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A Method for the Determination of Optimum Plot Size in Experiments with Rubber Trees (Hevea)

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On the account of scarcity of information about plot size and the great variability of genetic material, large plots are usual in experiments with rubber trees (Hevea). *This paper studies data from four experiments carried out in the Amazon Basin (Brazil) by a method that uses the intraclass coefficient of correlation among trees within plots, takes into account the guard rows, and defines as optimum plot size the number of test trees per plot, which minimises the estimate of variance of a treatment mean, for a fixed total number of trees per treatment. The first experiment (in the State of Pará) provided data on yield (averages of three years). The second one (in the State of Amazonas) furnished data on yield too. The other two experiments (in the State of Amazonas) furnished data on yield by microtapping (HMM), number of latex vessel rings, diameter of latex vessels, and stem girth at 1.30 m from the graft. The study leads to the following recommendations: for plots with a complete guard row: two test rows, with six to twelve trees, or three test rows, with nine to eighteen trees, or else four test rows, with sixteen to twenty trees. For plots with one half guard row: two test rows, with four to ten trees, or three test rows, with six to fifteen trees, or else four rows with sixteen to twenty test trees.*

When small plots are used, it is necessary to increase the number of replications. But this can be obtained even with a considerable reduction of area, and without loss of precision.

The problem of optimum plot size in experiments has been discussed for a long time, with numerous papers published and several methods proposed and recommended. Oliveira and Biava[1] published a bibliography on this subject, which includes papers from 1890 onwards. For perennials or tree crops, published papers are rather scarce. However, Pearce[2] says that 'to economize land, use small plots; to economize skilled labour, use large ones'. This is not correct in all cases.

For the rubber tree clones, information on optimum plot size is scarce. In some regions, especially in the Amazon Basin, the trees with rootstocks originating from seeds collected in the jungle are very heterogeneous and this leads to a high experimental error. The unequal incidence of diseases, caused, for example, by *Microcyclus ulei, Thanatephorus cucumeris* or *Ganoderma philippii* contributes also to the heterogeneity of trees. A similar problem occurs with reference to affinity between scion and rootstock.

The great variability among trees leads to the use of a large number of trees in the plots, with the objective of obtaining a 'representative sample'. It can be mentioned that Narayanan *et al.*[3] state that in Malaysia sixty to seventy-two trees per plot in large-scale trials, and six to seven in small-scale experiments, are used.

The use of big plots leads to experiments with very few replications, which is a serious handicap[4–8]. Several authors emphasise also the need of more replicates to ensure good precision to trials.

In the special case of rubber tree clone experiments, Paardekooper[9] and Narayanan[10]

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discuss the problem of plot size. The former mentions plots varying from nine to 168 plants.

This paper studies the problem of optimum plot size for experiments with rubber tree clones, with data collected in Brazil.

MATERIAL AND METHODS

The data used were collected in the States of Pará and Amazonas (Brazil). In Pará, the data on yield are averages of three years (1968/9, 1969/70, 1970/1) in an experiment started in 1956, with a 7.0 × 3.0 m spacing, and trees belonging to four clones (IAN 717, FX 3810, FX 3864, and FX 3899) on rootstock originating from seeds collected in the jungle. In the State of Amazonas, the data on yield refer to the year 1984, and come from an experiment of the Centro Nacional de Pesquisa de Seringueira e Dendê (CNPSD), started in 1975. Studies were also made of data of the variables: yield by microtapping (HMM), number of latex vessel rings, diameter of latex vessels, and stem girth at 1.30 m from the graft, collected from young planting on two other experiments in the State of Amazonas.

The data were studied by the methodology introduced by Pimentel Gomes[11] which uses the intraclass coefficient of correlation among test trees within plots, takes into account the guard rows, and defines as optimum plot size the number of test trees per plot, which minimises the estimate of variance of a treatment mean for a fixed total number of trees *(N)* per treatment.

In all experiments there was, for each of the four clones, one row of twelve trees. They were taken as belonging to three plots of $k = 4$ trees each. This model corresponds to a hierarchical classification and to an analysis of variance with mean squares V_1, referring to plots within clones, and V_2, referring to trees within plots. *Table 1* shows the corresponding analysis of variance.

It is easy to show that:

$$-\frac{1}{k-1} \leq \rho \leq 1, \quad (k > 1)$$

and also that an estimator of ρ is

$$\hat{\rho} = \frac{V_1 - V_2}{V_1 + (k-1)V_2} \quad (k > 1)$$

so that

$$V_1 \geq V_2 \rightarrow \hat{\rho} \geq 0$$

$$V_1 < V_2 \rightarrow \hat{\rho} < 0$$

Pimentel Gomes[11] proved that, for a fixed ρ, the coefficient of variation (C.V.) of the experiment

$$\text{C.V.} = \frac{100\sigma}{km} \sqrt{(1-\rho) + k\rho)}$$

where m is the mean per tree, is a decreasing function of k, so that the maximum value of (C.V.) occurs for $k = 1$. He proved also[12] that an estimate of $V(\hat{\rho})$ is given by the formula

$$V(\hat{\rho}) = \frac{2(1-\hat{\rho})^2 [1 + (k-1)\hat{\rho}]^2}{k^2} \left\{ \frac{1}{n_1 + 2} + \frac{1}{n_2 + 2} \right\}$$

where n_1 and n_2 are the numbers of d.f. of V_1 and V_2, respectively.

If a treatment includes N test trees, in r replicates, with plots of k test trees in n rows,

TABLE 1. ANALYSIS OF VARIANCE OF MODEL USED

Source	d.f.	Mean square	Expectation of mean square
Clone	(c-1)		
Plots within clone	c (p-1)	V_1	$\sigma^2 [1 + (k-1)\rho]$
Plants within plots	cp (k-1)	V_2	$\sigma^2 (1-\rho)$

it can be shown that the minimum value of $V(\hat{m})$ occurs, for n fixed and $\hat{\rho} > 0$, for

$$k = \sqrt{\frac{2bn\,(1-\hat{\rho})}{\hat{\rho}}} \qquad \hat{\rho} > 0$$

where $b = 1$, for a complete guard row, $b = \frac{1}{2}$, for a one half guard row, and $b = 2$ for a double guard row. This expression generalises results published by Pimentel Gomes[11]. The value of k should be, of course, a multiple of n. In any case, we have[13]

$$V(\hat{m}) = \frac{\sigma^2}{N}\,(1 + \frac{2b}{n})\,(1 + \frac{2bn}{k})\,[1 + (k-1)\,\hat{\rho}]$$

If both k and n are variables, the minimum of $V(\hat{m})$ occurs for

$$k = n^2, \quad n = \sqrt[3]{\frac{2b\,(1 - \hat{\rho})}{\hat{\rho}}} \qquad \hat{\rho} > 0$$

an expression which generalises results of Pimentel Gomes[12].

However, practical considerations frequently lead the scientists to prefer a 'rectangular' plot ($k \neq n^2$) rather than a 'square' plot ($k = n^2$), that is, a plot with the number of test rows equal to the number of test trees per row. But in this case, 'rectangular' plots should not be used too far, in form, from the 'square' ones. For example, a plot with twelve test plants in three rows (or may be two) leads to a lower value for $V(\hat{m})$ than a plot with twelve test plants in one row.

The total number of trees per plot is:

$$K = (1 + \frac{2b}{n}) \quad (k + 2bn)$$

so that the total number of trees per treatment is:

$$N = r(1 + \frac{2b}{n})\ (k + 2bn)$$

RESULTS AND DISCUSSION

The analysis of variance of the data are given in *Table 2*. It shows that in all cases studied one has $V_1 > V_2$, and thus $\hat{\rho} > 0$. For the case of yield, in the State of Pará, $\hat{\rho} = 0.0789$, with standard error $s(\hat{\rho}) = 0.1431$. So, for plots with a complete guard row ($b = 1$) the optimum number of test rows is:

$$n = \sqrt[3]{2(1 - 0.0789)/0.0789} = 2.86$$

that is, three, and, therefore, the optimum number of test plants per plot is $k = 3^2 = 9$. In this case we have, for a treatment with N plants on the whole:

TABLE 2. ESTIMATES OF MEAN SQUARES OF VARIABLES OF FOUR RUBBER TREE CLONES AND A YOUNG PLANTING

Source	d.f.	Mean squares: Yield (g/plant/tapping) PB	PM	HMM (g/plant/ 10 tappings)	NA	DV (μm)	CC (cm)
Clones	3	53 305 934	118 043.0000	55.3587	1.3668	0.1273	135.5660
Plots within clones	8	2 194 020	4 906.9500	48.5439	0.1385	1.1690	66.8098
Plants within plots	36	1 634 116	3 392.4779	26.3493	0.0554	0.7405	24 .8583

PB = State of Para
PM = State of Amazonas
HMM = Early yield test
NA = Number of latex vessel rings
DV = Diameter of latex vessels
CC = Stem girth

$$V(\hat{m}) = \frac{\sigma^2}{N}(1 + \frac{2}{3})(1 + \frac{2 \times 3}{9})(1 + 8 \times 0.0789) = (\sigma^2/N)\, 4.53$$

But *Table 3* shows that this variance increases very slightly if we take $k = 6$ plants in $n = 2$ rows, or $k = 8$ plants in $n = 4$ rows (which, of course, is equivalent to two rows of four). It will increase somewhat more if we take $k = 5$ plants in one row.

A similar situation is met for data from the State of Amazonas which show a value of $\hat{\rho} = 0.1004$, with $s(\hat{\rho}) = 0.1471$. So, for plots with a complete guard row ($b = 1$) the optimum number of rows is $n = 2.62$, that is three, and therefore, the optimum number of test plants per plot is also $k = 3^2 = 9$. The corresponding variance is $V(\hat{m}) = (\sigma^2/N)\, 5.01$, as shown in *Table 3*. But this variance changes very little when we take just one row with three or four trees, or three rows of two trees (which is equivalent to two rows of three trees), or four rows of two plants (that is, two rows of four).

Having in view the results shown on *Table 3* for all observed variables, it can be seen that the best solutions are given by the following cases:

With a complete guard row:

$n = 2, k = 6$ or 8

$n = 3, k = 9$

With one half guard row:

$n = 2, k = 4, 6$ or 8

However, we should note that the optimum plot size increases when $\hat{\rho}$, taken as positive, approaches zero. On the other hand, the estimates of ρ ($\hat{\rho} = 0.0789$ in the experiment of Parã, $\hat{\rho} = 0.1004$ in the experiment of Amazonas) are low and have a rather high standard error. So, in a pessimistic hypothesis, we may take $\hat{\rho} = 0.05$. The optimum number of rows calculated is then $n = 3.36$, that is, three rows, so that the optimum number of test trees is $k = 9$. Variances corresponding to several values of n and k of interest are shown in *Table 4*. These results suggest the recommendation for plots with a complete guard row, of plots with $n = 2$ rows with $k = 6$ to 12 test trees, or $n = 3$ rows with nine to eighteen test trees, or else four rows with sixteen to twenty test trees.

Plots with a half guard row should be somewhat smaller, as shown in *Table 5*.

It is important to have in view that, with $\hat{\rho} > 0$, when plots of a size above optimum have their number of test trees reduced, the number of replications should be increased to maintain a low variance of the estimates of treatment means. But this increase is such that, even with a greater number of plots, the total area of the experiment is reduced. When substituting an experiment with k' test trees in n' rows per plot for another with k test trees in n rows, the ratio between the corresponding areas is given by the expression:

$$\frac{A'}{A} = \frac{(1 + \frac{b}{n'})(1 + \frac{bn'}{k'})[1 + (k' - 1)\hat{\rho}]}{(1 + \frac{b}{n})(1 + \frac{bn}{k})[1 + (k - 1)\hat{\rho}]}$$

So, with $\hat{\rho} = 0.0789$ and for a complete guard row ($b = 1$), if we substitute a trial with plots of $k' = 6$ test trees, in $n' = 2$ rows, for another with plots of $k = 40$ test trees in $n = 4$ rows, the ratio between areas is:

$$\frac{A'}{A} = \frac{4.65}{8.15} = 0.57 = 57\%$$

Therefore we save 43% of the experimental area, without changing the precision of the trial.

The ratio between the number of replications (r and r') is:

$$\frac{r'}{r} = \frac{K}{K'} \quad \frac{A'}{A}$$

where K and K' are the total numbers of trees per plot in the two cases. In the example under discussion we have:

$$K = (n+2)\ (\frac{k}{n} + 2) = (4+2)\ (\frac{40}{4} + 2) = 72$$

$$K' = (2+2)\ (\frac{6}{2} + 2) = 20$$

$$\frac{r'}{r} = \frac{72}{20} \times 0.57 = 2.05$$

TABLE 3. ESTIMATES OF INTRACLASS COEFFICIENT OF CORRELATION, OF NUMBER OF TEST PLANTS, TOTAL NUMBER OF PLANTS PER PLOT WITH ONE TO FOUR TEST ROWS

Variables / plot sizes	ρ estimate	Plot	Half guard row 1 row	Half guard row 2 rows	Half guard row 3 rows	Half guard row 4 rows	Complete guard row 1 row	Complete guard row 2 rows	Complete guard row 3 rows	Complete guard row 4 rows
PB		k	3 or 4	4	6	8	5	6	9	8
Yield	0.0789	$V(\hat{m})$	(σ^2/N) 3.09	(σ^2/N) 2.78	(σ^2/N) 2.79	(σ^2/N) 2.91	(σ^2/N) 5.52	(σ^2/N) 4.65	(σ^2/N) 4.53	(σ^2/N) 4.65
(g/plant/tapping)		K	8 or 10	9	12	15	21	20	25	24
PM		k	3	4	6	8	4	6	6 or 9	8
Yield	0.1004	$V(\hat{m})$	(σ^2/N) 3.20	(σ^2/N) 2.93	(σ^2/N) 3.00	(σ^2/N) 3.19	(σ^2/N) 5.85	(σ^2/N) 5.01	(σ^2/N) 5.01	(σ^2/N) 5.11
(g/plant/tapping)		K	8	9	12	15	18	20	20 or 25	24
HMM		k	2	4	3	4	3	4	6	8
(g/plant/10 tappings)	0.1739	$V(\hat{m})$	(σ^2/N) 3.52	(σ^2/N) 3.42	(σ^2/N) 3.59	(σ^2/N) 3.80	(σ^2/N) 6.74	(σ^2/N) 6.09	(σ^2/N) 6.23	(σ^2/N) 6.65
		K	6	9	8	10	15	16	20	24
		k	2	4	3	4	2	4	3	4
NA	0.2728	$V(\hat{m})$	(σ^2/N) 3.27	(σ^2/N) 3.27	(σ^2/N) 4.12	(σ^2/N) 4.55	(σ^2/N) 7.64	(σ^2/N) 7.27	(σ^2/N) 7.73	(σ^2/N) 8.18
		K	6	9	8	10	12	16	15	18
		k	3	4	6	4	4	6	6	8
DV (μ m)	0.1264	$V(\hat{m})$	(σ^2/N) 3.34	(σ^2/N) 3.13	(σ^2/N) 3.26	(σ^2/N) 3.45	(σ^2/N) 6.21	(σ^2/N) 5.44	(σ^2/N) 5.44	(σ^2/N) 5.65
		K	8	9	12	10	18	20	20	24
		K	2	2	3	4	2	4	3	4
CC (cm)	0.2967	$V(\hat{m})$	(σ^2/N) 3.89	(σ^2/N) 3.89	(σ^2/N) 4.25	(σ^2/N) 4.72	(σ^2/N) 7.78	(σ^2/N) 7.56	(σ^2/N) 7.97	(σ^2/N) 8.50
		K	6	6	8	10	12	16	8	18

ρ = Intraclass coefficient of correlation

k = Number of test plants

K = Total number of plants

PB = State of Pará

PM = State of Amazonas

HMM = Early yield test

NA = Number of latex vessel rings (with square root transformation)

DV = Diameter of latex vessels

CC = Stem girth at 1.30 m from the graft

TABLE 4. VALUES OF n, k AND $V(\hat{m})$ FOR PLOTS WITH A COMPLETE GUARD ROW, WITH ρ = 0.05

n	k	V ($\hat{m}$)	n	k	V ($\hat{m}$)
2	6	4.17 σ^2/N	3	9	3.89 σ^2/N
2	8	4.05 σ^2/N	3	12	3.88 σ^2/N
2	10	4.06 σ^2/N	3	15	3.97 σ^2/N
2	12	4.13 σ^2/N	3	18	4.11 σ^2/N
2	14	4.24 σ^2/N	3	30	4.49 σ^2/N
2	20	4.68 σ^2/N	3	42	5.81 σ^2/N
2	30	5.55 σ^2/N	3	60	7.24 σ^2/N
2	40	6.49 σ^2/N	5	25	4.31 σ^2/N
4	16	3.94 σ^2/N	5	30	4.57 σ^2/N
4	20	4.10 σ^2/N	5	40	5.16 σ^2/N
4	24	4.30 σ^2/N	5	60	6.45 σ^2/N
4	32	4.78 σ^2/N			
4	40	5.31 σ^2/N			
4	60	6.71 σ^2/N			

TABLE 5. VALUES OF n, k AND $V(\hat{m})$ FOR PLOTS WITH A HALF GUARD ROW, WITH ρ = 0.05

n	k	V ($\hat{m}$)	n	k	V ($\hat{m}$)
2	4	2.59 σ^2/N	3	9	2.49 σ^2/N
2	6	2.50 σ^2/N	3	12	2.58 σ^2/N
2	8	2.53 σ^2/N	3	15	2.72 σ^2/N
2	10	2.61 σ^2/N	3	18	2.88 σ^2/N
2	12	2.71 σ^2/N	3	30	3.59 σ^2/N
2	14	2.83 σ^2/N	3	42	4.36 σ^2/N
2	20	3.22 σ^2/N	5	25	3.17 σ^2/N
2	30	3.92 σ^2/N	5	30	3.43 σ^2/N
4	16	2.73 σ^2/N	5	40	3.98 σ^2/N
4	20	2.92 σ^2/N			
4	24	3.14 σ^2/N			
4	32	3.86 σ^2/N			
4	40	5.16 σ^2/N			

therefore $r' = 2.05r$. If we have $r = 5$ blocks in the trial with $k = 40$ tree plots, the other experiment should have $r' = 2.05 \times 5 = 10.25$ blocks, that is, ten blocks approximately. But the total number of trees per treatment will be reduced from $N = 5 \times 72 = 360$ to $N' = 10 \times 20 = 200$, with a reduction of 44% in area.

CONCLUSIONS

- The data analysed suggest that under the conditions of the States of Para and

Amazonas (Brazil), experimental plots for rubber trees, adult plantings as well as young ones, with a complete guard row should have two rows with six to twelve test trees, or three rows with nine to eighteen test trees, or else four rows with sixteen to twenty test trees. Plots with one half guard row should be somewhat smaller: they should have two rows of four to ten test trees, or three rows with nine to fifteen test trees, or else four rows with sixteen to twenty test trees.

- Plots with only one test row, or with over four test rows are not recommended.
- Adequate plot size can reduce greatly the area of experiments with no decrease in their precision, or, alternatively, the precision can be enlarged without increase of area.

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