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RANDOMIZED-BLOCK ARRANGEMENT FOR INSECTICIDE EXPERIMENTS ON CITRUS TREES

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Since the end of World War II many new and promising insecticides have appeared on the commercial markets. Both State and Federal workers now have under way extensive experiments with these new materials on citrus trees, and a number of entomologists are employed by insecticide manufacturers to conduct grove experiments. In view of these developments, it seems appropriate to review an experimental design for comparing the effects of insecticides under orchard conditions, which consist of a randomized-block arrangement. This field-plot technique was adopted by the St. Lucie, Fla., laboratory of the Bureau of Entomology and Plant Quarantine more than ten years ago, after it was realized that variations in infestation between rows of trees were sometimes sufficient to invalidate the results of unreplicated row-treatment tests. Through repeated trials the randomized-block arrangement has proved to be a practical method for making true comparisons. Moreover, valid estimates of experimental error can be calculated readily from the data provided by this arrangement.

One of the problems which confront the experimentalist is natural variation in infestations. With scale insects, for example, no two citrus groves have exactly the same amount of infestation at any one time. Infestations differ in different localities, and in trees of different varieties, ages, sizes, and planted at different distances. Individual trees in a single

[1] K. W. Babcock, L. B. Reed, and especially F. M. Wadley, all members of this Bureau at the time this work was done, were very helpful in suggesting plot arrangements and methods of analysis.

grove may vary from no infestation to a heavy infestation; some tree rows may have more scales than others. Border trees adjacent to an older, heavily infested grove and along dusty roads usually have heavy infestations.

On the individual tree, scales are usually more numerous in the top, center, and skirt near the ground, generally as a result of poor spray coverage. Infestation on the north side may be quite different from that on the south side of a tree. One branch may have so many scales that it is killed, and another may have only a moderate infestation. Even the leaves on a single branch vary. Old leaves may have few scales, spring-flush leaves more, and new growth almost none. There is variation even among leaves of the same age and, indeed, in the number of scales on top surface and lower surface of the same leaf, or between right and left halves of a leaf.

The season of the year and the weather are important factors causing variations in infestation. A cold spell which causes defoliation may reduce infestations to a low level, and the most exposed trees may show the most effects. Excessive summer heat may influence the development of scales on one side of the trees more than on another side. Dashing rains and winds from one direction may change the pattern of infestation. Variations in the distribution of parasites, predators, and fungi affect the populations of their hosts.

The effect of this universal variability on the results of insecticide tests cannot be avoided completely by selecting locations for tests where the infestations are relatively constant. However, if a randomized-block arrangement with sufficient replications of the treatments is utilized and proper sampling techniques are followed, the effect of the factors that contribute to natural variation can be minimized.

In setting up a randomized-block experiment for comparing insecticides for control of scale insects on citrus, we include a set of unsprayed check trees to demonstrate the natural infestation. A standard insecticide, such as 1-percent emulsible oil, is also included for comparison with the new insecticides or combinations.

For a 10-treatment experiment a block of 120 trees or more is selected, with few skips, runts, or replants, and with a moderately heavy infestation, as nearly uniform as possible. After each tree has been examined, a map of the grove is prepared, and the missing trees, or those showing lack of uniformity, are indicated by symbols. There must be at least 100 uniform trees, excluding those in border and ditch-bank rows and those along dusty roads or on the outside edges of the block. On the map 10 compact blocks of 10 good trees each are marked off with circles. These blocks are numbered.

Code letters are assigned to each treatment, usually "O" for unsprayed, "A" for the standard spray, and "B", "C", "D", "E", etc., for the new insecticides or treatments to be compared. Ten corks marked with these code letters are put into a paper bag, and then drawn out one by one. The trees of block 1 are marked on the map with the letters as they are drawn. The corks are put back into the bag and then drawn again for a similar assignment of treatments to the trees in each of the other blocks. Thus the assignment of the treatments to the trees of a block is entirely by chance, and the blocks are randomized. The result is an experiment of ten blocks (replications), each of which contains one tree of each treatment. Each treatment is subjected equally to whatever variation in infestation exists between the different parts of a grove.

The trees are tagged with the code letter indicated on the map, to serve as a guide for the application of the different treatments. It takes longer to spray the scattered trees than to apply row treatments, but this disadvantage is more than offset by the elimination of possible effects on data from row variations or from variation between sections of the grove.

Sampling is done just before spraying, one month and three months after spraying, and at the end of the year. At shoulder level around each tree 20 or more leaves are cut off with scissors. New-growth leaves on which infestation has not had time to develop are not taken. The 20-leaf samples are put into separate 2-pound bags, which are marked with block and tree designations, and then folded and stuffed into 10-pound sirup cans. These cans are kept in a refrigerator at 45°-50° F. until the scale counts are made.

For making the counts a binocular microscope is used. The scales on the leaves are counted and then turned over to see if they are living. Records of total and living scales for each tree and each leaf are tabulated separately. If two men are making examinations, each examines 10 leaves from each tree; if there are three workers, each examines 7 from each tree, in which case the samples must comprise 21 leaves per tree. In this way the effect of variability between workers is minimized. If scales are numerous, only half of each leaf may be examined. The comparisons of the Florida red scale, mature females of which are present throughout the year, are based on living mature female scales per leaf, or per half-leaf.

When all samples have been examined, the tree totals, treatment totals, and block totals are calculated. Since the values so obtained are at best careful estimates, we must analyze the data statistically before drawing conclusions. Some idea of the within-treatment (between-tree) variations may be obtained by calculating the mean number of scales per tree-sample (or per leaf) and then the standard error for each treatment (Snedecor *1*, pp. 41 and 61). If the treatment values differ far beyond the limits of the standard errors, real differences due to the insecticide treatments may be assumed.

Much more information can be obtained about our data if we submit them to an analysis of variance (Snedecor *1*, pp. 214-317). Variance is a numerical measure of variation, and the error variance for a mean is the square of its standard error. From the analysis of variance we may estimate the relative importance of the variation between leaves on individual trees (sampling error) and the variation between trees. The variation between trees includes the following:

(a) Variation between blocks (replication)
(b) Variation between treatments
(c) Variation due to other factors (experimental error)

The calculation of variance does not require higher mathematics, but merely involves adding the squares of the items involved, subtracting a correction term easily calculated, and dividing by the number of items involved less one. Before this division is made, the different estimates of variation are reduced to a common denominator, and the estimate for error is obtained by subtracting from the total. If the experimental error is low in comparison with the treatment variance, we may be reasonably certain that the differences in treatment values in the table are real and not due to chance variation.

By use of simple formulas (Snedecor *1*, pp. 406, 426) it is possible to calculate the least significant difference and least highly significant difference between treatment values. If two treatments differ in living scale populations more than the calculated least highly significant difference, the chances are 99 to 1 that one was actually more effective than the other.

The method of sampling to determine the scale infestation in the different treatments of extreme importance. As only fractional samples can be examined, the infestation estimates based on them differ from the whole, and to the extent to which they have been subjected to other causes of bias. If small samples are taken from each of the trees that are treated alike, it may be expected that the means will reflect the total infestation more accurately than means of large samples taken from only one or two trees. Bias can be avoided to a large extent by taking the samples at random in the manner which has been described.

From the sampling error and experimental error it is possible to test the probable effects of increasing the size of the sample and number of blocks (replications) on the treatment values. In our experiments with scale insects many such calculations have indicated that little increase in accuracy (reduction of standard error) is to be gained by increasing the number of leaves per tree from 20 to 50 or even to 100. More gain in accuracy comes from increasing the number of replications, but beyond 10 or 12 the diminishing returns in accuracy do not justify the extra work involved. With 20 leaves per tree and 10 replications, results from recent randomized-block experiments with insecticides against scale insects have been entirely satisfactory. This arrangement has been used also for insecticide experiments with the citrus rust mite, the citrus red mite, whiteflies, grasshoppers, the little fire ant, and, indeed, has been found suitable for experiments with many deciduous fruits and vegetable crops (Kelsheimer *2*).

By adopting the randomized-block arrangement for insecticide experiments on citrus, we have reduced the interference of variations in infestation between parts of groves and between rows. We have been able to segregate and compare the magnitude of treatment variations, which are our main interest, and other sources of variation in results. Small differences in results can be determined as significant and due to the insecticides, or as chance differences due to experimental variation. Proper sample size and the most adequate number of treatment replications can be determined for the particular insect under experiment. The excessively large size of the samples formerly examined can be reduced without loss in accuracy of results. From the randomized-block arrangement, with its opportunities for critical analysis of data, we can draw conclusions with much more assurance than from former arrangements.

LITERATURE CITED

(1) SNEDECOR, G. W.
1946. Statistical methods, 4th ed. 485 pp. Ames, Iowa.

(2) KELSHEIMER, E. G.
1947. DDT treatments for control of mole-crickets in seed-beds. Fla. Agr. Expt. Sta. Bul. 434.

REPORT OF THE ANNUAL MEETING OF THE FLORIDA ENTOMOLOGICAL SOCIETY DECEMBER 12-13, 1947

The annual meeting of the Florida Entomological Society opened at 1:30 P.M., Friday, December 12, 1947, in room 404, Newell Hall, University of Florida, with president Max R. Osburn presiding.

President Osburn requested each person to rise and give his name, business connection, and address. It was then announced that all committees except the auditing committee had been appointed. Mr. Norman C. Hayslip was named to this committee with the privilege of selecting someone else to assist him in auditing the books of the treasurer-business manager. Following these preliminaries, the president gave a few words of greetings to the society and then asked the vice president, Dr. E. G. Kelsheimer, to take the chair. Dr. Kelsheimer then presented Mr. Osburn as the first speaker of the day. Mr. Osburn's paper was titled "Comparison of DDT, Chlordane and Chlorinated Camphene for Control of the Little Fire Ant." After the presidential address, the other papers were presented in the order listed below:

"Mosquito Collecting in the Vicinity of Fort Clinch." J. W. Decker.

"Notes on the Collection of *Larra americana* Sauss., a parasite of the Puerto Rican Mole Cricket." E. G. Kelsheimer.

"Laboratory Substitutions for Certain Types of Preliminary Field Tests." C. F. Ladeburg.

"Some Results of Recent Work on the Newer Insecticides at the Orlando Laboratory." W. V. King (presented by B. V. Travis).

"Border Influences of Serpentine Leaf Miner Infestations in Potato Fields." D. O. Wolfenbarger.