collection and disposal system. House fly breeding remained a problem but originated primarily from animal enclosures, pointing out the need for enforced ordinances to regulate livestock within the city. The difference in house fly indices between Cities I and II is due primarily to the house fly breeding in the open dump at City II. The spraying program in City II is believed to have been primarily effective in the control of the excrement-breeding fly, *Sarcophagula occidua.*

4. In addition to cost data the results of the study will yield data on the effects upon fly densities and human disease in: (a) City I with 3 garbage collections per week and an efficient sanitary land fill; (b) City II with 3 garbage collections per week but with inadequate disposal; and (c) City III with twice-weekly garbage collection subject to some variation and inadequate garbage disposal.

# CONSIDERATIONS IN SAMPLING FLY POPULATIONS

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In recent years many programs for the control of house-frequenting flies have been undertaken. As might be expected in such developmental programs, greater emphasis has been placed upon the methodology of control operations than upon the measurement of the fly populations. Various devices have been used in evaluating the effectiveness of the fly control measures, such as baited fly traps, tanglefoot tapes and sheets, and time counts over uniform attractants. The fly grill developed by Scudder* has been perhaps the most widely used of these devices in programs sponsored by the Communicable Disease Center. However, the application of the grill has varied between programs, and this lack of uniformity in the methods of taking and interpreting fly population samples has prevented really accurate direct comparison among programs.

The need for a standard method of sampling fly populations is indicated, not only to facilitate the evaluation of fly control operations but to make possible the correlation of the results with standard epidemiological measurements. A reliable sampling method should have the following characteristics:

1. It should be repeatable, and objective.
2. It should be useful in guiding control operations.
3. It should be economical to apply.
4. It should supply data which are suitable for statistical analyses.
5. It should be applicable over wide geographic areas.
6. It should be adaptable to a wide range of conditions.

The following discussion of the methods established and in use by the authors and their co-workers on an experimental study in south Georgia is presented for consideration by other programs.

## PREREQUISITES TO SAMPLING

The following steps were followed in establishing the sampling program on the experimental project under discussion.

1. Maps of the cities were obtained and brought up to date. Besides large office maps, small notebook-size reductions were made for field use.
2. All blocks in the cities were numbered for easy reference.
3. The cities were divided into sub-areas of about 10 blocks, or a 10 percent sample, except that in the high-income residential districts, sub-areas of 20 blocks, or a 5 percent sample, were used. Sub-areas which included industries with

*Scudder, H. I.: A New Technique for sampling the density of housefly populations. Pub. Health Rep. 62:681-686 (1947).

Placing fly grill over attractant to sample fly population.

high production of organic wastes never exceeded 10 blocks.

**SURVEY PROCEDURES**

**A. The Stationary Block Survey.** In each sub-area, the block with the highest fly potential was selected as a so-called STATIONARY SURVEY BLOCK. The stationary survey block was not changed during the survey unless another block in the sub-area showed, over a period of three consecutive weeks, a higher weekly average than the one selected. All the stationary survey blocks thus selected were inspected once a week, using the grill method. Although 10 grill counts were recorded, the average of the 5 highest counts was used as the index for each stationary survey block.

The stationary survey block method had several advantages and disadvantages. The advantages were: (1) the speed and low cost with which the surveys could be made; (2) continuous data on the same blocks throughout the season at weekly intervals; and (3) with a low index, the indication that a minimum number of flies was present in the whole area. The disadvantages were: (1) the dubious economy of re-treatment throughout a sub-area on the basis of the data from a single stationary survey block; (2) the possibility of a bias having been introduced into the data by the way in which the blocks were selected and by the methods of spraying used.

**B. The Visual Survey.** In order to prevent unnecessary expenditures of time and materials in re-treating a sub-area on the basis of the data from a single stationary survey block, a visual or reconnaissance survey was used in conjunction with the stationary block survey. It was found advisable to precede the stationary block survey with the visual survey by 2 to 3 days, thus using the stationary block survey as a check on the effectiveness of the control measures as indicated by the visual survey. Without using the grill, but based on experience with it, estimates of grill readings of each concentration of flies encountered in each block were made rapidly by the inspectors. The average of the five highest of these estimated grill counts for each block was used as the index figure for the block. The control operations section was then furnished with a map upon which the block index figures were shown. All blocks whose index figures exceeded three flies were designated to receive treatment. Treatment was applied to the designated blocks in relation to the index figure. Blocks with the highest indices were treated first. This type of survey proved very useful in providing data for directing the effective application of insecticides, but its standardization requires further study.

**C. The Random Sample and the Statistical Treatment of the Data.** Data obtained from stationary block surveys were subject to bias in that the blocks were inspected at different times, in different places, and by different persons. The visual survey enabled broad coverage to guide fly control operations. Its continued use holds promise as a tool for entomological evaluation and epidemiological investigations, but its development as a meaningful measure requires further study of the sources of bias and the magnitude of sampling error.

The methods of analytical statistics are based upon the theory of probability. In order to use these methods validly, data must be collected in such a manner as to allow the application of the mathematical model upon which the probability theory is based. A random sample will be defined, for the present subject under discussion, as a sample in which each variate is selected with known probability. If only the blocks with high fly counts are selected for sampling, any statistic derived from the data is representative of only a portion of the blocks, and no valid assumptions may be drawn with respect to the flies in the whole city unless one knows the relationship between the selected high blocks and all blocks in the area. This relationship depends on the local characteristics of each area and cannot be assumed invariant from one locality to another. It is therefore quite apparent that in order to insure generalization of inferences from one area to another, some form of random sample must be taken in all projects where fly indices are to be compared with epidemiological or other experimental data.

The random sample also serves as a check on change in the bias of stationary high count blocks. Bias is a conscious or unconscious allocation of greater probability of occurrence to some of the values of a variate. A mean calculated from biased data will not be an estimate of the true mean of the population being sampled. If, for example, only the stationary survey blocks are sprayed, a reduction in the city-wide fly index may not correspond to actual reduction of flies in the entire city, but only to the decrease of fly abundance in these blocks. A random sample, as used in this study, included one block out of each sub-area in the city at each sampling period, and was not biased in this particular respect, since the stationary survey block in each sub-area had only a 1-9 chance of being included in the weekly sample. The random sample also gave a similar check on the accurate selection of the highest block in the area.

The random sample therefore served a double purpose: It provided data by which to stabilize and check the operational surveys, and it supplied a valid statistical sample. This sample was drawn by selecting at random, with replacement, a block in each of the 10-block or 20-block areas in the city. The block thus drawn was sometimes surveyed by the same man who inspected the stationary survey block or by a different man altogether. For operational purposes the random survey was unsatisfactory if used alone. From this work it appears quite possible that with the use of a standardized visual survey as the principal guide to fly control operations the sampling procedure may be limited to the visual and random surveys.

### DETERMINING THE NUMBER OF INSPECTORS NEEDED

Both the number and the accessibility of alleys in a town were of great importance in determining the number of inspectors needed. In cities with few or no alleys, such as were encountered in this study, the maximum number of blocks which could

In setting up a fly sampling program, one of the most important steps is to obtain maps of the town and divide them into sub-areas, indicating a stationary survey block in each sub-area.

be surveyed per week by each inspector was approximately 125 for both the stationary and random surveys and approximately 275 for the visual type of survey. These figures were attained only under conditions permitting a full work day, and when travel time each day to and from the work area was 30 minutes or less. Based upon previous survey experience in other areas, where each city block was traversed by an alley, it has been determined that the stationary and random block surveys required one inspector for each 150 blocks to be inspected each week on each of the two types of survey. Time studies of the few blocks traversed by alleys, in the area of the experimental study under discussion, indicated a visual survey time per block of 4 to 5 minutes. Assuming that all of a city's blocks were so traversed by alleys and that 450 working minutes were available each day of a 5-day week, one inspector could conduct a visual survey through 450 to 560 blocks per week. Variations in these figures will result when working time is decreased by weather or other factors or when maximum efficiency is lost for any reason.

## FACTORS WHICH AFFECT VARIABILITY OF GRILL COUNTS

Various factors, which have been found in our experience to cause the greatest variability in grill counts of flies, are discussed in the following paragraphs.

**Inspectors.** In order to measure the accuracy of the different inspectors in estimating flies on the grill, certain tests were run. These tests involved the use of grills, to which dead flies had been fastened by means of an adhesive, and on which the fly distribution patterns were arranged to simulate field occurrence. In the lower density ranges, i.e., up to 50 flies, it appears that all the inspectors tested were reasonably accurate. The greatest errors of estimate were found to be in the vicinity of 175 to 250 flies. This readily may be explained by the fact that in this range of counts there were too many flies for the inspector to count on all parts of the grill; yet in this range, distributions of flies on the grill were quite uneven. Since the inspector counted the flies on a portion of the grill in order to estimate the total flies on the whole grill, an additional error, due to the uneven distribution of the flies on the grill, was introduced. This error reached a maximum in this interval. Below 50 flies the spread of the different readings was very small, but above 50 flies it was so great that special training of the inspectors in estimating large numbers of flies was indicated. The amount of time to be spent in training inspectors to use the grill will vary with the objectives of a fly control program. The authors recommend a minimum of 4 weeks' training wherever possible and frequent checks throughout the season. A time limit of 20 seconds should be used in all tests since it closely simulates the length of time in which flies can be expected to remain quiescent on the grill in the field.

**Temperature.** One of the early observations made by the inspectors was that the fly index on cold days was reduced to zero. This was to be expected since flies are poikilothermic. In determining the temperature threshold of fly activity from the grill records, it was found that the lowest air temperature at which countable flies were encountered was considerably lower than the observed temperatures at which flies would be immobilized. Field observations revealed that attractants in the sun had an environmental temperature about 24° F. warmer than the air temperature in the shade usually recorded. Counts made during those portions of the day when the air temperature is too low for normal fly activity will only reflect the number of attractants in the sun and the flies that were on or near them.

A preliminary laboratory test was conducted in 1949 in order to estimate more closely the threshold temperatures of various degrees of fly activity. Five house flies each were placed in large test tubes, 29 millimeters by 19 centimeters, which had been fitted with cork stoppers. A mercury bulb thermometer inserted through a perforation in each cork stopper allowed readings of the air temperature inside the test tube and within one-half inch of the flies. The test tubes of flies were cooled in a refrigerator for 30 minutes until the air temperature within each tube was approximately 27° F. After this cooling period, which immobilized the flies, the test tubes were brought into the laboratory where the temperature was 80° F. As the temperature within each test tube rose, the reading at the initiation of each level of activity was recorded. Fifty replications of this test were made, and a record was kept of the temperatures at which the following activities were initiated. The average temperatures at which the flies responded were: The first movement was made at 55° F.; at 62° F. they righted themselves; at 70° F. they crawled; and at 74° F. they became quite active. From this information, and from field observation data, the authors concluded that grill counts taken when the air temperature is less than 70° F. should not be used in the calculation of fly indices. This 70° F. temperature is only a preliminary threshold selection, however, since further investigations as to the length of time the fly is subjected to a gradual increase in temperature are needed, and the final results may indicate a different threshold of activity.

**Moisture.** Moisture is usually an important limiting factor only in areas of low rainfall. In Texas, New Mexico, and Arizona the limiting factor for fly production during the summer appears to be the availability of moisture. Data from Albuquerque, N. Mex., show that hog pens and some other animal enclosures with a constant supply of moisture were the main fly attractants (30 percent), and accounted for most of the high counts. In the southern region of the United States, from east Texas to the Atlantic, excessive moisture may sometimes inhibit rather than increase fly production; this may be due to the drowning of the larval stages and the mortality in the adult fly population caused by heavy rains.

**Shade.** Shade is apparently of least consequence among the factors selected, but is of importance in determining the distribution of flies in a block at different times of the day. Early in the morning or during a relatively cool day (70° to 80° F.) the majority of high counts are found in the sun. As the air temperature reaches the vicinity of 90° F., the flies will tend to move from the sun into the shade.

## METHODS EMPLOYED IN ORDER TO MINIMIZE THE EXPERIMENTAL ERROR INHERENT IN THE USE OF THE GRILL

Knowing that there is a certain inevitable variation in the ability of the different inspectors to count and estimate fly numbers, it was believed essential that a schedule for the systematic rotation of the inspectors from one inspection area to another be followed. In this manner the bias introduced by the individual inspectors will be equal in all cities or sections of a large city.

Of great importance in surveying small cities or sections of a large city, since they usually are surveyed during part of a working day, was the rotation of the time of day in which the inspection was made. It is quite obvious that the ideal procedure would be to have the inspections made at the same time of the day each week in all cities. Since this was not possible, the authors systematically rotated the inspection time in all inspection areas so that variability caused by the hourly fluctuation in numbers of flies was introduced to an equal extent in all areas when data from several inspection periods were combined.

In cities where fly inspections could not be completed in one day, the different parts of the city were inspected on different days in order to have one full inspection each week. Care was taken that no section was always inspected at the same time of the day. Blocks for each day's inspection were selected at random, without replacement, thus getting a full weekly sample from the town. The random selection of the stationary survey blocks to be inspected each day greatly increased the probability that all parts of the town were inspected at all the different times of the day.

## SUMMARY


1. Data from the sampling of fly populations served a two-fold purpose: They provided the operating personnel with information needed to determine when and where control measures were needed; and they were compared with epidemiological data on diarrheal disease.
2. A three-point survey was believed necessary to best serve both purposes:
   a. A visual survey was of great value in directing control operations and holds promise, once fully tested, of serving all inspectional purposes.
   b. A stationary block survey was of value in supplying continuous data from the most fly-productive block in each sub-area.
   c. A random survey was needed in order to permit comparison of fly indices with epidemiological data and served as a check on the bias introduced in the stationary block survey.
3. The most important variables influencing fly density readings by the grill or other methods were individual inspector capacities, temperature, moisture, and shade.
4. Comparability of fly inspection data was improved by the rotation of (1) the inspectors, (2) the order of inspection of the small cities or of the sections of the larger cities, and (3) the order of the inspections of the blocks within the cities.