GRASSHOPPER POPULATION SAMPLING*†

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Further illustration of the sampling principles discussed by Graham and Stark (1954) may be taken from problems encountered and methods used in sampling grasshopper populations. Unfortunately, many of the available examples are *still* problems, and manv of the methods in practical use have not been critically tested for suitability and adequacy. The author has drawn from his own experience and from published accounts only sufficient material to illustrate the principles involved and to provide a reasonablv comprehensive description of methods.

Purposes of Sampling Different Stages

The annual grasshopper survey is one of the most extensive projects in Canada and the United States requiring the use of specific sampling methods and techniques; the maps prepared from the survey data have proved to be of immense practical value to officials responsible for conducting control campaigns.

The maps are based on the numbers of eggs found in soil units of one square foot each, classified in terms of damage that might be expected during the following spring and summer; their accuracy in this respect varies with weather and crop conditions. The egg stage is used, rather than the adult stage, not only because it is "more stationary" and lends itself to "standard statistical procedures" (Graham and Stark, 1954) but also because adults can, and sometimes do, migrate from one area to another after an estimate has been made of their numbers and before egg-laying has been completed. Moreover, the number of eggs laid under various combinations of conditions by a given number of females has not been accurately determined. However, estimates of adult grasshopper populations are made before surveys of egg deposits in order (1) to provide rough indications of the area that may be omitted from the egg survey and (2) to provide data that can be used where necessary to supplement data obtained from the egg survey. The latter use is particularly important when autumn rains make completion of egg surveys impossible. Estimates of nymphal populations are made in restricted, representative areas to indicate the accuracy of the preceding egg survey.

Estimates of egg and nymphal populations are also required in experiments on control by means of cultural practices; estimates of nymphal and adult populations are required in experiments on chemical and biological control; and estimates of populations of all three stages are used in studies of population dynamics and the effects of ecological factors.

Methods of Sampling Different Stages and Species

Melanoplus mexicanus mexicanus (Sauss.), being the most widespread, persistent and generally injurious species in North America, has been the one most commonly used in studies of sampling methods. Probably the fact that it is more uniformly distributed than the other pest species, and more amenable to statistical procedures, has also influenced workers in this field.

Eggs of *Melanoplus m. mexicanus*

In sampling for eggs of *M. m. mexicanus* in the prairies of Western Canada, it is standard practice to sift samples of soil through a six-mesh screen, watching for eggs and egg pods in the soil as they fall through the screen, and finally for those left on the screen itself. Five sample units of one square foot, or ten of one-half square foot each, are taken per field. The units are distributed, without selection, over a distance of about 100 yards, but not necessarily at random.

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Routes of travel are adjusted to average about one field in one and one-half townships. Most of the samples are taken in grain fields, but if roadsides are in suitable condition for oviposition by this species they are also sampled.

Davis and Wadley (1949) found that sparse populations of eggs of *M. m. mexicanus* in the Great Plains area of the United States came very close to the Poisson, or truly random, distribution; as populations increased in density the variance increased more rapidly than the mean, indicating a departure from the Poisson series in those categories. However, they went on to explain that analyzing the data "as if the fields had been taken at random . . . does not lead to serious mistakes . . . What inaccuracy there is will be on the conservative side."

Considering practical as well as theoretical aspects, Davis and Wadley decided that sample units within fields need not be taken according to a fixed pattern nor by a strictly random method, but they should extend well into the field and be free from personal choice; fields to be sampled should be selected by systematic (stratified) methods. Allowing an error of 0.125 of a pod per square foot, they found that five square feet in each of ten fields would be sufficient for a county, or group of similar counties, containing uniform populations in the lower categories. Denser and/or less uniform populations could be brought within the limits of accuracy more economically by increasing the number of fields rather than the number of sample units within fields; 30 fields was the maximum number per county or group of similar counties required for any of the conditions studied by Davis and Wadley.

On the basis of these conclusions, more than 30 fields in 3000 square miles would rarely be required in a practical survey of eggs of *M. m. mexicanus* over large areas of country; considerably fewer than 30 would have to be sampled under conditions more commonly encountered. This suggests that Canadian surveys of the same species at the rate of five square feet in each of five or six fields per 324 square miles (nine townships) should be highly accurate in the main, the accuracy increasing directly with the size of an infested area and inversely with the intensity of infestation.

Eggs of *Camnula pellucida*

The methods used to sample nymphal and adult populations of *M. m. mexicanus* differ greatly from those used to sample egg populations of that species, and the methods used to sample egg populations of *Camula pellucida* (Scudd.) also differ from those used to sample egg populations of *M. m. mexicanus*. These differences serve as excellent illustrations of the truth of the statement that "the sampling of a particular insect population must be resolved about the distribution and life cycle of the insect involved" (Graham and Stark, 1954).

Egg deposits of *C. pellucida* tend to be concentrated in restricted areas of sod of relatively few species of grass. To obtain an accurate estimate of such a variable population by strictly random means is impractical in general surveys (King, 1939). An attempt is made, therefore, to estimate the size and number of egg beds in a given number of miles of roadside or pasture, doing just enough detailed sampling to obtain a reasonably accurate estimate of the classes, or categories, into which the egg beds fall. Unlike the oviposition sites of *M. m. mexicanus*, the sod in the favoured sites of *C. pellucida* is usually too tough to make soil sifting possible; it must be shaved with a sharpened trowel or lifted with a spade, placed in a screen, and teased apart by hand. Where populations fall into the same category over a considerable area it may be possible to make as few as three stops in two townships, but in irregular infestations, or those lying close to the boundaries of a category, an observer may be obliged to make up to six stops per township. It is doubtful whether the data obtained by these methods are amenable to standard statistical procedures; at best, considerable experience is required to interpret the results satisfactorily in terms of requirements for control.

Nymphs and Adults

Nymphal and adult populations are sampled by various methods, depending upon the use that is to be made of the resulting data and the accuracy required. During the annual survey, where it is desired to know the population per unit area but where economy of time is a major consideration, observers attempt to count the numbers of grasshoppers that leap or fly from quadrats of one square yard, the size of the quadrat being estimated visually. This is more difficult in nymphal populations because nymphs are less easily seen than adults and are usually present in much larger numbers per quadrat. The number of quadrats per field is not usually specified; an observer is required only to satisfy himself that he has obtained a reasonably accurate measure of the mean for the field. He rarely takes fewer than 10 quadrats or more than 25. The number of fields per municipality (Canada) or county (United States) closely approximates the number sampled during the egg survey. In experiments on chemical control, where it is necessary to know relative population densities only, comparisons are sometimes based on the number of specimens collected in a given number of strokes of a sweepnet (Hinman and Cowan, 1947). Smith and Stewart (1946), studying grasshopper populations in relation to biological control, developed a cage method that proved highly satisfactory for sampling nymphs and adults. These methods are all being compared at present with a method involving the dilution of a population with radio-active specimens. The work is being done under the leadership of L. G. Putnam, Grasshopper Research Co-ordinator for Canada, Entomology Laboratory, Saskatoon, Sask., and F. T. Cowan, Officer-in-Charge, United States Department of Agriculture, Agricultural Research Service, Entomology Research Branch, Bozeman, Mont.; the results have not yet been published.

Effects of Undesirable Variables

The subjects "patterns of variability within a sampling universe" and the desirability of sometimes "sampling with respect to the known environmental variates" have been covered by Graham and Stark (1954). Where sampling is done to compare population levels under different sets of conditions (much sampling is done for this purpose), undesirable variables must be carefully noted, and removed if at all possible. For example, estimates of adult grasshopper populations in an adequate series of weedy grain fields might logically be compared with estimates in an adequate series of clean fields, providing one wishes to gain some information on the relative attractiveness of the two types of fields to the species concerned; but a comparison of the data would be of little value, probably quite misleading, if the estimates in the series of weedy fields were made on a clear, hot, dry, calm day by observer A and those in the clean fields on a cloudy, cold, wet, windy day by observer B. The example is obviously extreme, but most workers are guilty at times of overlooking or ignoring one or more variables that might seriously affect the reliability of their samples.

Vegetation

Examples of the effects of the variations in vegetation density are available in reports on experiments with poisoned baits: The mean percentage mortality in baited cages placed in relatively dense vegetation was 21.1 per cent lower than in an adjacent series in which the same crop had been artificially thinned; the difference necessary for significance at the five per cent level was only seven per cent (Handford, 1941). In two sets of experiments designed for other purposes, but differing in several factors including density of vegetation, the treatments in dense vegetation gave an average of 23.2 per cent mortality whereas those in sparse vegetation gave an average of 87.7 per cent (Handford and Putnam, 1942.) These are, of course, merely illustrations of the effects of variations in density of vegetation; in practice every effort is made to find uniform stands of vegetation for experiments in which treatments are being compared.

Weather

York and Prescott (1951), after making direct comparisons of sweep-net and cage methods of collecting grasshoppers at different times of the day and under different conditions, concluded that "the sweep-net method of sampling shows great variation in the catch of grasshopper nymphs and many other insects due to time of day and meteorological conditions, thus making it of little value in population studies. Its use in evaluating control on field plots seems valid if the treated and comparable untreated plots are sampled within a short period of time.

"Sampling with a cage is at least as fast as with the net, but is more 'back breaking'. It shows less variation from meteorological conditions than the insect net, and as a consequence is probably more advantageous when the weather is unfavorable for sweeping."

Personnel

That important differences can occur in the results obtained by different workers under almost identical conditions may be illustrated by a third example: Methods used in comparing the effects of different grasshopper baits on nymphs, described by Hinman (1939), required that a number of nymphs be collected from each plot and held in cages for 72 hours before mortality was determined. It was frequently necessary to have the collections made by two or three men in order to keep within uniform time limits. Using Student's *t* test on differences between pairs (Goulden, 1929), Handford and Putnam (unpublished report for the year 1940, on file at the Entomology Laboratory, Kamloops, B.C.) found that mean mortality difference of 12 per cent, in collections made by different workers, were significant at the 2 per cent level. The obvious correction was to have the collectors work as a group, each making part of the collection from each plot.

Removing Effects of Undesirable Variables

Where it is impractical or impossible to eliminate undesirable variables, every effort should be made to design techniques that will remove their effects. The six-mesh screen, for example, samples eggs of *M. m. mexicanus* satisfactorily under most conditions. However, where soil is gravelly, or moist, or full of debris, better results are obtained by using a four-mesh screen either alone or nested in the six-mesh screen. If soil is too moist to be handled this way, a good quality plasterer's trowel, well sharpened on the edges, serves to cut away thin layers of soil and expose the eggs. Soil that is baked can be cracked by means of a wooden mallet or block; if eggs are present the lumps usually break along the course followed by the ovipositor, thus exposing the pod.

Bias Resulting from Personal Selection

That "personal selection" may easily "bias the estimation of the mean" (Graham and Stark, 1954) may be illustrated by work done on sampling grasshopper egg populations. The author, in sampling fields at Piapot, Sask., in 1943, obtained a mean of 1.03 pods of *M. m. mexicanus* and *M. packardii* Scudd. per square foot in non-selected, stratified sampling, and a mean of 2.5 pods per square foot for selected, stratified samples; the difference, analyzed by Student's *t* test on differences between pairs, was significant at the 1 per cent level. The only criterion for selection was that the area from which a sample was taken should have been subjected to the maximum effects of the sun's rays; each selected sampling point was limited to a distance of three paces from the corresponding non-selected sample.

The Experimental Area as a Sample

Experimental procedures, as such, do not commonly come to mind when one is discussing the sampling of insect populations. Nevertheless, a set of experimental plots is a sample of a particular universe and should be subject to the same standards and the same restrictions as any other type of sample. As a rule an area used for an experiment on control forms a very small sample as compared with those taken in ecological studies or for purposes of estimating present or future damage; yet if the design and procedures satisfy the mathematical requirements, and the data have been analyzed statistically, conclusions are likely to be drawn with considerable confidence. The possibility that the site and conditions may not be truly representative, or that undesirable variables may not have been removed, is sometimes overlooked. A great deal of thought should be given to the selection of a site for field experiments and to the possible need for duplicate sites in important minor habitats. Failure to remove undesirable variables can increase the error variance to such an extent that true differences, which would otherwise be statistically significant, are effectively masked. At the other end of the scale one should never forget the possibility that his data may have come from the one experiment in 20, or one in 100, in which analysis indicated differences between treatments when, in fact, further work might have reduced the differences to the point where they were not significant. These and several related points are more clearly and adequately covered by Beveridge (1951).

Sampling Problems Awaiting Solution

As will have been gathered from the foregoing remarks, few, if any, of the sampling problems encountered in grasshopper studies have been completely solved. Although estimates of egg populations have been subjected to formal mathematical tests, still too little is known about the "patterns of variability" of even the major pest species. Mr. L. G. Putnam is now giving this problem further attention. Work under his direction and that of Mr. F. T. Cowan, on methods of sampling nymphal populations, has already been mentioned. The statistical adequacy of methods in general use for sampling adult grasshopper populations is still to be determined. There are innumerable minor problems within these major fields. It is recognized, for instance, that relatively more adults of *M. bivittatus* than of *M. mexicanus* are picked up in a net from a mixed population of these species when one walks slowly than when one moves more rapidly. The same is probably true to a lesser degree in mixed populations of *C. pellucida* and *M. m. mexicanus*. However, the differences have not been critically measured and compared. It also appears that nothing at all is known about the relationship between the true proportions of parasitized and non-parasitized grasshoppers in a given infestation and the proportion picked up in a sweep-net, one of the standard tools for doing such sampling. The effect of weather conditions on the results obtained in sampling adult and nymphal populations requires further study. These and many other problems in sampling grasshopper populations await the attention of workers interested in this very important field.

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INSECT POPULATIONS IN CARIBOO POTATO FIELDS[1]

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From late June to early September of 1951, 1952, and 1953, insect populations were sampled regularly on 3 farms, 10 miles apart, near Soda Creek, B.C. The objective was to correlate the populations, particularly of leafhoppers, with the natural incidence of witches'-broom virus disease of potato. Little or no disease appeared, but the populations and their dynamics are of interest both in themselves and economically.

Comparative freedom from virus-carrying insect pests has led to the establishment of a thriving seed potato industry in the Cariboo district. The montane climate and swift grow-season ensure that the greatest pest of all, *Myzus persicae* (Sulz.), the green peach aphid, is not often seen and seldom becomes numerous. Most of the growers have never had occasion to use insecticides, nor do they own the necessary equipment.

Since 1949 the western potato flea beetle, *Epitrix subcrinita* (Lec.), has become established in the area, moving northward to Quesnel in 1954. From occasional specimens taken at Soda Creek in 1950 (Prof. G. J. Spencer, University of British Columbia, personal communication), it has increased almost to outbreak proportions, at one site killing the vines and severely damaging the tubers in 1952. In the 3 seasons under review, 85 samples of insects were taken, totalling upwards of 84,000 specimens, of which 91 per cent were adults of *E. subcrinita.* Randomized subsamples of flea beetles, submitted to Dr. L. G. Gentner, Medford, Oreg., confirmed the identity of the species. Of the remaining 7,200 insects, representative samples only were determined.

During the period, recorded monthly temperatures showed very little variation from year to year or from the long-term average, although in 1951 precipitation was below normal.

Methods

An average of about 2.5 samples were taken per week, each of 100 strokes with a standard 15-inch net. The samples were stratified into 4 subsamples of 25 strokes, taken in each quarter of a field. The subsamples were put together in the killing bottle and the total catch, including immature forms, was counted into the broad, easily recognized categories shown in Figs. 1 and 2.

Exploratory plotting suggested that populations of the insects were similar for corresponding weeks in the 3 years. With this in mind, the 3-years' data in each insect category were grouped into weekly subtotals, divided by the number of samples and

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