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How to Perform an Agricultural Experiment

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Volunteers for International Technical Assistance

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HOW TO PERFORM AN AGRICULTURAL EXPERIMENT

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by

G. Stuart Pettygrove

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July 1971

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FOREWORD

Local technicians in developing countries are increasingly being called upon to test innovative measures developed by researchers at the national or regional level. Improved varieties, new fertilizer practices, irrigation, pesticides, new feed mixtures, and improved harvest procedures are just a few of the more important innovations which must be thoroughly tested at the local level before they are passed on to the farmer by extension methods.

Often, local research is carried out, not by trained research personnel, but by extension agents, teachers and training center workers, community development agents, foreign technicians, fertilizer and seed distributors, and large farmers.

The purpose of this paper is to provide local agriculturalists with an understanding of the basic considerations in the design, execution, and measurement procedure of an agricultural experiment. This paper does not cover statistical analysis, and it is assumed that trained statisticians are available for this purpose.



CONTENTS

FOREWORD	
LIST OF FIGURES	
SECTION I. SOME BASIC CONCEPTS	
I. The Need for Local Research	
II. Experiment versus Demonstration 2	
III. Some Basic Concepts in Statistics2A. The normal distribution3B. The null hypothesis4C. The "significant difference"4	
SECTION II. HOW TO PERFORM AN AGRICULTURAL EXPERIMENT	
I. Preliminary Research 5	
II. Designing the Experiment6A. Replication6B. Randomization6C. Selection of treatments10D. Selecting the location11E. Plot size and shape12	
III. Execution of the Experiment	
IV. Measuring and Recording the Results	
APPENDIX: A Table of Random Numbers	,
RTRI TOGRAPHY 23	5

LIST OF FIGURES

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. .

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•:

Ι.	Normal Curve	3
II.	Normal Curves with and without Fertilizer	3
III.	Completely Randomized Design	7
IV.	Randomized Complete Block	8
۷.	Randomized Complete Block Suitable for Demonstration	8
VI.	How to Randomize the Latin Square Design	9
VII.	Split-plot Design	10
VIII.	Plot Shape	13
IX-A.	Laying Out a Right Angle	15
IX-B.	Laying Out a Right Angle	16
IX-C.	Laying Out a Right Angle	16

SECTION I

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SOME BASIC CONCEPTS

I. The Need for Local Research

Many countries today are experiencing what is called "agricultural development". Basically, this means three things for agriculture: (1) it has become technically more complex; (2) it has become less oriented to home consumption and more oriented to the market; (3) it has become dynamic, that is, it is not simply moving to a new, more efficient level of operation, but it is in a continuous state of flux. In many countries, research facilities have been established at the national and regional level. New varieties and innovative practices are being successfully tested at these facilities, but before they can have any effect on farm production, they must be thoroughly tested at the local level at research stations, schools, and on farms.

The basic problem facing local experimenters is the following: "Will the use of a new or different practice affect the outcome of some particular segment of agricultural enterprise in our area, and, if so, to what extent?" If farmers fail to adopt a beneficial practice because it has not been tested locally, or if they adopt a harmful practice because it has been improperly tested, local extension agents and all those who have carried out experiments must share the blame. Local personnel have a great responsibility to become skilled in testing and evaluating new practices so that they may avoid such mistakes. If great care is exercised, untrained personnel can become sufficiently expert in experimentation to bring many benefits to the local farmers and hence the entire community.

II. Experiment versus Demonstration

What is an experiment?

An experiment is a test of more than one treatment carried out in an unbiased manner. No assumptions are made regarding the outcome; the results must always be accepted. If we suspect that the results are not typical, we still must accept them, but we should perform the experiment again. In an experiment, treatments are <u>replicated</u>, or repeated, and they are arranged in test plots or units in a procedure of randomization.

An <u>observation trial</u> is not used to draw any experimental conclusions, but is a device that may be used to determine if a practice is worth testing.

A <u>result trial</u> on a farm is the testing or demonstration of a single practice which has been proven elsewhere, but which in the farmer's mind is still unproven.

What is a demonstration?

A demonstration shows a response which has already been proven in an experiment. It is not conducted according to the specifications for an experiment, and therefore it cannot be used to draw conclusions. If it does not demonstrate the expected results, it is ignored, perhaps plowed up, and it may be run again.

III. Some Basic Concepts in Statistics

The statistical analysis of results is beyond the scope of this paper, but we must have an understanding of some basic concepts if we want to be able to interpret the statistician's analysis of our experiment. The three concepts briefly described here are the normal distribution, the null hypothesis, and the significant difference.

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A. The normal distribution

If a very large acreage of wheat is grown under uniform conditions and then harvested in plots of 100 square feet, the yields recorded for each of these plots will vary from a very low figure to a very high figure; but more of the plots will yield close to a middle figure, and as we move away from this median to either a higher or lower yield figure, we will find successively fewer plots. If the yield is plotted against the number of plots giving a particular yield, the familiar bell-shaped normal curve is obtained.



FIGURE I. NORMAL CURVE

If the same crop is grown under identical conditions with the addition of a fertilizer treatment, there will still be a wide range of yields for the 100 square feet plots, but the entire curve will have shifted somewhat to the right.



FIGURE II. NORMAL CURVES WITH AND WITHOUT FERTILIZER

Note that the two curves overlap in the crosshatched area; some plots with and without fertilizer will yield the same. If only a small number of the fertilized plots had been measured, it is possible that just by chance

alone the plots selected would have been those in the hatched area. We could not know from our measurements whether the fertilizer had really increased the yield.

The purpose of proper experimental design is to allow us to determine whether the treatments have actually shifted the normal curve, or whether the effect we observe is simply due to chance and the treatment has not shifted the normal curve. This brings us to the next concept.

B. The null hypothesis

The statistician begins his analysis by assuming that, in fact, the treatments had no effect, and that any effect observed was simply due to chance. This assumption is known as the <u>null hypothesis</u>. If we flip a coin and get four heads in a row, we assume this to be due to chance, and not some special quality of the coin.

Next, the statistician processes the data to determine the validity of the null hypothesis. He may reject the null hypothesis, which indicates that the observed effect of the treatment was <u>significant</u> and probably not due to chance, or he may not reject the null hypothesis and conclude that the observed effect of the treatment was probably due to chance.

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C. The "significant difference"

The term <u>significant</u> will be found in the results of many experiments. This may also be indicated by an asterisk (*) or by the phrase "significant at the 5% level". These all indicate that the statistician has determined that there is only a 5% chance that the observed difference was due to chance. If the results are found "highly significant", also indicated by a double asterisk (**) or by the phrase "significant at the 1% level", this indicates that there is only a 1% probability that the observed effect of the treatment was due to chance.

This discussion indicates that a single experiment, no matter how carefully designed and executed, cannot conclusively "prove" that a treatment has a significant effect. This points to one reason for repeating experiments.

SECTION II

HOW TO PERFORM AN AGRICULTURAL EXPERIMENT

I. Preliminary Research

A thorough job at this stage of the experiment, including a search of the available literature and interviews of experienced persons, will save a great deal of trouble later. The experimenter should not be afraid to ask for help now; help may be of no use once the experiment has been laid out. The preliminary research should cover the following points:

- (1) A careful study of the crop should be made. Also in fertilizer and irrigation experiments, the local soil should be studied. For pest control experiments, information on the life cycle of the pest should be obtained.
- (2) Economic factors should be studied, especially if a new crop is being introduced. Will treatments affect the market for this crop? What is the cost of treatments?
- (3) Has this experiment been performed already? Quite likely, a similar experiment has been carried out. Were the results clear, and do they bear on the planned experiment? Have similar experiments been carried out in other districts?

The preliminary research should be recorded, so that it may be included in the final report.

II. Designing the Experiment

In any experiment, there is error introduced by factors beyond the control of the experimenter: soil heterogeneity, plant variability, which is due to genetic variability, plant competition within and between plots, variation in moisture content of harvested plants, climate variations (when experiments are run for more than one year), and the size and shape of plots. This error cannot be eliminated, but it can be reduced, primarily by the replication and randomization of treatments, by the correct selection of treatments, by careful selection of the location, and by the proper design of plots.

A. Replication

Replication is the repetition of a treatment several times to obtain a mean value or yield. In field experiments, a single replicate generally is planned to contain one plot of each treatment in a rather compact block. Replication is accomplished by repeating blocks. A non-replicated trial is not an experiment.

The number of replications depends upon the degree of precision desired and the degree of soil heterogeneity. Generally, two replications is not enough. The American Society of Agronomy suggests 3-6 replications for field plots. The smaller number will suffice where average rather than annual results are desired. For corn yields, 4-6 replications are often used. For small nursery plots, 5-10 are recommended.

B. Randomization

Randomization simply means that treatments are assigned to plots in a random fashion or are randomized within a block. The reason for randomization is the elimination of any bias which might occur if we assigned treatments to plots in either a haphazard or a regular fashion.

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The randomization procedure should be completely objective and may be accomplished by flipping a coin, drawing cards from a deck, or by using a specially prepared table of random numbers, such as the one found in the appendix of this paper.

Following are four types of randomized design.

1. The completely randomized design

This design consists quite simply of the assignment of treatments to a previously determined number of plots. It is useful for some types of treatments on animals, but it is not the most efficient design for field trials with plants. Its main advantage is its simplicity and flexibility. Treatments may be assigned to plots by drawing cards from a deck, drawing slips of paper from a container, or by using the table of random numbers in the appendix.

Example: A, B, and C represent three different levels of nitrogen

tested on wheat. 4 reps X 3 treatments = 12 plots.

Plot no.	1	2	3	4	5	•
Treatment	В	А	В	С	С	
	6	7	8	9	10	Row direction
	Α	с	A .	С	В	
	14	12				
	A	В				↓

FIGURE III. COMPLETELY RANDOMIZED DESIGN

2. The randomized complete block

In this design, treatments are assigned at random within a block, and the blocks are replicated. The blocks should be kept as compact as possible, and the number of treatments kept as low as possible consistent with the objectives of the trial. The main advantage of the randomized complete block design is the high reliability of the data obtained from it and its high suitability for demonstration (as seen in Figure V.).

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Example: A-F are 6 different fertilizer treatments on sugar beets. Note that each treatment occurs once in each block. 6 treatments X 5 replications = 30 plots.



FIGURE IV. RANDOMIZED COMPLETE BLOCK



FIGURE V. RANDOMIZED COMPLETE BLOCK SUITABLE FOR DEMONSTRATION

The Latin square design

In this design, treatments occur once in each column and once in each row, and treatments are randomized in both directions. Thus, the Latin square removes variability in two directions while the randomized complete block removes it in only one direction. The number of replications always equals the number of treatments in a Latin square design. It is more precise than the randomized complete block, but it becomes very cumbersome for more than eight treatments.



FIGURE VI. HOW TO RANDOMIZE THE LATIN SQUARE DESIGN

In the above figure, columns and rows are first numbered from 1 to 5, and treatments are assigned to the plots in regular alphabetical order, simply rotating the order one place in each row or column. In the middle square, we have the same square after the columns have been rearranged by randomizing the numbers at the heads of the columns. In Step 3 on the right, we have now randomized the rows by the same method, and the procedure is completed. Note that in the right-hand square, treatments appear only once in each row and column.

4. The split-plot design

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This design is used to test two factors in combination. It is not the most precise design for this purpose but is often used to facilitate physical operations. For example, some field treatments, such as irrigation, are more conveniently applied to relatively large strips through the experimental area. If different dates of harvest are one of the factors being tested, it may be easier to harvest in strips through the experimental area rather than harvest a few feet of row and then skip across rows for another small harvest area.

There are many split-plot designs which vary in precision. If at all possible, an experienced person should be contacted for advice before one uses this design. The basic design involves assigning one factor to <u>main plots</u>

which are arranged in randomized complete blocks or in a Latin square. Assign to the main plots those treatments for which you are willing to sacrifice precision. The treatments of the second factor are assigned at random to <u>sub-plots</u> within each main plot.

Example: Planting dates and fertilizer treatments on tomatoes.
3 planting dates (main plots) X 4 fertilizer treatments (sub-plots) X 3 replications = 36 plots

	3/]5 			4/	15			4/	1		
D	A	C	B	В	С	A	D	В	A	с	D	Block I
4/1				4/15					3/15			n
A	D	В	C	A	С	B	D	В	D	С	A	Block II
	4/1				3/15				4/	15		
C	В	D	A	D	С	В	A	С	В	A	D	Block III

FIGURE VII. SPLIT-PLOT DESIGN

C. Selection of treatments

Many factors which influence the farmer's profit can be applied in an experiment as contrasting practices. Rate of seeding, date of planting, spraying and dusting treatments, fall vs. spring plowing, method of seed-bed preparation, surface vs. furrow application of irrigation water, weed control by herbicides vs. cultivation, fertilizer treatments, pasture grass-legume mixtures, and crop rotations are only a few of the more important ones.

In selecting fertilizer treatment rates, it is desirable to use rates which differ by equal intervals, such as 20, 40, 60, 80, and 100 pounds of nitrogen per acre. We may have an idea of what rate would be inadequate

and what rate would be well in excess of optimum. We should test the entire range, including two or three levels between the minimum and maximum. An untreated control plot is not necessary in a fertilizer plot where it is understood that the crop needs some minimum level of fertilizer to grow well. However, the demonstration value of any experiment will be enhanced if we designate a control plot which represents the local practice.

In a <u>factorial</u> experiment, the effect of more than one factor is studied. For example, we may study the effects of 4 levels of nitrogen and 3 levels of phosphorus. This would give 3X4 or 12 treatment combinations. You should try to keep the experiment simple and not try to study too many factors at once.

D. Selecting the location

This is a highly critical step in the performance of an experiment. The most important consideration in selecting a location is soil heterogeneity. It was formerly believed that "the experimental field should contain many different soil types to be representative." This is a misconception. The soil should be representative of that generally found in the area, but within the experimental area the land should be as uniform as possible with respect to topography, fertility, the subsoil, and previous management.

The causes of soil heterogeneity are the following:

- (I) topography: hillsides will result in gullies and washing down of nutrients; low spots or variation in the texture of the subsoil will cause plant variation.
- (2) moisture content variation from foot to foot.
- (3) variability in the penetration of irrigation water.
- (4) wide variation in available soil nutrients.

- (5) competition and shading from trees and hedgerows.
- (6) past use of the soil, including previous varietal and cultural trials, and previous applications of organic matter, fertilizer, and crop refuse.

What steps can we take to reduce the soil heterogeneity?

- Select land with a slight (1-2%), uniform slope. Avoid the use of draws, lowlands, and other irregularly-shaped pieces of land.
- (2) Where previous trials have been run which might affect soil uniformity, grow one or more "blank trials" before experimenting. A blank trial is a single crop--preferably a small grain--grown as uniformly as possible over the entire field to "smooth out" soil variations.
- (3) Place new plots at a right angle to previous plots.
- (4) Select land at least 20-30 yards from trees, hedgerows, and roads.
- (5) All information concerning the past history and present condition of the land should be recorded and included in the final report. This will assist others in interpreting the results.

E. Plot size and shape

1. Plot size

In most local experiment stations or schools where land is limited, size and shape of the plot is a matter of convenience; but there are considerations to take into account.

There are two basic plot sizes: (a) nursery plots, cared for by hand, often with multiple short rows 10-22 feet long, and (b) field plots, adapted to the use of standard farm machinery. Commonly, larger plots are used for corn, sugar beets, and hay rather than for small grains. Small plots may be necessary where many varieties or strains are being tested, where the amount

of seed of a new variety is limited, or where funds are short. There seems to be agreement among researchers that up to about 1/40 acre (100 square meters), an increase in plot size will reduce the error, but that above that size, the decrease in error is less than would be provided by an increase in the number of replications. Small plots are more variable due to (a) fewer plants, (b) losses in harvest or errors in measurement, and (c) competition and border effects are greater.

2. Plot shape

Plot shape generally makes no difference, except that relatively long narrow plots with the long dimension in the direction of the greatest soil variation overcome soil heterogeneity.



FIGURE VIII. PLOT SHAPE

There are two other practical considerations in plot shape. First, plots should be sufficiently wide to allow for removal of border strips or to render borders not important when not removed. Second, field plots should be of shape and size to accomodate farm machinery.

 Suggested plot sizes and shapes for various crops (from Field Plot Technique by E. L. Leclerg, et al)

> Small grain: 3-4 rows X 10-20 feet (center rows harvested) Corn: 3-4 rows X 10-12 hills; 2 rows X 10 hills Soybeans: 1-4 rows (2-3 ft. apart) X 16 feet

Sorghum: 2-4 rows X 30 feet (center rows harvested in 3 and 4 row plots) Alfalfa: 7 feet X 60 feet (center 5 ft. harvested with mower) 5-8 drilled rows 7" apart with 12-14" alley between border rows 3-5 drilled rows 12" apart with 18" alley and entire plot harvested Sugarbeets: 4 rows (20-24" apart) X 30-60 feet (plants thinned to

12" apart in row)

4. Border rows and guard areas

When there is competition between adjacent rows of different varieties, especially where they differ in growth habits, serious error may be introduced. In semi-arid or sub-humid areas where plants compete for water, small grain yields are greatly affected by plant competition, and for this reason, single row plots are not used. In many crops 3-5 row plots are grown and the two outside rows are not harvested for yield. Where alfalfa rows are spaced 7" apart, interplot competition is a serious factor. If alleys between plots are widened to 14", border rows should still be removed because the alley itself may allow border rows to grow more vigorously than the plants on the inside rows.

Fertilizer application often requires the use of machinery, and the flow of such fertilizer may not be precisely controlled on the ends of the field. Therefore "guard areas" 1-2 feet wide at the ends of the plot are thrown out.

III. Execution of the Experiment

A. How to lay out a right angle

If the corners of the plots are not laid out at exactly 90 degrees, plots will cover a different area than we imagine they do. The following

procedure is based on the fact that a triangle with sides in a 3:4:5 ratio forms a perfect right angle.

Equipment needed:

50 ft. cloth tape measure or heavy string or wire marked at 30, 40, and 50 ft. Stakes

String

Procedure:

- Lay out a baseline with stakes and string. This should be as long as the desired total plot width, that is, the two stakes (A and A') should be two corner posts.
- (2) Place a third stake (B) next to the string exactly 40 ft. from one of the original corner stakes.



FIGURE IX-A. LAYING OUT A RIGHT ANGLE

- (3) Have a co-worker hold the end of the tape on corner stake A while you draw an arc in the dirt with a 30 ft. radius on the tape. You should swing the extended tape to draw this arc across the approximate place the side boundary will pass.
- (4) Have the co-worker hold the end of the tape at stake B; and following the same procedure as in step (3), draw an arc with a radius of 50 feet. Where the two arcs cross, place a stake (C).



FIGURE IX-B. LAYING OUT A RIGHT ANGLE

(5) Tie a string from stake A to stake C. This forms a right angle at A, and you can now repeat the process at A'.



FIGURE IX-C. LAYING OUT A RIGHT ANGLE

B. Labeling and mapping

Accurate mapping and labeling is a simple procedure; but to overlook it may mean losing the entire experiment, if, for example, someone pulls up your marker stakes before the experiment is completed, and you have made no map for your records.

You must draw a map because field markers are often obliterated by weather or tractor drivers. The map should refer to permanent structures, such as fence posts, standpipes, building corners, etc. You should be able to locate each separate treatment exactly, even if all stakes, strings, and labels are removed from the field. Also at this stage, the planned treatments should be listed and described; and the map should indicate which plot received which treatment.

Field markers should be written in grease pencil, which will not be washed off in the rain or by irrigation water. Stakes may be used to label plots; cardboard tags are often used in orchards. At the time treatments are applied, make sure your application, the field markers, and the map all agree.

C. Uniform application

Failure to apply treatments uniformly is an extremely common type of mistake which greatly decreases the value of the experiment. Extreme care should be taken to see that fertilizer, pesticides, seed treatments, etc., are applied exactly as specified and uniformly over the plot.

Application equipment should be cleaned between trials. Seeds must be swept out when different varieties are being planted.

If more than one worker is applying treatments, do not have the same worker apply the same treatment over more than one replication.

Do not inadvertently add factors. For example, when fertilizer is side-dressed on a row crop, the shoes on the applicator may prune some of the roots, and this will affect plant growth. The proper untreated check would consist of a plot through which the fertilizer rig had been pulled without the material. Seed soaked in a chemical should be compared with seed soaked in water, not dry seed.

Carefully weigh all materials used where this is required and calibrate application equipment to make sure you are putting on the amount you think you are. Fertilizer elements should be mixed several weeks ahead of the application to allow time for any chemical reactions to take place.

Obtain a uniform stand. Small grains will tiller where adjacent plants are missing, but on corn and many row crops, the existing stand will not "fill in". One solution is to plant thick, then thin down to the desired stand.

Uniform care of plots is important. Weeds greatly influence crop yields and should be removed early in the trial.

IV. Measuring and Recording the Results

Considerable time and expense has been spent thus far, yet many experimenters fail in the end because they improperly measure and record the results. The experimenter may take measurements at the wrong time; or he may take measurements at the right time but fail to put all results in numerical terms; or he may measure at the right time, in numerical terms, but fail to measure all the affected attributes, or he may do all these things correctly but not record the results in simple, but complete form. When should measurements be taken?

Different varieties mature at different times, and therefore should not be harvested all at the same time. The experimenter must watch closely and harvest each variety as it matures. He must record the total days to maturity for each variety.

The rate at which results are reached is sometimes important. For seed germination, the rate of emergence as well as the percentage should be recorded.

B. What should be measured?

This is an extremely important question and one not adequately considered by inexperienced experimenters. In some experiments, workers may simply harvest and weigh the crop with no consideration for other factors which are important on the market and may have been affected. The market and nutritional value of the product must always be kept in mind. Even at a local experiment station or a school, where there is no sophisticated measuring equipment, there are many attributes that can be measured. For example, fertilizer treatments on tomatoes may affect not only the total yield, but also the time to maturity, the color, the size and shape,~and susceptibility to diseases. For corn, the number of ears

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should be counted, and if facilities are available, moisture percentage measured, using a sample of ears representing all sizes and with kernels from 1 or 2 rows on each ear.

The following are some further examples of measurable attributes of field and horticultural crops:

Sugar content of sugarbeets Specific gravity of potatoes Grade of peaches Oil and protein content of soybeans Coumarin content of sweetclover Hulling percentage and milling quality of oats Ginning and fiber properties of cotton Pithiness of carrots

In short, when deciding what to measure, we should always keep in mind the value of the product on the market.

C. Put all observations in numerical terms

Many attributes of quality do not readily lend themselves to measurement in numerical terms. For example, after pesticide treatments we may want to measure the amount of insect damage on the leaves. It may seem easiest to judge damage in terms of "light", "moderate", and "heavy". But unless we put everything in numerical terms, a statistician cannot make use of our results.

In the case of disease or insect damage, a convenient numerical scale should be set up. For example, to measure potato scab, set a scale ranging from 0 to 10. 0 represents a potato completely free of scab, and 10 represents a potato entirely covered with scab. In some places, standard scales have been established--1-5 or 1-7--and photographs representing each step are used as a method of standardization. In general, the following recommendations may be made:

(I) Try to design the scale so that observations are normally distributed, that is, the middle number is the most frequently observed.

- (2) There should be as many steps in the scale as an experienced observer can distinguish.
- (3) Where any individual judgment is involved in making observations, try to avoid having more than one person make the observations. If this is not poss^{ib}le, do not have each person collect the data from a single treatment.
- D. A report procedure

Research, even at the local level, is a continuous process. Single experiments seldom determine new farming practices, but rather the results of experiments have a cumulative effect. For this reason, as well as others, the written report of our experiment must receive some attention. It must be complete, but not overly complex. It must convey clearly and concisely what the experimenter tested, under what conditions the test took place, and the results. If the report is to be placed in a file with similar reports, there may already be a standard format. If there is no sample format, the following is generally acceptable:

- (1) Title page: the title page should clearly indicate the nature of the experiment. The experimenter's name, the date, and location must be included.
- (2) Introduction: this must include a review of literature or basic background information, including all similar experiments previously carried out, and a definition of the problem.
- (3) Procedure: this must include pertinent soil and climatic conditions and a careful description of the treatments and how they were applied.
- (4) Results: these should be in both tabular and graphic form with the results of the statistical analysis.

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- (5) Conclusion and recommendations: as a minimum, any further experiments called for by the results should be mentioned.
- (6) Appendix: this may include a plot map and the statistician's calculations.

APPENDIX: TABLE OF RANDOM NUMBERS

To randomize any set of ten items or less, begin at a random point on the table and follow either rows, columns or diagonals in either direction. Write down the numbers in the order they appear, disregarding those which are higher than the numbers being randomized and those which have appeared before in the series. If you wish to randomize more than ten numbers, pairs of columns or rows can be combined to form two digit numbers and the same process followed as that described above.

¹Thomas M. Little, and F. J. Hills, <u>Experimental Methods for Extension</u> <u>Workers</u>. (Davis, California: University of California Agricultural Extension Service, 1966), p. 55.

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