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Clonal Nursery Studies in Hevea I. Nursery Yields and Associated Structural Characteristics and their Variations

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Yields of young trees and other characters associated with the size of the laticiferous system have been studied on eighty clones from different provenances. The means and coefficients of variation of these characters and their clonal distribution patterns have been examined. The number of trees necessary for detecting significant clonal differences of different magnitudes has been determined.

A reliable method of predicting at the nursery stage the likely yield performance of the tree would considerably reduce the time required to produce a clone for commercial use. The most direct method of forecasting the yield is to relate the yields of mature trees with those of young plants or with other correlated characters that can be measured readily at the nursery stage. Relatively low correlations can be useful in early selection, especially if the dependent variable (*i.e.* mature yield) is correlated with two or more independent variables (*i.e.* nursery characters) which are not highly correlated with each other. The multiple regressions of mature yield on several nursery characters independent of each other can account for more of the variance than any simple correlation, and the reliability of the selection technique will thereby be improved.

In this paper a study is made of the variation in the yield and the main physical and structural characteristics of young nursery buddings which are thought to determine the size of the laticiferous system. The number of plants necessary to detect significant differences in these characters are considered in this paper. Later papers will be concerned with the degree of independence of the characters and the correlations between them and the yield of the tree when mature.

EXPERIMENTAL

Eighty of the 1954 international exchange clones, from five different provenances, were used in the study (see Appendix). These were budded in three replications of six trees each, with a planting distance of about 1.8 × 1.8 m, and were cut back in June 1964. Yield determinations using the principle of the Haemaker-Morris-Mann technique (RUBBER RESEARCH INSTITUTE OF MALAYA, 1933) were done on S/1.d/1 tapping for ten days at heights of 71 and 61 cm at 33 and 56 months from budding respectively, by taking the aggregate yield for the last six tappings (thirty-three months) or four tappings (fifty-six months) for each tree. Girth measurements were taken at the same time at 102 cm height, and bark samples were removed as plugs at thirty-three months. The bark thickness was determined by the Schlieper gauge. Longitudinal sections were taken and stained with Sudan III to determine the number of latex vessel rings and the distance between them; transverse sections were stained with osmic acid to measure the diameter of the latex vessels and sieve tubes.

RESULTS AND DISCUSSION

Figure 1 shows the frequency distribution of the mean for each characteristic over

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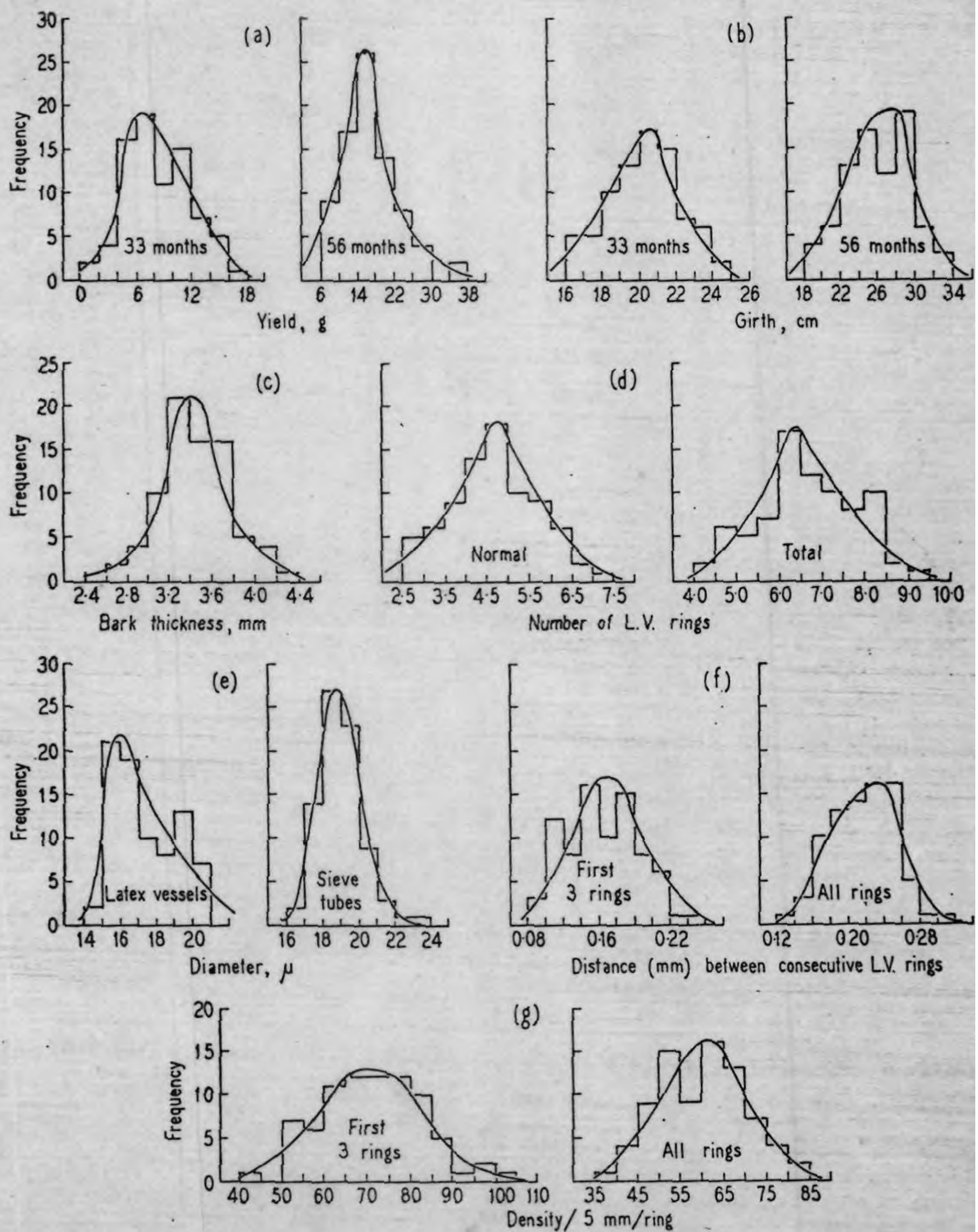

Figure 1. Frequency distribution (between clones) of nursery yields and other associated structural characteristics.

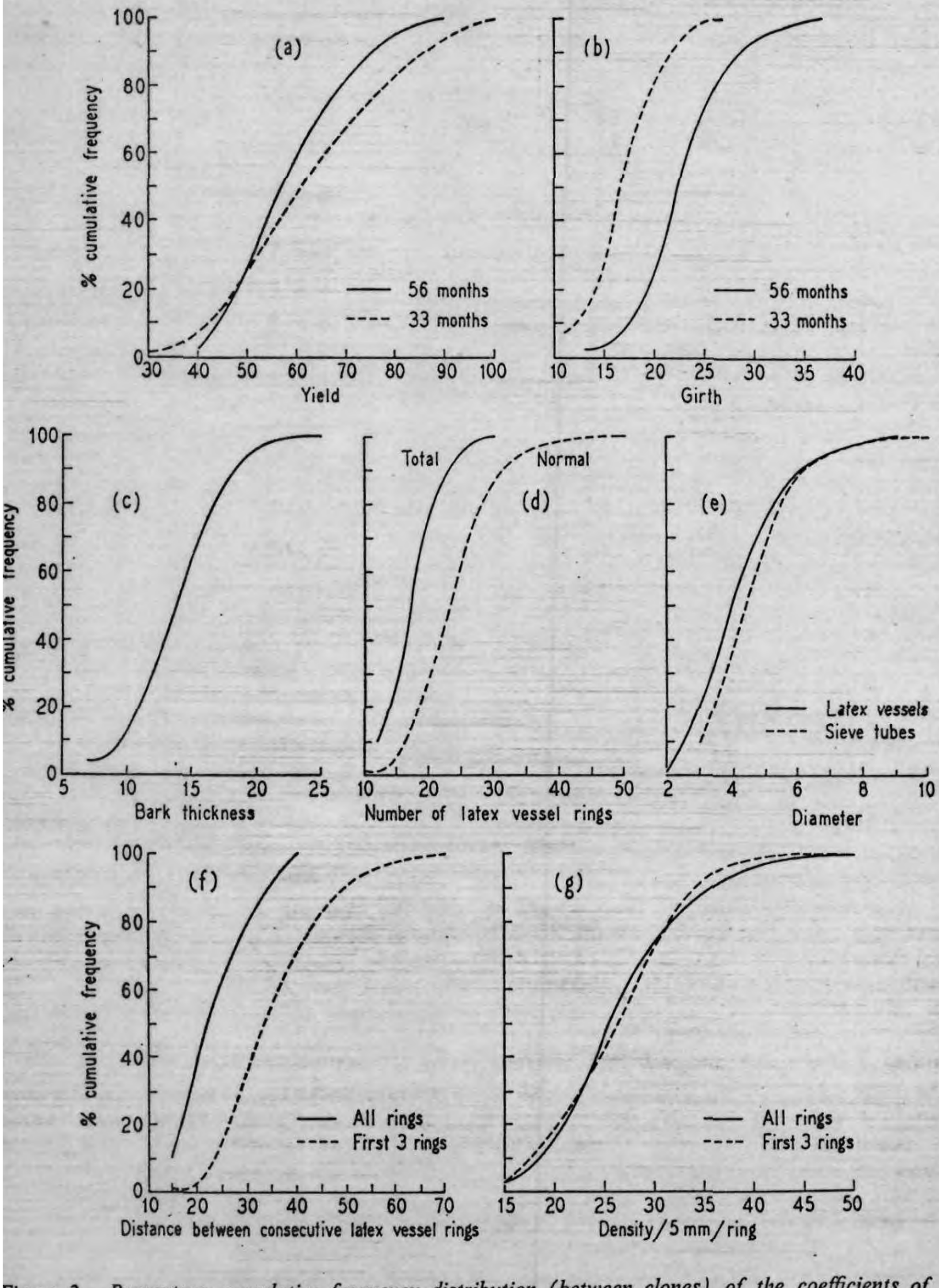

Figure 2. Percentage cumulative frequency distribution (between clones) of the coefficients of variation (c.v.) for nursery yields and other associated structural characteristics.

the eighty clones. (The number of trees per clone varied between eleven and eighteen.) *Figure 2* illustrates the percentage cumulative frequency distribution of the coefficients of variation of the different characteristics.

Yield. The total yield from six tappings at thirty-three months and four tappings at fifty-six months, averaged over the eighty clones, were 8.4 and 16.7 g per tree respectively. The yield at thirty-three months shows a range in mean from 2.5 to 16.4 g per tree whereas the yield range at fifty-six months is from 6.4 to 35.7 g per tree. *Figure 2a* shows that the pattern of variation in yield between trees within clones for the two periods at which tapping was carried out is similar.

Girth. In mature trees more than half the variation in yield within clones is accounted for by differences in girth (PAARDEKOOPER, 1961; NARAYANAN AND HO, 1970). Between clones, however, girth is important in the initial years of tapping and also when tapping renewed bark (PAARDEKOOPER, 1965; RUBBER RESEARCH INSTITUTE OF MALAYA, 1969). At thirty-three months of age the mean girths for the different clones varied between 16.2 and 24.3 cm (average 20.3 cm). The mean girth of the same trees increased to 26.0 cm at fifty-six months and had a wider range (18.4 – 32.8 cm) *(Figure 2b)*. The wider variation is the result of within- and between-clone competition through overshadowing of less vigorous trees by more vigorous ones at such close planting distances.

Bark thickness. The bark thickness, girth and latex vessel ring number are interrelated in mature trees and are in turn correlated with yield (PAARDEKOOPER, 1965; NARAYANAN *et al.*, 1973). In the thirty-three-month-old nursery buddings the mean bark thickness varied between 2.5 and 4.2 mm (average 3.4 mm). The order of variation was slightly less than that of girth *(Figure 2c)*.

Number of latex vessel rings. In mature trees the number of latex vessel (LV) rings is an important structural yield determinant. Between clones it accounts for as much as 25 – 50% of the variation in yield (RUBBER RESEARCH INSTITUTE OF MALAYA, 1966; WYCHERLEY, 1969) whilst within clones the number of LV rings and girth explain most of the variation in yield (RUBBER RESEARCH INSTITUTE OF MALAYA, 1967). The normal rings (*i.e.* excluding the outer disorganised rings) and the total number of rings were examined for all eighty clones at thirty-three months of age. The total number of LV rings varied from 4.3 to 9.1 (average 6.7 rings). This is in agreement with the rate of latex vessel formation of about 2.5 rings per year obtained from the linear relationship between LV ring number and age up to fifteen years (RUBBER RESEARCH INSTITUTE OF MALAYA, 1966; GOMEZ *et al.*, 1972). The variation between trees within clones in the total number of LV rings is intermediate between that for girth and bark thickness.

Diameter of latex vessels and sieve tubes. A relationship between latex tube bore and yield was reported by ASHPLANT (1928), and between yield and sieve tube diameter by GUNNERY (1935). FREY WYSSLING (1930) and RICHES AND GOODING (1952) discussed the theoretical relationship of LV diameter to the rate of flow. In their considerations of tapping efficiency GOMEZ *et al.* (1972) considered the association between the yield and the cross-sectional area of the latex vessel system which was, *inter alia*, a function of the square of the radius of the latex vessel. MENDES (1969) and SHEPHERD (1969) working on young putative polyploid plants reported improved yields and larger latex vessel diameter respectively following colchicine treatment.

The diameter of latex vessels and sieve tubes was studied in the thirty-three-month-old nursery buddings. Measurements of LV diameter were taken only on undistorted and well-defined vessels — *i.e.* those in the three LV rings closest to the cambium. Similarly, only undistorted sieve tubes selected at random under the microscope were measured. The diameter of latex vessels varied between 14.7 and 20.5μ, and of sieve tubes between 16.7 and 23.3μ. The mean diameter of the latex vessels (eighty clones) was 17.3μ, compared with 25.6μ (mean of eight clones) at nine years from budding (GOMEZ *et al.*, 1972). Both these characters have low coefficients of variation *(Figure 2e)*.

In a study on the components of variation in ten clones it was found that the variation due to the number of observations within trees was between 8 and 25 times larger than that between trees for latex vessel diameter, and between 4 and 33 times larger for sieve tube diameter (*Table 1*). The standard error of a clone mean based on '*n*' trees and '*c*' observations per tree is given by

$$\sqrt{\frac{\sigma^2 c(n)}{cn} + \frac{\sigma^2 n}{n}}$$

where $\sigma^2 c(n)$ and $\sigma^2 n$ are estimates of the true variation of the 'number of observations within trees' and variation 'between trees' respectively. Since $\sigma^2 c$ is small, the standard error is only slightly reduced by an increase in the number of trees. *Table 2* shows the coefficient of variation (c.v.) of a clone mean for these two characters with different numbers of trees and observations within trees.

Distance between latex vessel rings. There are clonal differences in the distribution of LV rings in virgin bark, and the concentration of LV rings is generally highest nearest the cambium (RUBBER RESEARCH INSTITUTE OF MALAYA, 1966). The distribution also changes markedly with age (GOMEZ *et al.*, 1972). The differences between nursery and mature yield performance may be partly attributed to the rate of laying down of LV rings, since mature yield from virgin bark is correlated between clones with the number of latex vessel rings but not with girth. The distance between LV rings is a continuous variable, whereas the number of latex vessel rings is a discontinuous variable and, in nursery plants, is small. It was therefore, hoped that the distance between LV rings might be more easily determined and prove more discriminating than a count of LV rings in nursery plants. The distance between consecutive rings was therefore measured, (WYCHERLEY, 1969) to test this hypothesis and to study the correlation between the distance between consecutive LV rings and the rate of LV formation, and the number of latex vessels in the bark of mature trees.

TABLE 1. COMPONENTS OF VARIATION OF DIAMETER OF LATEX VESSELS AND SIEVE TUBES

Clone	Latex vessel				Sieve tube			
	$\sigma^2 c(n)$	$\sigma^2 n$	$\sigma^2 c(n)/\sigma^2 n$	Mean	$\sigma^2 c(n)$	$\sigma^2 n$	$\sigma^2 c(n)/\sigma^2 n$	Mean
AV 1191	8.33	0.87	9.6	20.0	10.48	0.32	32.8	19.8
GT 1	6.79	0.51	13.3	17.5	10.48	0.84	12.4	18.6
PB 5/51	7.16	0.68	10.6	19.2	6.44	0.39	16.6	19.2
PB 28/59	5.57	0.68	8.2	19.5	7.51	0.34	22.2	20.2
PR 107	2.00	0.16	12.5	15.0	17.12	0.62	27.8	20.8
PR 255	3.10	0.20	15.3	16.8	8.41	0.28	29.6	19.9
PR 261	3.02	0.12	24.7	15.5	9.01	0.47	19.2	20.9
RRIM 600	8.95	0.43	20.7	20.2	9.98	0.69	14.4	18.4
RRIM 614	5.67	0.32	17.9	20.3	9.50	2.41	3.9	17.5
RRIM 701	2.47	0.23	10.7	15.7	10.01	0.52	19.1	18.6

$\sigma^2 c(n)$ is an estimate of the true variation 'between observations within trees.'
$\sigma^2 n$ is an estimate of the true variation 'between trees.'

TABLE 2. COEFFICIENTS OF VARIATION OF A CLONE MEAN FOR DIAMETER OF LATEX VESSELS AND SIEVE TUBES BASED ON 5 OR 10 TREES, WITH 5 OR 10 OBSERVATIONS PER TREE

No. of trees and observations per tree	Coefficients of variation of a clone mean							
	Latex vessel				Sieve tube			
	5		10		5		10	
Clone	5	10	5	10	5	10	5	10
AVROS 1191	3.6	2.9	2.5	2.1	3.5	2.6	2.5	1.9
GT 1	3.5	2.8	2.5	2.0	4.1	3.3	2.9	2.3
PB 5/51	3.4	2.8	2.4	2.0	3.0	2.4	2.1	1.7
PB 28/59	3.1	2.5	2.2	1.8	3.0	2.3	2.1	1.6
PR 107	2.2	1.8	1.6	1.3	4.3	3.3	3.1	2.3
PR 255	2.4	1.9	1.7	1.4	3.2	2.4	2.2	1.7
PR 261	2.5	1.9	1.8	1.3	3.2	2.5	2.3	1.8
RRIM 600	3.3	2.6	2.3	1.8	4.0	3.2	2.8	2.2
RRIM 614	2.6	2.1	1.9	1.5	5.3	4.7	3.8	3.3
RRIM 701	2.4	2.0	1.7	1.4	3.8	3.0	2.7	2.1

The mean distance between rings for the first three rings and for all rings together is highly correlated between clones ($r = 0.763$). The distance between three rings nearest the cambium is smaller — indicating a higher concentration of LV rings — and is also more variable than that between the other rings *(Figure 2f)*. *Table 3* shows the mean distance between consecutive latex vessel rings up to the eighth ring and the 95% confidence limits for individual clones. The average distance between consecutive LV rings is smaller in young buddings than in mature trees, averaging 213μ. The distance between the first and second ring is smaller than that between outer consecutive rings. Rings beyond the third ring are more uniformly spaced. This is further illustrated by the cumulative frequency curves in *Figure 3*. The distance between the second and third ring is more variable than that between other rings *(Table 3)* and may therefore be a better measure of the rate of latex vessel formation. The correlation between the total number of LV rings and the distance between rings is highest with the second and fourth ring ($r = -0.709$). The distance between the cambium and the second ring is small and is variable within clones, so that from practical considerations, such as convenience and clarity of structure, the distance from the second to the fourth ring is the most suitable for purposes of measurement.

Density of latex vessels per 5 mm within rings. The density of latex vessels within the rings is an important feature of the laticiferous system. GOMEZ *et al.* (1972) reported a higher density closer to the cambium.

The mean density of latex vessels in the three rings closest to the cambium and in all rings taken together were highly correlated between clones ($r = 0.843$), with the same

TABLE 3. MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION OF THE MEAN DISTANCES BETWEEN CONSECUTIVE LV RINGS FOR DIFFERENT CLONES

Item	Cambium and 1st ring	1st and 2nd ring	2nd and 3rd ring	3rd and 4th ring	4th and 5th ring	5th and 6th ring	6th and 7th ring	7th and 8th ring
No. of clones	80	80	80	80	74	62	43	18
Mean	0.16	0.11	0.23	0.31	0.30	0.30	0.28	0.28
S.D.	0.04	0.04	0.09	0.09	0.08	0.10	0.07	0.07
C.V. (%)	24.10	35.90	40.70	30.10	25.90	32.00	25.70	24.10
Mean − 2 S.D.	0.08	0.03	0.05	0.13	0.14	0.10	0.14	0.14
Mean + 2 S.D.	0.24	0.19	0.41	0.49	0.46	0.50	0.42	0.42

order of variation *(Figure 2g)*. The mean density over all rings averaged 9.4 per millimetre over eighty clones, compared with an average of 13.6 per millimetre for mature trees from three clones RRIM 501, 623 and 625 (GOMEZ *et al.,* 1972). *Table 4* shows for each ring the mean LV density per 5 mm of ring over all clones, and the 95% confidence limits for individual clones. The density is higher in the second ring than in the first, followed by a progressive decline with distance from the cambium, as was observed in mature trees by GOMEZ *et al.* (1972). This is also shown by the cumulative frequency curves in *Figure 4.*

Number of Trees Necessary to Establish Significant Differences Between Two Clones for each Character.

Table 5 shows the 50 and 90% values obtained from the percentage cumulative frequency distributions of the coefficients of variation within clones for the different characters shown in *Figure 2.* These values have been used to derive the number of trees necessary for detecting significant differences between two clones for each of the characters. At the 50% point the corresponding coefficients of variation are about 60% for yield, 4% for diameter of latex vessels and sieve tubes, and

TABLE 4. MEANS, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION OF THE MEAN DENSITY OF LATEX VESSELS PER 5 MM OF RING FOR DIFFERENT CLONES

Item	1st ring	2nd ring	3rd ring	4th ring	5th ring	6th ring	7th ring	8th ring
No. of clones	80	80	80	80	74	62	43	18
Mean	74.7	82.1	54.7	37.2	29.7	25.7	23.4	21.1
S.D.	23.2	20.4	13.4	10.6	10.3	8.9	9.7	10.6
C.V. (%)	31.0	24.9	24.5	28.6	34.7	34.8	41.5	50.4
Mean − 2 S.D.	28.3	41.3	27.9	16.0	9.1	7.8	4.0	0
Mean + 2 S.D.	121.1	122.9	81.5	58.4	50.3	43.6	42.8	42.3

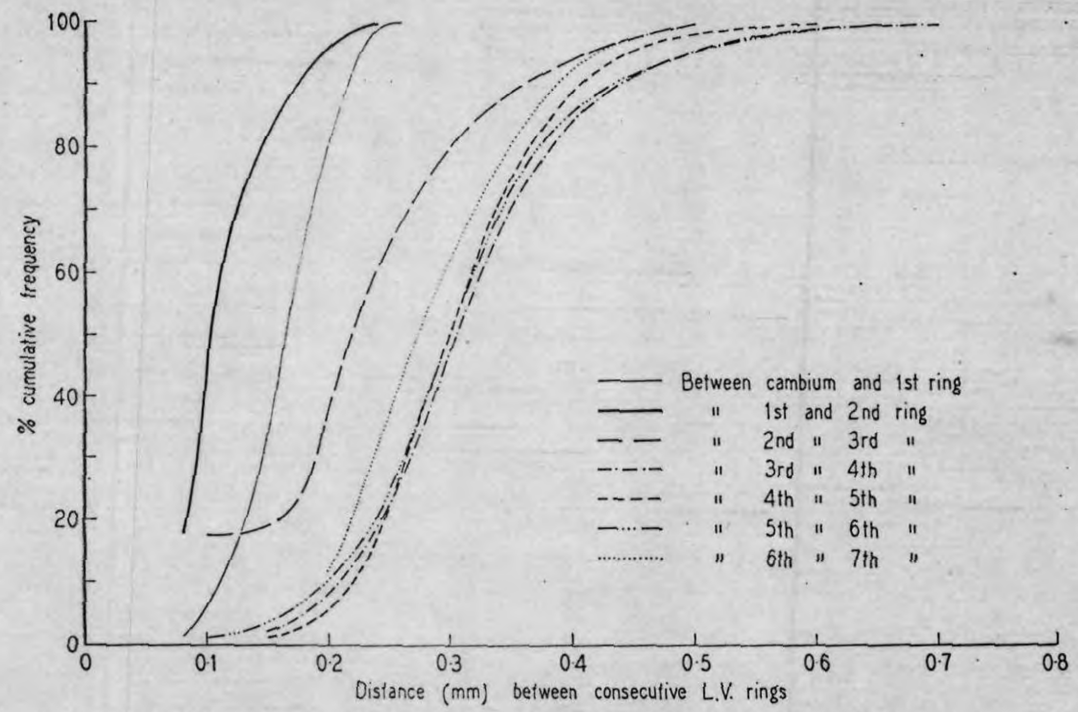

Figure 3. Percentage cumulative frequency distribution (between clones) of the mean distance between consecutive latex vessel rings.

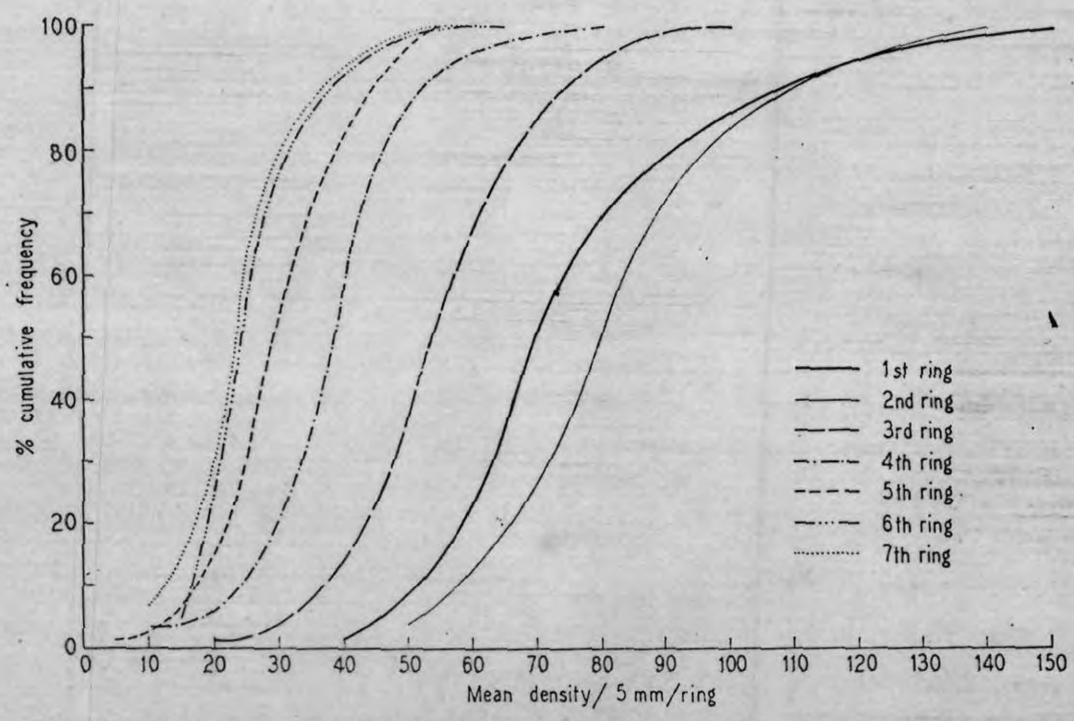

Figure 4. Percentage cumulative frequency distribution (between clones) of the mean density of LV per 5 mm of ring for different rings.

TABLE 5. PERCENTAGE VALUES OF DISTRIBUTION OF COEFFICIENTS OF VARIATION FOR DIFFERENT CHARACTERS

Character		C.V. percentage value	
		50	90
Yield (g)	33 months	60.0	86.0
	56 months	57.0	75.0
Girth (cm)	33 months	16.8	21.4
	56 months	22.8	29.4
Bark thickness (mm)	33 months	14.0	18.6
No. of LV rings (33 months)	Functional	23.0	30.6
	Total	17.3	23.0
Density of LV per 5 mm of ring (33 months)	Over three rings	26.6	34.5
	Over all rings	25.5	35.6
Distance (mm) between consecutive LV rings (mm) (33 months)	Over three rings	33.6	49.2
	Over all rings	22.3	34.0
Diameter (μ) (33 months)	Latex vessels	3.8	6.0
	Sieve tubes	4.3	6.3

Note: These values have been read from the graph of the percentage cumulative distribution of each character.

between 14 and 34% for other characters. The clonal differences to be detected at the 5% level of significance have been taken as 20, 30 and 40% of the mean for yield, as 2, 4 and 6% of the mean for diameter and as 10, 20 and 30% of the mean for the other characters *(Table 6).*

The relationship between the number of trees and the c.v. is given by

$$n \geq \frac{2s^2t^2}{d^2} \qquad ...(1)$$

where d is the desired observed difference to be detected, s^2 is an estimate of the true variance, and t is the appropriate Student's t value at 5% level of significance. The values of n given by Equation 1 are shown in *Table 6.* About thirty and twenty trees respectively are necessary for an even chance of detecting clonal differences in yield of the order of 30 or 40% of the mean. Likewise, between five and twenty-five trees are required to detect clonal differences of the order of 20% of the mean in the other characters

TABLE 6. NUMBER OF TREES[a] NECESSARY FOR DETECTING SIGNIFICANT DIFFERENCES BETWEEN TWO CLONES

Percentage value		50%				90%			
Character	Size of *d* (% of mean)	10	20	30	40	10	20	30	40
Yield (g)	33 months		70	32	19		143	64	37
	56 months		64	29	17		109	49	28
Girth (cm)	33 months	23	7	4		37	10	5	
	56 months	41	11	6		68	18	9	
Bark thickness (mm)	33 months	16	5	3		28	8	4	
No. of LV rings (33 months)	Functional	42	11	6		73	19	9	
	Total	24	7	4		42	11	6	
Density of LV per 5 mm of ring (33 months)	Over three rings	56	15	7		93	24	11	
	Over all rings	51	14	7		99	26	12	
Mean distance between consecutive LV rings (mm)	Over three rings	88	23	11		187	48	22	
	Over all rings	40	11	6		90	23	11	
	Size of *d* (% of mean)		2	4	6		2	4	6
Diameter (μ) (33 months)	Latex vessels		29	8	4		70	19	9
	Sieves tubes		37	10	5		77	20	10

[a]*r* is the number of trees such that the difference of size *d* between two clones (expressed as a percentage of the common mean) will be significant at the 5% level, assuming that the populations are normal and have the same variance.

(diameter excepted) with a 50% degree of confidence. Between eight and ten trees are sufficient for an equal chance of detecting clonal differences of 4% of the mean diameter of latex vessels and sieve tubes, while twenty [illegible] to detect the same difference with about 90% confidence. About sixty measurements per tree were taken to estimate the diameter of latex vessels and sieve tubes as shown in *Table 2,* but fewer measurements per tree will be adequate for future work.

CONCLUSIONS

Within the clones studied in the nursery, yield is the most variable parameter with a c.v. of about 60%; the diameter of latex vessels and sieve tubes is the least variable with variations under 5%. The girth, bark thickness, number and density of LV rings and the distance between consecutive rings are intermediate in this respect with c.v. around 20%. Measurements of the last three characteristics are generally less variable when all (instead of some) LV rings are considered. Between twenty and thirty trees are sufficient to allow an even chance of detecting clonal differences in yield of the order of 30 – 40% of the mean. With the same number of trees, smaller clonal differences in other characters can be detected. The distance between the second and fourth LV ring is more variable (between clones) than between outer rings, and is also highly correlated with the total number of rings. It therefore provides the best indicator of the rate of latex vessel formation. The more variable girth at fifty-six months compared with thirty-three months may be attributed to the intensification of competition within and between clones in the closely planted nursery. Any nursery selection should therefore be applied as early as possible not only to save time but also to avoid errors due to competition.

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APPENDIX

SOURCE OF CLONES

Malaysia	*Ceylon*	*Java*	*Sumatra*	*Vietnam*
RRIM 501	RRIC 1	LCB 1320	AVROS 308	IRCI 1
RRIM 513	RRIC 3	GT 1	AVROS 385	IRCI 2
RRIM 519	RRIC 4	WR 101	AVROS 427	IRCI 3
RRIM 600	RRIC 5	PR 107	AVROS 1191	IRCI 5
RRIM 605	RRIC 6	PR 226	AVROS 1279	IRCI 9
RRIM 607	RRIC 7	PR 228	AVROS 1349	TR 1406
RRIM 610	RRIC 14	PR 231	AEROS 1350	TR 1512
RRIM 612	RRIC 16	PR 247	AVROS 1447	TR 1514
RRIM 614	RRIC 21	PR 248	AVROS 1502	TR 1542
RRIM 615	RRIC 22	PR 249	AVROS 1518	TR 3702
RRIM 623	RRIC 36	PR 251	AVROS 1734	
RRIM 632	RRIC 37	PR 252	AVROS 1735	
RRIM 633	RRIC 41	PR 255	AVROS 2012	
RRIM 636	RRIC 42	PR 258	AVROS 2037	
RRIM 638	RRIC 45	PR 259		
RRIM 701		PR 261		
RRIM 707				
ES 4				
ES 5				
ES 8				
PB 86				
PB 5/51				
PB 5/63				
PB 28/59				
Ch 30				