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## ADVANTAGES OF STATISTICAL METHODS FOR THE PRACTICAL ENTOMOLOGIST


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During the past few years statistics has joined other branches of science in the rapid march of progress. Fifteen years ago most research men and some of the leading statisticians recognized that the statistical methods available were not suitable for practical research problems. The elaborate methods of that day were based upon the theory of infinitely large samples and were not sufficiently accurate for practical data obtained from limited observations. Those who attempted to apply statistics to practical problems usually became so involved in complicated mathematics that they either lost sight of the practical aspects of their problem or gave up statistics as hopeless. One authority said of the traditional machinery of statistical processes: "Not only does it take a cannon to shoot a sparrow but it misses the sparrow!" The author of this statement, Professor R. A. Fisher, recognized the trouble and successfully introduced a remedy. He developed a system by which data from small experiments could be analyzed and judged on their own merits by means of ordinary arithmetic. His application of the analysis of variance method to small experiments revolutionized this phase of statistics and furnished to the agricultural science worker a practical tool which enables him to evaluate properly the results of his undertakings.

The object of this paper is to point out the value of this relatively new Analysis of Variance method in ordinary entomological experiments. It is unnecessary to tell a group of entomologists that individual insects of the same species will not always react to changes in environment in exactly the same manner. Their actions will vary according to sex, age, health, weather conditions, and other factors. For example, a recent investigation determined that female house flies are about twice as hard to kill as male flies of the same age and breeding, also that young flies of either sex are harder to kill than older ones. The results of our simplest experiments vary, depending upon numerous conditions that we cannot hope to keep constant. Even if it were possible to keep these conditions constant, our experiments would be so limited in their application to practical problems that they would lose much of their significance. Thus for ordinary experiments we must accept the fact that the results will be variable and must make allowance for that variation, which tends to cause incorrect conclusions.

In some simple experiments where wide differences are exhibited between treatments, and the discrepancy is small, correct evaluation may be made by simple examination without statistical analysis. However, unless recognized methods of analysis are used, there is danger that different investigators will draw different conclusions from the same simple set of data. The method of analysis of variance aids the investigator in making precise evaluations, and it inspires confidence in his results because others are informed of the degree of precision with which his experiments were conducted.

The analysis of variance places numerical values on the variation arising from the several sources. These numerical values are known as "variances". Thus, the analysis of variance is just what the name implies. If we analyze a sample of fertilizer, we weigh the sample, then separate and weigh the different plant foods or active ingredients; we then subtract the weight of the active ingredients from the total weight and we have left the weight of the inert ingredients. In analyzing data, we calculate the total variance, then we separate and measure the variances due to treatments or other controlled factors. We subtract those variances that are under our control from the total and have left the error variance which we could not control. By comparing the treatment variance with the error variance, we can tell immediately whether the experimental results are reliable or not. Or by use of the error variance and a table of odds, one can estimate the odds in favor of any one treatment in comparison with any other treatment. Each experiment is judged on its own merits, and the degree of confidence placed in it is automatically adjusted according to the number of replicates made and the error encountered.

However, one should not get the idea that the statistical method is a mill through which data may be passed and the final interpretations produced mechanically without use of personal judgment. This would be as foolish as expecting an unskilled laborer to build a fine house just because he had the best available carpenter tools. Even the best carpenter needs all of his senses, proper building materials, and a well-planned design. Nevertheless, a carpenter can use his tools without being able to manufacture them. The statistical method is merely a tool and does not interpret data, neither does it do our thinking. Yet we can make practical use of this tool without understanding all of the complicated theory upon which its operation is based.

Statistical methods have done more for us than make it practical to measure the reliability of results. They have provided methods of testing technique and have made possible the design of more efficient and more comprehensive experiments. In this connection it should be emphasized that the form of analysis appropriate for any data depends upon the design of the experiment producing them. If the requirements for replication and randomization are not fulfilled, the standard methods of analysis are not at all applicable. At least two replicates must be provided in any experiment and it is preferable to have four or more. The test plots or test insects should be assigned to the treatments by some method of deliberate randomization. Any deviation from absolute randomization invalidates the analyses unless the effects of such departures are measured and due adjustments made. A third requirement is that the experiment contain checks or controls in order to avoid the possibility of unseen accidents causing apparently positive results. Thus, if a single insecticide is tested, its effect must be compared either with the effects of a well-known insecticide, or with the effects of some suitable control; otherwise there is no certainty whether the results are due to the insecticide in question or due to an accident.

The simplest experiment that employs the best principles of experimentation consists of pairing the test plots or insects so as to get the members of a pair as nearly alike as possible. Then one random member of each pair is exposed to the condition to be tested, while the other member is either observed as an undisturbed check or is exposed to some alternative. In such an experiment the variation between pairs is not error because it affects the two individuals of any one pair alike. Therefore, a separate item is provided in the analysis of variance for pairing and the variance due to pairing is thereby excluded from the estimate of error. This method is very effective in reducing experimental error and employs in simplified form the chief principles embodied in the more complex designs. However, it is limited to the testing of only two treatments.

In field experiments with three or more treatments, the "randomized block" arrangement of plots is popular. This consists of dividing the experimental area into as many equal sized blocks as there are to be replicates, with an attempt to get the blocks as uniform within as possible and to place the major differences between the blocks. Each block is divided into as many plots as there are treatments to compare. The treatments are then assigned at random to the plots with the restriction that each treatment appear once and only once in each block. In this arrangement the differences between blocks have similar effects upon all treatments and do not tend to cause erroneous results. Thus, a portion of the usual experimental error loses its effect and ceases to be error. Skill in the arrangement of blocks to conform with the variations in soil, plants, and insects results in greater precision. The randomized-block design was developed for field experiments but the principle of grouping exemplified by it is being used with equal success in laboratory experiments.

When from four to seven treatments are to be tested and it is convenient to use the same number of replicates, the "latin square" design is appropriate and will as a rule be more efficient than randomized blocks. If more than six or seven treatments are to be tested, the latin square becomes too cumbersome and is not recommended. A four by four latin square requires 16 plots arranged in a rectangle with 4 plots on each side. The four experimental treatments are assigned at random to the plots except that each treatment must occur once and only once in each row of plots in two directions across the field. This design can be employed in laboratory experiments but it is chiefly used in small-plot field experiments. The double grouping of plots featured by it provides for a double reduction of error. It also insures a representative distribution of treatments throughout the experimental area and thereby prevents the chance placement of like-treated plots side by side as is sometimes done in randomized blocks. The latin square is especially appropriate in the situation so often encountered in which a reduction of error is greatly needed but little is known of its source or distribution. In other words, the rigidness of the latin square makes it safe and more foolproof for general use. However, this quality also limits its adaptability to special conditions, and situations may arise in which it is less efficient than the more flexible randomized blocks. The latin square is preferred for the arrangement of field plots unless situations do arise that definitely indicate some other arrangement.

Often it is desirable to test in the same experiment several control schedules and several different insecticides and to determine what influence the insecticides have upon the schedules. If there are four schedules and four insecticides, a total of 16 combinations will be possible and unless all 16 are tried there is no certainty just which would be best. The "factorial design" is appropriate for experiments of this type and can be used with or without randomized blocks. It is especially efficient in laboratory experiments where the natural distribution of plants, soils, and insects does not limit the number of treatments that can be effectively handled at one time. For example, at Sanford, Fla., last fall all combinations of 3 poisons, 3 rates of application, 2 diluents, and 2 dosages were tested simultaneously, there being in all 36 treatments. The large blocks ordinarily required to conduct such an experiment in the field are a disadvantage. However, if circumstances make it sufficiently important to do so, there are ways of handling a large number of treatments with small blocks.

In investigations of insects affecting winter vegetables we have found the randomized blocks, the latin square, and the factorial design all to be useful and easy to put into operation. Any one who confines himself to the simpler experiments can with little outside aid learn to analyze results obtained from these experiments. Simple instructions are given by Fisher and Wishart (1930) or by Paterson (1933), while tables for making tests for significance are given and explained by Snedecor (1934). The bulk of the calculations consists of adding numbers from a table of squares and can be done by any careful assistant that can operate an adding machine. The chief requirement, then, is the use of common sense in designing the experiments and in making interpretations.

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## LABORATORY STUDIES OF POISONED BAITS FOR THE CONTROL OF THE SOUTHERN ARMYWORM

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The southern armyworm, formerly called the semitropical armyworm, *Prodenia eridania* (Cram.), is at times one of the most destructive pests on truck crops in Florida. It feeds on a great variety of host plants, notably celery, cabbage, tomato, sweetpotato, Irish potato, pepper, beet, carrot, and cowpea. In the younger stages the larvae have habits similar to those of the true armyworms, as indicated by the common name, but as they near maturity they take on the characteristics of cutworms, such as a tendency to feed alone, to hide during the day, and to cease migrating any great distance.

Quite often this insect appears in large numbers on vegetable crops just prior to harvest, causing a definite loss unless controlled by artificial means. To date the only successful control measures have been applications of arsenical or fluorine dusts and sprays, which may be responsible for dangerous residues on the marketed product. For the past several years studies at the Sanford, Fla., laboratory of the Bureau of Entomology and Plant Quarantine have been directed towards the development of control measures that would employ materials least objectionable from the residue standpoint. As a part of this program, poisoned baits were investigated because (1) arsenical baits are less dangerous from a residue viewpoint than are arsenical sprays or dusts, (2) they are readily fed upon in cages by the larger larvae, and (3) they have not been entirely satisfactory in practical use under field conditions.

## OBJECT

The hypothesis upon which this investigation was based was the belief that poisoned baits would be effective in the control of the southern armyworm under field conditions, provided they were made more attractive than the natural food by combining sufficient quantities of the proper poisons, foods, and attrahents. The ultimate objective, of course, was to find this ideal combination and to prove its effectiveness. The two experiments discussed herein have necessarily been limited to the testing of only a few of the many possibilities. The purpose of this report is not merely to make known the results of these tests but also to describe and illustrate an experimental method that is widely applicable to entomological problems.

The mortality caused by a bait is affected by several different factors, chief of which are the availability of normal food, the kind of poison, the quantity or rate of poison, the kind of food used as bulk in the bait, and the kind of attrahent. A poison may be more effective with certain foods or attrahents than with others; likewise, an attrahent may be more effective in certain combinations than in others. Thus the objective was not only to test the influence of each factor upon the mortality but also to test the influence of each factor upon the effectiveness of each of the other factors. The first experiment was devoted to the study of the mortality as affected by the availability of unpoisoned normal food and the kind and rate of poison in the bait.

## METHOD

The usual method of testing different kinds of poisons is to change them while keeping all other conditions as nearly the same as possible. The rates at which poisons are used are usually tested in the same manner. Such a method has its advantages but it will not satisfy the objectives of this investigation because it does not provide for a study of the relationship between poisons and rates. This relationship cannot be studied unless every combination of poison and rate be tried simultaneously. In other words, rates and poisons must both be varied at the same time and in the same experiment. It was found that if this were done in an orderly manner according to a definite scheme, and not haphazardly, approximately twice as much information could be obtained per unit of effort. This is due to the fact that the total data obtained can be utilized in studying poisons and can again be utilized in studying rates. This principle of experimentation has been described by Fisher[1] as the "factorial design", evidently because several *different factors* are tested simultaneously and according to a definite design. The factorial design was used in each of the experiments considered in this report. This type of experiment is not new except for the fact that until within the last few years research men have seldom recognized and taken full advantage of it, evidently owing to the difficulty in interpreting the complex results. Fisher has largely solved this difficulty by his method of analysis of variance.

The effectiveness of each treatment was determined by caging five larvae, which were half grown or larger, singly in salve tins each of which contained 1/20 gram of bait. After 18 to 20 hours' exposure to the bait each larva that remained alive was transferred to a fresh salve tin containing only sweetpotato leaves, as these were known to be a favored natural food. After being confined with unpoisoned food for a period of four days the larvae were discarded and records made of the mortality. A group of five larvae constituted a sampling unit. All treatments were tested simultaneously. In order to insure representative results, the tests were replicated on several different dates with freshly-mixed baits and with new broods of larvae.

Usually it was necessary to employ several rearing jars to supply sufficient larvae of the same size for a test. In preliminary experiments this was found to be a frequent cause of bias, evidently due to unpredictable differences in larval vigor or resistance in the different jars. In order to insure an equal distribution of these and similar variations and thereby to eliminate bias, the following method was adopted for assigning the individual larvae to the treatments: A group of larvae of uniform size equal in number to the number of treatments tested was selected from a single jar. These were assigned, in a haphazard manner, one to each treatment. This process was repeated until the required five larvae per treatment were obtained.

[1]Fisher, R. A. 1935. The Design of Experiments. Oliver and Boyd. London. Chapter 6.

As an aid in the interpretation of the resulting data, Fisher's method of analysis of variance was used as described by Fisher and Wishart.[2].

**Experiment I. Poisons and Rates.** The first experiment was devoted to a study of the mortality as affected by the availability of unpoisoned normal food and the kind and rate of poison in the bait.

The four materials chosen for this study were paris green, synthetic cryolite, phenothiazine (thiodiphenylamine), and powdered cube. Paris green and cryolite were known to be toxic to this insect when applied to its natural food, but were objectionable because of possible dangerous residues. For this reason the other two materials were tested with the idea that a concentration or rate of one might be found that would be effective.

Three rates for each material were used, viz., 1, 2½, and 7 pounds per 100 pounds of bait, incorporated in the following standard bait mixture:

| | |
|---|---|
| Bran | 50 pounds |
| Cottonseed meal | 50 pounds |
| Molasses | 1 gallon |
| Water | As needed to moisten |

Bait alone was supplied for food in one-half of the treatments. In the other half an abundance of sweetpotato leaves was included with the bait. The mortality in the absence of natural food was expected to show the toxicity of the materials as compared with starvation checks, while the mortality in the presence of natural food should show the degree of attraction of the various baits. Thus there were the following three factors in this study: (1) Poisons, with four alternatives (paris green, synthetic cryolite, phenothiazine, and cube); (2) rates, with three alternatives (1, 2½, and 7 pounds per 100 pounds of bait); and (3) food, with two alternatives (either present or absent).

Six replications of the tests were conducted on different dates, in which a total of 30 larvae were exposed to each of the 28 treatments. The mortality data have been summarized in Table 1. An analysis of variance of the data from all treatments showed that the standard error of a treatment total was 2.19 and that a difference of 6.2 was required between treatment totals to show significance at odds of 19 to 1.

[2]Fisher, R. A., and J. Wishart. The arrangement of field experiments and the statistical reduction of the results. Imperial Bureau of Soil Science, Technical Communication No. 10. 1930.

TABLE 1.—MORTALITY OF SOUTHERN ARMYWORM LARVAE IN TESTS WITH POISONED BAITS.

| Material and Concentration | Number of Dead Larvae | |
|---|---|---|
| | Bait Alone | Bait and Food |
| Control[1] | 0 | 0 |
| Control | 0 | 0 |
| Paris green, 1 pound per 100 | 23 | 8 |
| Paris green, 2½ pounds per 100 | 22 | 12 |
| Paris green, 7 pounds per 100 | 28 | 21 |
| Cryolite, 1 pound per 100 | 26 | 12 |
| Cryolite, 2½ pounds per 100 | 28 | 22 |
| Cryolite, 7 pounds per 100 | 29 | 29 |
| Phenothiazine, 1 pound per 100 | 3 | 7 |
| Phenothiazine, 2½ pounds per 100 | 15 | 12 |
| Phenothiazine, 7 pounds per 100 | 23 | 14 |
| Cube, 1 pound per 100 | 0 | 0 |
| Cube, 2½ pounds per 100 | 1 | 0 |
| Cube, 7 pounds per 100 | 6 | 0 |

A difference of 6.2 is required between any two figures, irrespective of position, to show significance at odds of 19 to 1.

[1]There were two control treatments without any bait at all and two with unpoisoned bait.

A more detailed analysis was later necessary for the precise study of the interrelationships of the three treatment factors. For this purpose the data from the six cube treatments and the four control treatments were omitted in order to avoid the large number of zero values which would tend to bias the estimates of error and interaction variances. The complete analysis of variance of the 18 remaining treatments is given in Table 2 because it summarizes so precisely the effects and the interrelationships between the various things studied. For example, the significant variance for between tests not only shows that the data vary from test to test more than can be explained by chance but also tells how much more. This is worthwhile information because it indicates that the treatments studied were compared under widely different sets of conditions and are therefore more representative and more widely applicable than if they had been obtained under uniform conditions.

The highly significant variances for poisons, rates, and foods prove that some of the alternatives of each were more effective than others. The examination of the total mortalities indicates that cryolite with 146 dead worms out of a possible 180 was more effective than paris green with 114, and that both were much more effective than phenothiazine with 74. Likewise the

7-pound rate with 144 dead larvae was more effective than the 2½-pound rate with 111, and both were more effective than the 1-pound rate with 79. When unpoisoned host food was absent, 197 larvae died out of a total of 270, whereas in the presence of such food only 137 died.

TABLE 2.—ANALYSIS OF VARIANCE FOR STUDY OF POISONS, RATES, AND PRESENCE OR ABSENCE OF FOOD.

| Source of Variation | Degrees Freedom | Sum of Squares | Variance |
|---|---|---|---|
| Total (Between sampling units) (5 larvae) | 107 | 305.074 | |
| 1. Between treatments | 17 | 195.074 | 11.47** |
| 2. Between tests | 5 | 17.296 | 3.46* |
| 3. Remainder (error) | 85 | 92.704 | 1.091 |
| 1. Between treatments | 17 | 195.074 | 11.47** |
| (a) Between poisons | 2 | 72.296 | 36.15** |
| (b) Between rates | 2 | 58.685 | 29.34** |
| (c) Between foods | 1 | 33.333 | 33.33** |
| (d) Remainder (interaction) | 12 | 30.760 | 2.56** |
| (1) Poisons and rates | 4 | 4.760 | 1.19 |
| (2) Poisons and food | 2 | 8.001 | 4.00* |
| (3) Rates and food | 2 | 1.167 | .58 |
| (4) Poisons, rates, and food | 4 | 16.832 | 4.21* |

**Indicates a highly significant variance when compared with the error variance.
*Indicates a significant variance.

The lack of a significant interaction[3] for rates times poisons indicated that the increase in rates affected all poisons alike and that the apparent differences in this respect were due to chance. Likewise the interaction variance for rates times foods was too small to be significant, indicating that the addition of sweetpotato leaves attracted the larvae away from one rate of poison as much as from any other rate. On the other hand, there was a significant interaction variance for poisons times foods, proving that the sweetpotato leaves attracted the larvae away from some poisons more than from others. Or, to express it another way, some of the poisons acted as if they might have been repellent, while others did not. This can be studied in more detail by examining the following mortalities:

| | *Mortality without food* | *Mortality with food* |
|---|---|---|
| Cryolite | 83 | 63 |
| Paris green | 73 | 41 |
| Phenothiazine | 41 | 33 |

[3]The reversible interrelationship between two factors is usually expressed as the interaction of one times the other.

Since a difference of 13 is required for significance, the data show that although there was as a rule a decrease of mortality in the experiment as the result of the presence of sweetpotato leaves, this decrease did not prove to be significant when phenothiazine was used. Likewise the data show that although paris green was as a rule more effective than phenothiazine, this was not demonstrated to be true in the presence of sweetpotato leaves. This is interpreted as probably due to the low mortality obtained with phenothiazine as a whole rather than to a lack of repellency. This might not have been the case if stronger concentrations had been used, as indicated in the results obtained with the highest concentration of phenothiazine shown in Table 1.

The variance for the triple interaction was significant, showing that there was an interrelationship between the various factors due to something apart from their own actions. This is demonstrated in Table 1 by a general tendency for the presence of sweetpotato leaves to have less effect upon the mortality of the lower rates of phenothiazine and the higher rates of cryolite and paris green.

**Experiment II. Constituents of Baits.** While the first part of this study showed that phenothiazine was inferior to both cryolite and paris green, yet in the absence of natural food its strongest concentration was quite toxic. It therefore seemed desirable to study the effect of this material in connection with various bait ingredients to determine if some combination would not be sufficiently attractive to cause the insects to feed upon the bait rather than the natural food. Both paris green and phenothiazine were used at the low rate of 2½ pounds per 100 pounds of bait to see if mortalities could not be obtained which were as good as those secured with cryolite in the first part of this study.

Each factor selected for Experiment II had two alternatives, viz.:

(1) Poisons; either paris green or phenothiazine, at the rate of 2½ pounds per 100 pounds of bait.

(2) Bulk; either corn meal or cottonseed meal, at the rate of 50 pounds per 100 pounds of bait.

(3) Syrup; present at the rate of 2 gallons per 100 pounds of bait, or absent.

(4) Lemons; present at the rate of 4 pounds per 100 pounds of bait, or absent.

All baits contained 50 pounds of bran as a basic material, to which all possible combinations of the other ingredients were added, resulting in 16 treatments. These were tested only in the presence of abundant natural food, as the main object was to determine those combinations capable of overcoming the attraction of the natural food.

Eight time replicates of each test were conducted with different broods and fresh baits, the results of which are presented in Table 3. No treatment resulted in a really satisfactory number of dead larvae, showing that no combination was sufficiently attractive to overcome the attraction exerted by the presence of natural food, since the greatest mortality (18 larvae) was only 45 per cent of the 40 larvae used for each treatment.

TABLE 3.—MORTALITY FROM COMBINATIONS OF INGREDIENTS.

| Poison | Bulk Ingredients | | | Number of Larvae Dead |
|---|---|---|---|---|
| Paris green | Corn meal | Syrup | Lemon | 10 |
| Phenothiazine | " | " | " | 5 |
| Paris green | Cottonseed meal | " | " | 13 |
| Phenothiazine | " | " | " | 5 |
| Paris green | Corn meal | No syrup | " | 13 |
| Phenothiazine | " | " | " | 2 |
| Paris green | Cottonseed meal | " | " | 5 |
| Phenothiazine | " | " | " | 5 |
| Paris green | Corn meal | Syrup | No lemon | 18 |
| Phenothiazine | " | " | " | 6 |
| Paris green | Cottonseed meal | " | " | 14 |
| Phenothiazine | " | " | " | 7 |
| Paris green | Corn meal | No syrup | " | 7 |
| Phenothiazine | " | " | " | 3 |
| Paris green | Cottonseed meal | " | " | 9 |
| Phenothiazine | " | " | " | 3 |
| Difference required for significance, odds of 19 to 1 | | | | 7 |

As this part of the study had a fourth factor, the analysis of variance (Table 4) is more complex than that for Experiment I. It contains three triple interactions and one quadruple interaction.

It is quite probable, as indicated by the data, that paris green was better than the phenothiazine. The summary total for the mortality arranged by factors is presented in Table 5. This shows that for the experiment as a whole there were no signficant differences between cottonseed meal or corn meal as a bulk material for these baits, nor between the presence or

TABLE 4.—ANALYSIS OF VARIANCE FOR STUDY OF BAIT CONSTITUENTS.

| Source of Variation | Degrees Freedom | Sum of Squares | Variance |
|---|---|---|---|
| Total (between sampling units) | 127 | 122.93 | |
| I. Between treatments | 15 | 39.805 | 2.654** |
| II. Between tests | 7 | 10.242 | 1.463 |
| III. Remainder (error) | 105 | 72.883 | .694 |
| I. Between treatments | 15 | 39.805 | 2.654** |
| a. Bulk | 1 | .071 | .071 |
| b. Poisons | 1 | 21.946 | 21.946** |
| c. Syrup | 1 | 7.508 | 7.508** |
| d. Lemons | 1 | .633 | .633 |
| Remainder (interaction) | 11 | 9.647 | .877 |
| Interactions: | | | |
| Bulk × poisons | 1 | .945 | .945 |
| Bulk × syrup | 1 | .070 | .070 |
| Bulk × lemons | 1 | .008 | .008 |
| Poisons × lemons | 1 | .195 | .195 |
| Poisons × syrup | 1 | .945 | .945 |
| Syrup × lemons | 1 | 1.758 | 1.758 |
| Bulk × poisons × syrup | 1 | .383 | .383 |
| Bulk × poisons × lemon | 1 | .195 | .195 |
| Bulk × syrup × lemon | 1 | 1.320 | 1.320 |
| Poisons × syrup × lemon | 1 | .383 | .383 |
| Bulk × poisons × syrup × lemon | 1 | 3.445 | 3.445* |

**Indicates a highly significant variance when compared with the error variance.
*Indicates a significant variance.

absence of lemon. However, the difference between the total mortality for all combinations containing paris green as the toxic agent and for those containing phenothiazine was highly significant. The presence of syrup resulted in a definite increase in mortality over the absence of syrup.

(To be continued)