimenting with relatively small quantities of rubber seed.

DISCUSSION

The removal of cotyledons should be expected to slow down development ; increased growth in the case of RRIM 513 seedlings thus treated is most unusual. Oil is a storage food product, therefore normally there should be a positive correlation between oil content and growth and the significant negative correlation obtained in the case of Tjir 1, RRIM 513 and 506 is again most unusual. White (1951) maintained that the growth of seedlings should be influenced by the cotyledons since growth rate is decreased when cotyledons were removed. Hale (1969) showed that *Arachis* seedlings can be grown without cotyledons to mature plants but maturity was delayed : the vitamins niacin, thiamin, and riboflavin and the auxin 2, 4,-dichlorophenoxyacetic acid were found to be effective in replacing these substances normally present in cotyledons. It is significant that, of the three clones showing abnormal behaviour in this regard, two, RRIM 513 and Tjir 1, are known to be very satisfactory seed parents and one, 506, is of Tjir 1 parentage, though untested with respect to stock suitability. Perhaps this significant inverse relationship in the case of these clones is indicative of positive selection from a population for high-yielding and vigorous stocks which would be compatible with high-yielding scions.

Experiments by Prokofiew (quoted by Arreguin, 1958) have indicated that, in plants other than *Hevea*, as rubber formation increased essential oil formation decreased. Seasonal determinations of rubber of the desert shrub guayule have also indicated that as rubber formation increased essential oil formation decreased. It has also been suggested by Beadle & Tatum (1941) that both isoprenoids are polymerized from a common precursor, which in the presence of a specific enzyme genetically controlled, can be converted either to rubber or essential oils. However this relationship between oil and latex may not be the same in *Hevea* as the oil in *Hevea* is a triglyceride and not an isoprenoid. But it is possible that there may be some molecular rearrangement at an early stage with genetical control intervening. The relationship of oil content to growth is too far removed from the natural to admit a direct connection and it is possible that a third substance, stimulatory or inhibitory, is involved. Available evidence on the different components of the oil do not however offer a suitable explanation at present. Dorrel (1970) has pointed out that genetic studies on the inheritance of fatty acids in flax seeds would be clarified if study could be limited to the cotyledons, avoiding the 2n maternal testa and 3n endosperm (of which 2/3 is maternal).

In the field of clone selection the 9.6% trees mentioned earlier by Whitby (1919) as giving three to six times the yield of the other trees in a field could be isolated with considerable certainty by this method at a very early stage. From known mother trees, such as IAN clones resistant to *Dothidella*, this method would enable early evaluation of the higher-yielders with resistance evaluation to follow. Up to now the trend is to first screen for disease resistance and then select for yield but it is obvious that now we can isolate high-yielders and work more extensively on further evaluation of their secondary characters.

For breeding purposes plants of early evaluated positive characters could be stimulated to flower and even open pollinated progeny could be further evaluated for the next stage of selection. In short the discovery of these significant correlations connected within the new parameter of oil content would make a drastic change in our breeding and selection policy and enable two concurrent streams : one, short-term for immediate distribution, and a second long-term for genetical study, to be carried out simultaneously.

In Fig. 4 the mean oil content of the Tjir 1 seedlings examined is 260.7. The plant corresponding closest to this is shown at A. The growth is poor and latex content is small. The plant with the highest micro-tapping is shown at B with twenty times the latex and better growth, combined with a much smaller oil content. In Fig. 5 showing RRIM 513 seedlings plant A representing average oil content had a high latex content as well, but the better growth of B was associated with a smaller oil content.

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QUESTION AND ANSWERS

Question: Is there any relation between oil content of cotyledons and age of seeds at sowing? (Dr. Y. D. A. Senanayake).

Answer: We have not studied the variation of oil content with age of the seed at sowing. Studies of the variation of oil content with age of the seedling showed no variation for the first eight weeks. As a comparatively inert drying oil not much variation could be expected with age of the seed at sowing.