AMERICAN SOCIETY FOR HORTICULTURAL SCIENCE
1957 69:208-216

Number of Replications and Plot Sizes Required for Reliable Evaluation of Nutritional Studies and Yield Relationships with Citrus and Avocado[1]

W. W. JONES, T. W. EMBLETON, and C. B. CREE
University of California Citrus Experiment Station,
Riverside, California

There are many reports in the literature (1-17) on the planning of field nutritional studies with tree crops, but no attempt will be made to review these here. We have been asked to report on our procedure for handling field experiments. In order to place reasonable confidence in conclusions drawn from such experiments, it is clear that the original plan of an experiment must permit adequate sampling procedures. In studies with tree crops such as citrus and avocados there are three major problems related to sampling: (A) yields, (B) leaf composition, and (C) fruit composition. We have found more variability in yields than in leaf or fruit composition. The number of trees per plot, or the number of replications per treatment required to achieve a given precision varies greatly and depends on which factor is of major interest.

The present paper shows in graphic form the number of replications of mature citrus or avocado trees required in a randomized block experiment in the field to give a reliable evaluation of the significance of differences associated with differential treatments. Trees just coming into bearing have higher variability for yields than mature trees. Replication requirements for yields are compared for several plot sizes. Other factors are compared with yields in a given plot size. The shapes of the plots were not evaluated. These data are based on our experience with oranges, lemons, and Fuerte avocados in California, and are averages from a large number of field plots over a period of several years. It is hoped that these data will prove helpful in setting up other experimental field plots so that the design chosen will accomplish the desired objectives with adequate, but not excessive, precision.

The formulas used in calculating these relationships are those proposed by Cochran and Cox (3). Our calculations are based on plot means. In each chart (Figs. 1-10) the vertical axis represents the critical difference between means expressed as a percentage of the mean of the experiment; and the horizontal axis represents the number of replications required to give significance at the 5 per cent level, nine out of ten times. The calculations are based on a randomized block with four treatments. The values on the graph would change very little if there were more than four treatments (3). However, if there were fewer than four treatments it would be advisable to recalculate the number of required replications.

If one knows the coefficient of variability, in percent (obtained by dividing the square root of the error variance of the experiment by the mean of the experiment and multiplying by 100), the foregoing calculations can be made. The coefficients of variability for some common measurements made on oranges, lemons, and avocados from individual plots are presented in Table 1, and were used in calculating the data shown in Figs. 1-10. It can readily be seen that the variability for yields is greater than for the other factors in question. Moreover, the avocado yields are more variable than the citrus yields. Table 2 shows that the yield variability for citrus is similar to that of several other tree crops. In our experimental work we have found coefficients of variability for avocado yields ranging from 58 to 102 per cent for single-tree plots for one year, and from 27 to 41 per cent for 4-tree plots, using the mean yield for two years.

Figures 1-10 show the plot size and required number of replications for some common measurements made on oranges, lemons, and avocados.

Table 1. Coefficients of variability (in per cent) for factors measured on oranges, lemons, and avocados.[a]

Factor	Oranges (4-tree plots, 1 year)	Lemons (4-tree plots, 1 year)	Avocados (1-tree plots, 1 year)
Yield	13.0	14.9	81.0
Brix of juice	3.0	1.4	-----
Acid in juice (per cent)	6.0	3.4	-----
Vitamin C in juice (mg/100 ml)	6.0	4.6	-----
Juice (per cent)	4.0	5.5	-----
Leaf N (per cent)	4.2	5.5	7.8
Leaf P (per cent)	4 9	5.9	10.5
Leaf K (per cent)	10.0	6.8	21.1
Oil in fruit pulp (per cent)	-----	-----	10.7

[a]Based on averages from large numbers of field plots

ORANGES

Yield:--Fig. 1 shows the relation of plot size and replication to precision of estimating orange yields. An increase in the number of trees per plot and/or an increase in the number of replications results in greater precision. Inherent variability and practical field considerations make it difficult to measure with confidence small differences due to treatment, and we usually accept plot designs which permit critical evaluation of 20 per cent (or even more) difference from the general mean. From Fig. 1 it may be seen that this requires four replications with 8- and 16-tree plots, or ten replications with 4-tree plots.

Leaf Analysis:--Fig. 2 shows that yield of citrus trees varies more than the concentration of nutrients in the leaves. There is also more apparent variability in potassium concentration than in phosphorus in such leaves, and more in phosphorus than in nitrogen, in agreement with the findings of others (10). Critical studies have not been made to indicate whether variability in samples or in chemical analysis is responsible for these differences. Thus if the field experiment design is adequate for measuring treatment effects on yields, one can be confident that effect of treatment on nutrient element concentration in the leaves can be determined more precisely. This, of course, is desirable, since small differences in leaf composition may result in large differences in yield. If only nutrient uptake, as measured by leaf analysis, is to be determined, a smaller field experiment may be used than when yields are also a consideration. But with us, yield is usually of primary importance and the experiments are so designed.

Fruit Quality:--Like nutrient elements, fruit quality factors (Fig. 3) can be measured more precisely than yield on any given experimental design.

Table 2.--Coefficients of variability for yields of crops reported in the literature from different countries.

Crop	Size of plot	Location	Coefficient of variability (per cent)	Citation
Navel oranges	4 trees	U.S.A. (California)	32	(1)
	4 trees	U.S.A. (California)	22	(1)
Valencia oranges	4 trees	U.S.A. (California)	28	(1)
	5 trees	U.S.A. (Florida)	17	(19)
Eureka lemons	4 trees	U.S.A. (California)	23	(1)
Jonathan apples	4 trees	U.S.A. (Utah)	26	(1)
	3 trees	Australia	11	(16)
Baldwin apples	-----	U.S.A. (New York)	21-56[a]	(4)
Cox's Orange Pippin apples	1 tree	England	26-47[a]	(6)
	6 trees	England	28-47[a]	(6)
Lane's Prince Albert apples	6 trees	England	14-67[a]	(6)
Worcester Pearmain apples	4 trees	England	27-151[a]	(6)
Seedling walnuts	4 trees	U.S.A. (California)	30	(1)
Smith's Seedling peaches	4 trees	Australia	14	(16)
Grapes	4 vines	Australia	17	(16)
	6 vines	Argentina	17	(7)

[a] The high coefficient of variability per cent is for young trees apparently just coming into bearing. The low value is more typical of the variability of yields of mature trees.

LEMONS

Figs. 4, 5, and 6 present the data on yield, leaf analysis, and fruit quality for lemons. The results are very similar to those for oranges. As with oranges, leaf composition and fruit quality factors are less variable than yields.

AVOCADOS

Even in a relatively uniform-appearing block of avocado trees, yields from tree to tree are extremely variable. We think this is due, in part, to the variability in the seedling rootstocks. Fig. 7 shows that to measure a 20 per cent difference based on the general mean at the 5 per cent level with P = 90 per cent, eight replications of 16-tree plots and 38 replications of 4-tree plots are required. In southern California individual avocado orchards are relatively small and planted, in many instances, on the warmer sloping lands. This makes it difficult to find large, uniform blocks suitable for precise nutritional studies. If one is willing to accept less precision (P = 75 per cent), the number of replications of four-tree plots (Fig. 10) can be reduced to 25. This is still not a practical working arrangement for most field studies.

Leaf analysis and fruit quality are much less variable than yield and can be determined with a reasonable number of replications, except for leaf K, as indicated in Figs. 8 and 9.

METHODS TO INCREASE PRECISION

The data previously shown indicate that it may be difficult to obtain the desired information by using randomized blocks, unless exceptionally large and costly experiments are used. To get around this we are using, wherever possible, factorial, Latin square, and split plot designs, with and without confounding.

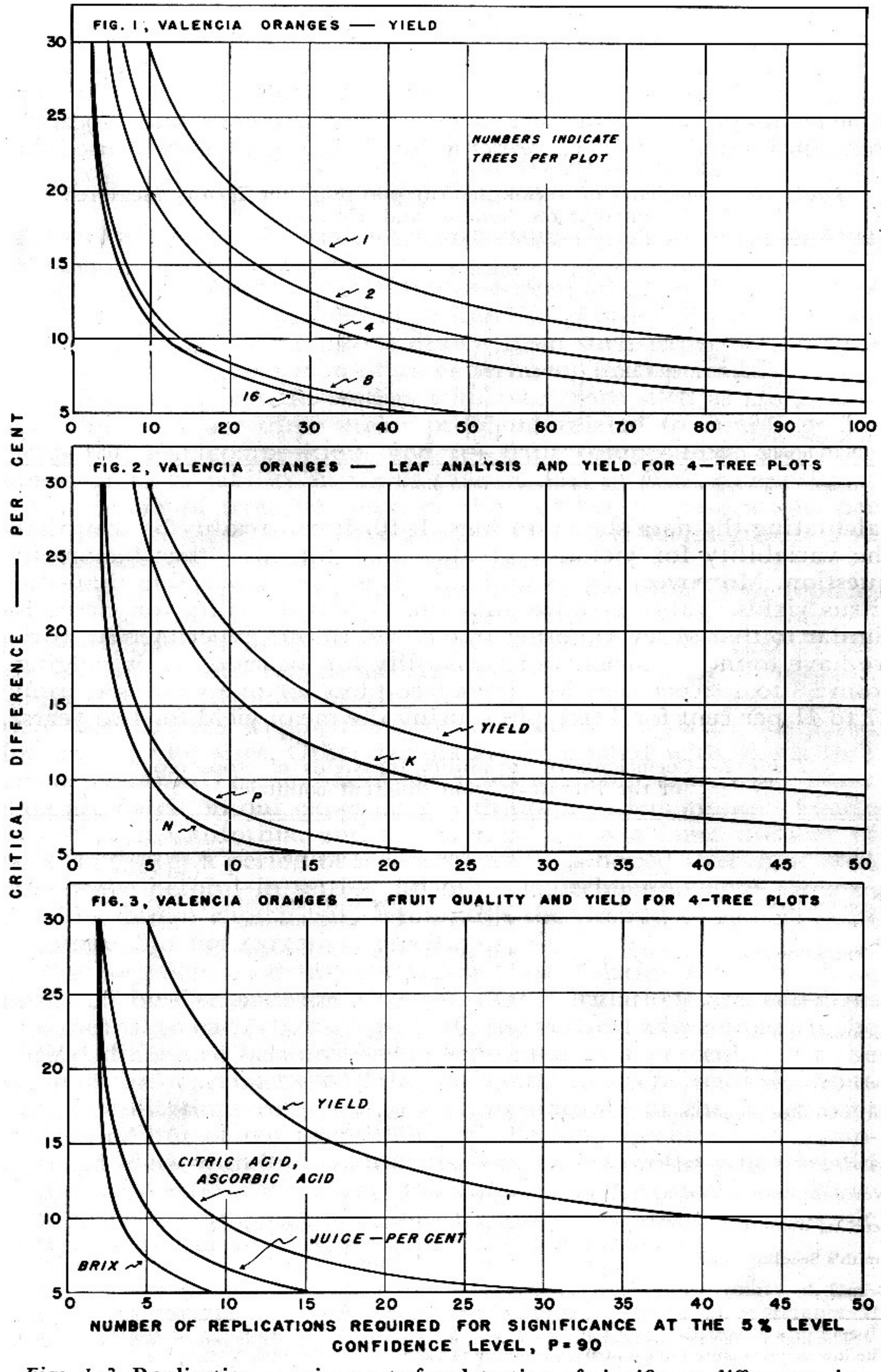
Some increase in precision for yields may be obtained through uniformity trials and continuity plots (plots that are uniformly treated and located at frequent intervals throughout the experiment). This has been done with the long-term fertilizer experiment with Washington Navel oranges at Riverside (1, 5). The results of the continuity plots have been used to adjust the yields of the treatment plot by covariance analysis. To some extent this adjustment is based on the assumption that the annual variations in climatic conditions affect the yields of the continuity plots and the treatment plots equally. This does not appear to be entirely true, since the reduction in error variance varies from period to period, as shown in Table 3.

This procedure is time-consuming, and a more practical approach is needed, especially for short-time experiments in cooperators' groves. In other experiments we have reduced the error variance from 0 to 48 per cent by covariance adjustment of the yields of oranges on the trunk circumference of the trees at the time of initiating the experiment. Similar adjustments with avocados have not as yet been beneficial.

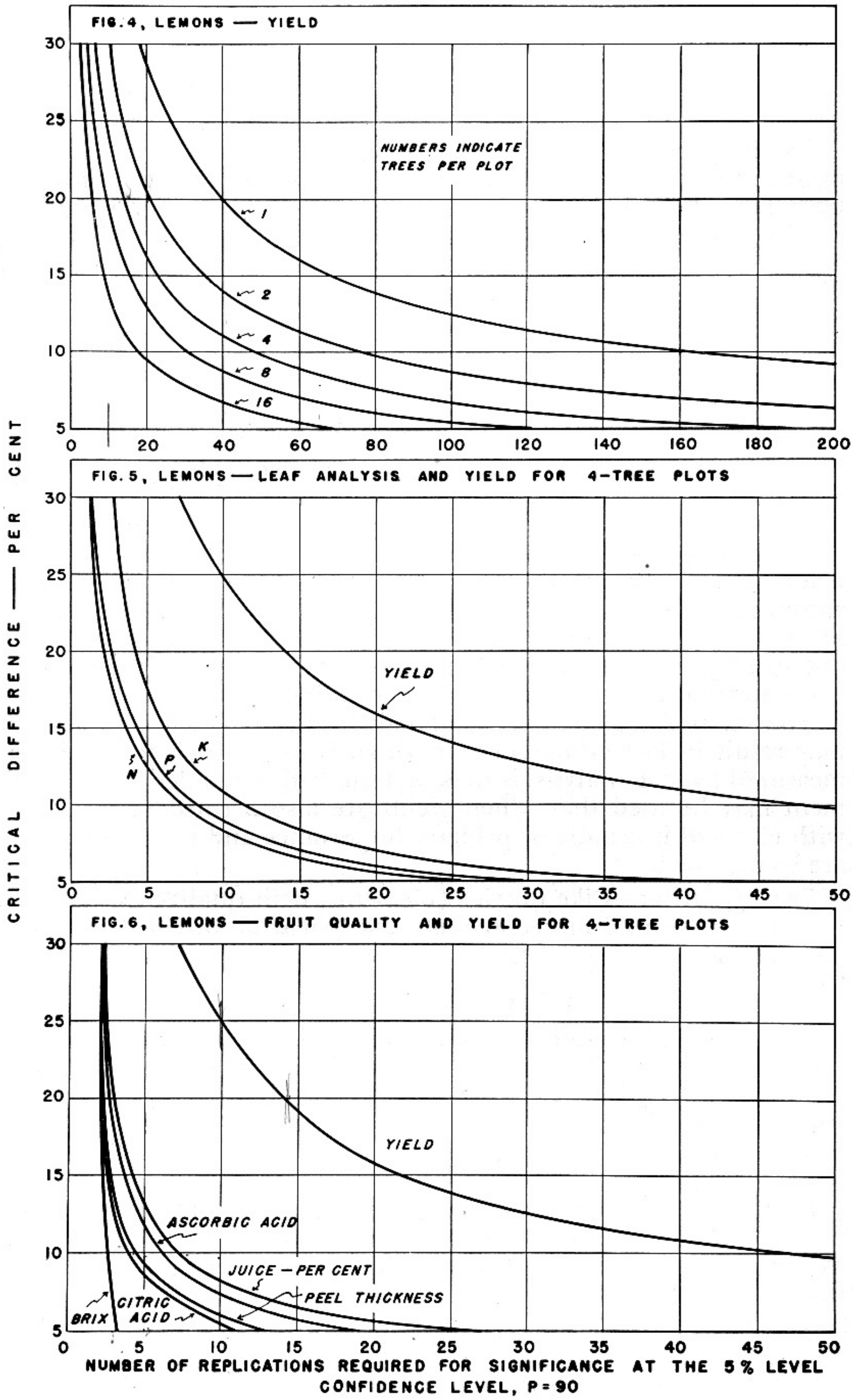
Table 3. Adjustment of Washington Navel orange yields by covariance analysis with continuity plots. Long-time fertilizer experiment, Citrus Experiment Station, Riverside, Calif.

Period	Mean yield (lb/tree)	Coefficient of variability (per cent)		Reduction in error variance due to adjustment (per cent)
		A[a]	B[b]	
1928-1931	113	10.92	15.38	49.60
1932-1935	147	13.27	16.75	37.21
1936-1939	115	16.72	20.37	32.50
1940-1943	168	15.74	15.97	2.82
1944-1947	172	15.70	16.69	11.43
1948-1951	186	15.05	15.30	3.32
1952-1955	183	15.12	18.30	31.72

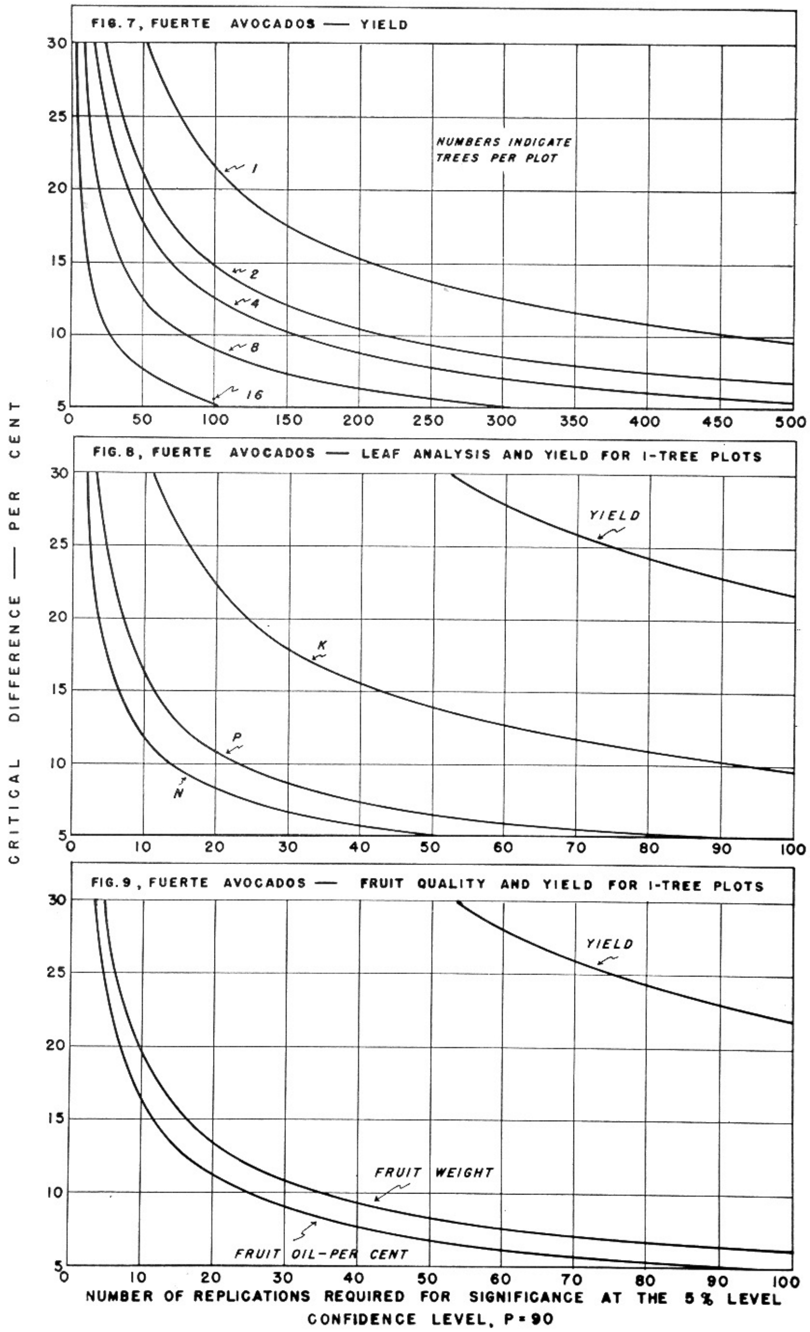
[a]Calculated on adjusted yields.
[b]Calculated on unadjusted yields.



Figs. 1–3. Replication requirements for detection of significant differences in yield (Fig. 1), leaf analysis and yield (Fig. 2), and fruit quality and yield (Fig. 3) in Valencia oranges.



Figs. 4–6. Replication requirements for detection of significant differences in yield (Fig. 4), leaf analysis and yield (Fig. 5), and fruit quality and yield (Fig. 6) in lemons.



Figs. 7–9. Replication requirements for detection of significant differences in yield (Fig. 7), leaf analysis and yield (Fig. 8), and fruit quality and yield (Fig. 9) in Fuerte avocados.

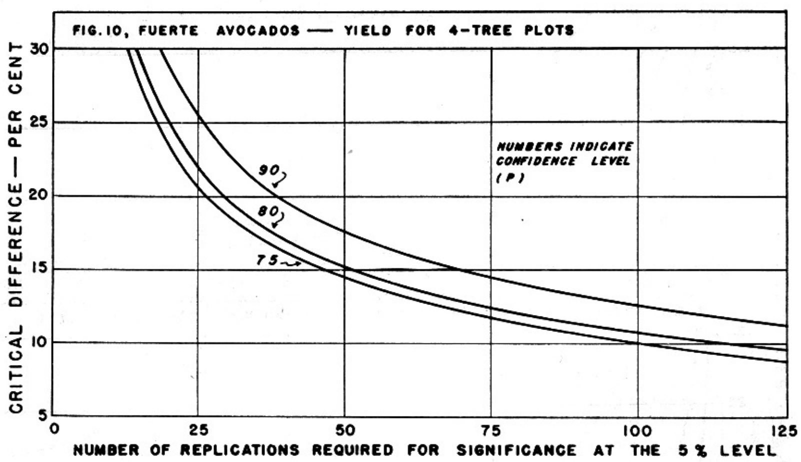


Fig. 10. Replications required to detect significant differences in yield in Fuerte avocados in 4-tree plots, with P at different levels.

SUMMARY

In field nutritional studies in citrus and avocado orchards, we have found that fruit yields from individual plots are much more variable than leaf composition or certain fruit quality factors. The data emphasize that in the planning of orchard experiments, the probable variability of the factors to be measured should be known.

On the basis of our experience with oranges, lemons, and avocados, we have presented a series of curves which should be helpful in designing field experiments with these and other tree crops.

LITERATURE CITED

1. BATCHELOR, L. D., and H. S. REED. 1918. Relation of the variability of yields of fruit trees to the accuracy of field trials. Jour. Agr. Res. 12: 245-283.
2. CAMERON, S. H., R. T. MUELLER, A. WALLACE, and E. SARTORI. 1952. Influence of age of leaf, season of growth, and fruit production on the size and inorganic composition of Valencia orange leaves. Proc. Amer. Soc. Hort. Sci. 60:42-50.
3. COCHRAN, W. G., and G. M. COX. 1950. Experimental designs. John Wiley and Sons, Inc., New York.
4. COLLISON, R. C., and J. D. HARLAN. 1930. Variability and size relations in apple trees. N. Y. Agr. Exp. Sta. Tech. Bul. 164.
5. PARKER, E. R., and L. D. BATCHELOR. 1932. Variation in the yields of fruit trees in relation to the planning of future experiments. Hilgardia 7:81-161.
6. PEARCE, S. C. 1949. The variability of apple trees I. The extent of crop variation and its minimization by statistical means. Jour. Hort. Sci. 25(1):3-9.
7. ___________. 1950. The interpretation of Uniformity trials. East Malling (Kent) Res. Sta. Annu. Rept. 1 Jan. 1949-30 Sept. 1949: 91-92.
8. POTTER, G. F., and B. G. SITTON. 1954. Experimentation with orchard trees. Chapt.

17. Fruit Nutrition. N. F. Childers, ed. pp. 810-834. Horticultural Publications, Rutgers University, New Brunswick, N. J.

9. REUTHER, WALTER, and P. F. SMITH. 1950. A preliminary report on the relation of nitrogen, potassium, and magnesium fertilization to yield, leaf composition, and incidence of zinc deficiency in oranges. Proc. Amer. Soc. Hort. Sci. 56:27-33.

10. __________ and _________. 1954. Leaf analysis of citrus. Chap. 7. Fruit Nutrition. N. F. Childers, ed. Pp. 257-294. Horticultural Publications, Rutgers University, New Brunswick, N. J.

11. SCHULTZ, E. F., JR., and G. W. SCHNEIDER. 1955. Sample size necessary to estimate size and quality of fruit, growth of trees, and per cent fruit set of apples and peaches. Proc. Amer. Soc. Hort. Sci. 66:36-44.

12. SINCLAIR, W. B., and E. T. BARTHOLOMEW. 1944. Effects of rootstock and environment on the composition of oranges and grapefruit. Hilgardia 16:125-176.

13. SITES, J. W., and H. J. REITZ. 1949. The variation in individual valencia oranges from different locations of the tree as a guide to sampling methods and spot-picking for quality. I. Soluble solids in the juice. Proc. Amer. Soc. Hort. Sci. 54:1-10.

14. __________ and _________. 1950. The variation in individual valencia oranges from different locations of the tree as a guide to sampling methods and spot-picking for quality. II. Titratable acid ratio of the juice. Proc. Amer. Soc. Hort. Sci. 55:73-80.

15. STADLER, L. J. 1929. Experimental error in field plot tests. Proc. Internatl. Cong. Plant Sci. 1:107-127.

16. STRICKLAND, A. G. 1935. Error in horticultural experiments. Jour. Dept. Agr. Victoria, Australia 33:1-9.

17. WALLACE, A., R. T. MUELLER, and M. G. SQUIER. 1952. Variability in orange leaves of the same age and collected from a single tree. Proc. Amer. Soc. Hort. Sci. 60:51-54.