Sampling of grasshopper populations
by George T York

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the degree of Master of Science in Entomology
Montana State University

Abstract:
Sampling of grasshopper populations was carried on at Broadus, Montana during the summer of 1947 to develop accurate and dependable methods.

The method used most was a cylinderical sampling cage with a ground coverage of one square foot. A flask with carbon tetrachloride as a killing agent greatly increased the speed and accuracy of counting.

As no direct method was found for determining the population the following two points indicate the cage was obtaining an accurate sample of the population; (1) Counts made by two operators gave essentially the same results, (2) Counts by the same operator at different times of the day gave results that differed only by expected variation.

Visual estimates of nymphs were much lower than counts with the cage.

Sampling of adult populations with the cage was tried at night and found satisfactory for accurately determining the population present.

It has several advantages over other methods.

The sampling of live adults in comparison to counts after the use of a dust which stupified the grasshoppers gave essentially the same results under late season conditions.

Visual estimates of adult populations by two observers failed to give consistent results.

Collections of nymphs by the sweep net and cage for determining the percentages of the various instars were not taken in sufficient quantities for statistically sound results.

The cage, as used during this work, was limited to vegetation not over 24 to 30 inches high.

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ABSTRACT

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INTRODUCTION

Sampling of various insect populations has been, and will continue to be a matter of considerable concern to entomologists engaged in field studies. The evaluation of chemical, cultural, or biological control measures are based on population counts which must have a certain degree of accuracy before confidence can be placed in the results. Ecological studies involving fluctuations in populations are likewise dependent on sampling in such a fashion as to give correct and reliable data.

The first consideration in sampling populations is the accuracy or correctness of the sampling method. Without an accurate method, sampling is of little value regardless of the amount that is done. In other words, if sampling results are influenced by such conditions as time of day, temperature, or other climatic factors the data would not be reliable or comparable from time to time. If, for instance, one series of counts has a standard error of ten per cent of the mean and a similar series taken under different conditions shows a 100 per cent increase, this difference having been induced by climatic factors, the results are obviously wrong and misleading even though the same degree of reliability has been achieved in the sampling. Statistical analysis of such data would only lead the uninformed to place more confidence in an erroneous set of data.

This emphasis on proper experimental technique is not intended as a new or novel idea, but is only to reemphasize what practically every author of statistical textbooks has pointed out, but which so many experimenters still fail to heed. For example Goulden (1939) states, "The

problems of statistics are, therefore, not entirely mathematical problems; in fact they are very largely problems based on the technique and requirements of the research worker." Waugh (1943) expresses much the same viewpoint when he states, " -- statistical methods cannot, in themselves, solve any problem. The original data must have been accurate, the methods must have been properly applied, and the results must have been interpreted by one who understands both the methods themselves and the field to which they have been applied."

The determination of the validity of a method is often the most difficult problem and in many cases can only be approximated by indirect methods. Once the system is established the variability can be reduced to the desired degree of accuracy by adequate sampling.

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REVIEW OF LITERATURE

The problems encountered in sampling insect populations are many and varied, thus no attempt has been made to cover the entire field. A few notations are given to show the diversity of conditions and problems, while the rest deal in one way or another with the problems of sampling grasshopper populations.

As evidence of the difficulties encountered in various fields of entomological research might be mentioned the work of Smith and DeBach (1942) who, in discussing the proof of biological control and the "-- multiplicity of factors and the complexity of their interrelations." have the following point to contend with, "There remains, also, the general difficulty of developing population-sampling techniques which will satisfy the statistical requirements." Baten and Hutson (1943) list some of the problems encountered in sampling red spider populations and mention the discarding of eight months of sampling data due to poor techniques. Huffaker and Back (1943), in making mosquito population studies list a number of methods and their variations due to various factors. However from the study they conclude, "Results have revealed that none of these methods can be depended upon for an adequate, non-selective analysis of a heterogeneous mosquito population."

The sweep net method has been used for many years as a means of evaluating insect populations. In most cases the experimenters were aware of the inadequacy of the method but seldom had anything better to offer. Lockwood (1924) in studying grasshoppers makes the following statement, "Sweepings made at different times of day, under varying cli-

matic conditions, in different crops, together with the varying speed of operators upset the uniformity of such reports." As a substitute he suggested early morning observations, but as a drawback to this method he reports, "Young grasshoppers are inclined to shelter themselves under close-growing foliage and in cracks and rubbish during the nights and colder parts of the first daylight hours." Another suggestion was to observe moving hoppers as they crossed a certain line, counting the numbers over a known distance of the line and as they moved a certain distance, thus getting the number on a square yard or square rod basis. This has a very restricted use as 'hoppers are seldom moving in such a manner as to be counted.

DeLong (1932) using the sweepnet in working with leafhoppers discusses several factors including wind and temperature as influencing the catch.

Gray and Treloar (1933) made a special study, including statistical analysis, of the insect population by the use of the sweep net in an apparently uniform stand of alfalfa. From a study of the correlation between successive sweepings they concluded they were not sampling from a homogeneous, but rather a heterogeneous population. As their sampling was done in groups of 25 sweeps per sample they concluded that individual sweeps would have shown even greater heterogeneity. In addition to this analysis they showed the number of samples of 25, 50, 100, and 200 sweepings that would be required for reliable results. They state, " -- the large actual variation between yields of 200 sweeps each will impress the observer with the fallacy of attempting the enumeration of insect

populations in such a habitat as an apparently uniform field of alfalfa by making anything less than a very large number of sweeps throughout the entire field." In addition to this they state, "Indeed the number of sweeps required for even a 50 per cent error range (as of the true mean) is so large as to preclude the usefulness of the technique for census purposes under most practical conditions."

Beall (1935) compared the sweep net with the cylinder method. Sweep collections were made during the day, and cylinder collections at night. The cylinder collections were, "taken as a standard". From these data he figured the number of sweeps necessary to provide a sample equivalent to the population on one square meter. This varied a good deal depending on the particular insect that was being studied. In addition, he calculated the number of sweeps necessary to give a specified degree of accuracy. This was considerably less than that given by Gray and Treloar (1933), but corresponded to a modification made later by Treloar and Gray (1935).

Romney (1945) gives a brief review of insect sampling with a reference to sweep net sampling as far back as 1846. In his work with the beet leafhopper (Eutettix tenellus Bak.) he compared the catch in a cylinder with two sweep-net methods. He concluded that, "This quantitative method (the cage) was found to be satisfactory for a detailed study of the insect fauna on herbaceous plants up to 12 inches in height, although it is limited to use in sandy soils." The basis for this statement was the fact that adult and nymph counts did not follow the temperature curve. With adults there was a more or less uniform catch regardless

of time of day or temperature, indicating that the catch was independent of physical factors. The curve for nymphs showed a drop in catch as the day progressed but this was explained as a movement from the particular plants which he was sampling to large plants for protection from excessive heat. A similar experiment with a uniform host plant condition did not show this drop, so it may be assumed his explanation was correct.

Hills (1933) was probably the first to use a cage for routine field counting. This cage consisted of two metal circular bands held 20 inches apart by four upright metal supports. The framework was covered with sheeting, fitted with a celluloid top, and attached to a handle, such as a pitchfork handle. By means of the handle the cage could be suddenly set in place over the plants and the enclosed insects counted. Methods for collecting the insects from the cage were also given. The author states, "The method herein described makes possible accurate counts of the insects on a square foot basis whenever temperatures are high enough to cause slight activity." However he gives no data to support this view so one must accept of reject the accuracy of the method merely on his statement.

The fact that this method was generally accepted by beet leafhopper workers, as evidenced by the work of Douglas et al (1939) and Romney (1943) indicates that it was the most reliable method available.

Gray and Treloar (1933) object to the use of a cage because of (1) "-- practical difficulty of placing the chamber over the vegetation without -- disturbance of the insect population --." (2) " -- if fumigation is to be effective the chamber must fit the contours of the ground

quite closely." (3) " -- difficulty of collecting all the insect forms from among the vegetation and debris of the substratum." and (4) " -- to render conclusions drawn from such small areas applicable to field plots it is necessary to replicate the samples a large number of times. These area analyses are necessarily slow and frequently tedious procedures."

Hills (1933) overcame, at least to some extent, all of these objections. By using a handle he reduced the disturbance factor, the circular cage could be forced into the ground, and he did not fumigate. By not fumigating he speeded up the process so that many more samples could be taken.

Shotwell (1935) described methods for making a grasshopper survey. For adult populations the observer walked thru the field and estimated the average number per square yard. He reports that, "Numerous trials in which different workers have estimated separately for the same field have shown that the counts checked very closely, and this justified the placing of considerable confidence in the accuracy of the method."

In a later publication Shotwell (1942) discussed the use of several methods for determining mortality of grasshoppers from bait tests in the field. Two of these, the pan-bait method and the plot and cage method are not applicable to field sampling of populations, but merely determine rate of feeding or mortality from the poisons. As to the use of a cage he states, "Counts of live hoppers per unit area are sometimes obtained by dropping a cage over them in the field and then gassing the hoppers that are caught. This method is unsatisfactory because many individuals may escape by flight or on foot where the cage does not make contact with

the ground owing to unevenness of the surface or heavy vegetation." He also discusses a special method for sweep net collecting for observing differences in populations. As to its effectiveness he states, "Only gross differences in population are measurable by this method, but it is believed more accurate data are obtainable in this way than by mere visual estimates of populations."

Mr. C. C. Wilson (unpublished reports from Sacramento, Calif.) used a cage for determining grasshopper mortality. In field population studies where comparisons of the cage and visual estimates have been made, the cage almost always gave the higher count.

Hinman and Cowan (1945) report the use of the insect net for evaluation of treatments for grasshopper control. Their procedure was to make counts in the treated and untreated plots before and after treatment. They state, "The ratio of the average number of grasshoppers per sweep on the treated plot to the number per sweep on the check plot would be expected to remain constant except for the effect of the treatment." This method should be satisfactory for checking insecticides but has very limited use for population studies.

METHODS AND PROCEDURES

All experimental work was carried on at Broadus, Montana. Sampling of nymphs was done in a 35 acre dry land alfalfa field which was planted in rows three feet apart with the rows running north and south. There was considerable volunteer alfalfa between the rows. The field was practically free of weeds but had a moderate growth of cheat grass, (Bromus tectorum L.) which however, was dry at the time the sampling was done. The work with adults was conducted on a very poor stand of newly planted alfalfa, hereafter referred to as idle land, and on wheat stubble. The idle land was approximately 5 acres in size, well covered with a growth of Russian thistle (Salsola pestifer A. Nels) 2 to 6 inches high, and with the scattered plants of alfalfa nearly defoliated by the high grasshopper population. The wheat stubble was approximately one foot high, with small scattered Russian thistle thruout.

One of the objectives in this study was the development of a method of determining the actual number of grasshoppers present in a given area. Once such a method was established then the accuracy of various types of sampling could be determined. Likewise, the ecological factors as they effected the sampling methods could be evaluated and the proper correction factors determined. In accordance with this plan small areas were to be dusted with a material to give a quick "knock-down" and samples taken to determine the population while in this state. In addition, cages were to be placed over vegetation at night and counts made the following day, thus getting a double check on the population present. In attempting the first procedure, PCH dust (2% piperonyl cyclohexanone, 0.2 % pyrethrins, plus

cohutta talc) was used to give a quick "knock-down" of the grasshoppers. Previous work by Parker and Hinman (Unpublished reports, U.S.D.A. Bur. of Ent. and Plant Quarantine, Bozeman Laboratory) had shown this material to be effective for this purpose. Areas approximately 50 feet square were dusted. A small metal hoop with an area of one square foot was used for sampling. It was soon realized that the counts were not accurate when sampling small grasshopper nymphs as they had a very pronounced tendency to crawl under debris or into cracks in the soil and therefore could not be found. This difficulty had been mentioned by previous investigators. The second method of using wooden framed cages placed over vegetation at night was not satisfactory due to the difficulty of properly sealing around the ground to prevent escape.

With these methods proving impractical, it was decided to try the sampling cage as described by Hills (1933), with slight modifications as used by Douglass et al. (1939). This cage consisted of two cylinderical metal bands of 3/16 by 3/4 inch steel, with an inside diameter of 13.56 inches to give an area of one square foot. The two bands were held 30 inches apart by welding to six metal uprights. Small holes 1½ inches apart in the bottom band permitted sewing cloth to the metal, while with the top band the cloth was folded over the top and sewed just beneath the band. A handle was welded to the side to facilitate manipulation of the cage.

In operating the cage, it was carried well above the vegetation to prevent entrance of any insects. The operator walked along briskly and then suddenly set the cage down at arm's length. In case the ground

was uneven the cage could be forced down by grasping it by the top and giving it a twist, thus sealing off the bottom. Grasshoppers were collected and counted as they jumped onto the side of the cage and started crawling toward the top. Some would occasionally drop to the ground or remain in the foliage where they could soon be located and captured. In early work a test tube with a small amount of cotton in the bottom saturated with carbon tetrachloride was used to collect the specimens. In later work a short-necked flask with the same killing agent was used which speeded up the collecting considerably. On placing the neck of the flask over the grasshopper the insect would almost invariably jump and land back in the flask. In case it did not jump, the operator could tap the cloth from the outside of the cage and force it into the flask. The killing action of carbon tetrachloride was so rapid that only one or two jumps were made, hence escape was virtually impossible. Counts of 10 to 20 nymphs per cage could be made very rapidly and accurately using the above technique. Autotomy, particularly of the hind legs, was common with carbon tetrachloride as the killing agent.

A square, wooden framed cage with an inside area of 3 square feet was built for sampling relatively low adult populations.

RESULTS

A comparison of visual estimates with cage sample methods:

Visual estimates were made by two workers in the same portion of the alfalfa field on July 14, 1947. Each worker estimated and recorded his results independently so there would be no chance of his being influenced by the other worker. In both cases the estimates were 6 grasshopper nymphs per square yard. Immediately following this, 144 samples covering the same portion of the field were taken with the square foot cage. The tabulation of these counts is given in table I. The cage counts gave a mean of 3.0902 grasshoppers per square foot with a standard error of .2085. The visual estimate if reduced to a square foot basis gave only .67 grasshoppers per square foot, or only 21.7 per cent of the cage sample.

In a similar experiment conducted July 16, 1947 the visual estimates were 5 and 7 per square yard, giving the same average as in the first experiment. The mean for the cage method was 3.2951±.0508 per square foot based on 288 samples taken by two operators. Cage counts from this work are given in table II. The resulting difference in estimating populations using the two methods of sampling remained nearly constant; the visual estimate gave only 20.4 per cent of the population obtained by the use of the cage method as compared to 21.7 in the previous experiment. A big difference was apparent between the cage counts and visual estimates.

A comparison of two operators using the cage method:

The data of the July 16, 1947 experiment, given in table II, was taken by two operators working simultaneously over the same area.

TABLE I

Grasshopper nymphs collected by the square foot cage method on July 14, 1947

Number of grasshoppers per sample							
5	4	0	5	8	4	1	5
3	0	1	1	4	3	3	4
1	3	1	0	4	1	2	1
4	3	1	1	3	2	0	2
5	2	5	2	2	2	4	2
1	0	1	1	11	2	1	1
11	1	2	2	4	1	4	4
3	6	3	3	6	6	4	1
7	0	2	4	3	1	0	1
4	3	2	0	8	4	16	1
7	5	2	4	1	4	1	4
2	6	1	0	4	4	1	1
2	1	1	4	7	6	3	1
2	4	8	5	1	3	6	4
8	1	3	1	4	0	5	4
3	5	1	3	6	4	0	0
2	1	5	1	4	3	6	1
3	2	8	4	7	1	2	3

Total samples 144

Total nymphs 445

Mean number of nymphs 3.0902

TABLE II

Grasshopper nymphs collected by the square foot cage method on July 16, 1947, by two collectors

Collector A															
Number of grasshoppers per sample															
0	0	0	0	2	13	1	4	3	3	2	1	2	4	1	3
8	4	3	0	4	3	2	0	1	2	2	1	2	5	10	4
4	0	3	0	8	2	7	4	2	2	1	0	8	4	5	2
2	0	1	1	7	0	6	3	3	1	2	0	12	2	0	7
7	1	0	1	10	2	1	0	4	2	7	6	5	5	3	3
2	3	2	3	0	1	2	1	2	2	0	0	2	6	4	2
4	1	1	10	7	4	1	1	1	3	4	2	3	1	5	4
5	4	4	2	5	1	4	3	6	3	9	4	4	7	8	4
1	3	3	3	5	5	5	5	1	7	1	2	1	3	2	0

Total samples 144
Total nymphs 450
Mean number of nymphs 3.1250

Collector B															
Number of grasshoppers per sample															
3	5	2	2	5	7	1	1	2	2	0	2	8	0	1	2
0	2	0	0	4	7	3	5	2	0	0	5	3	8	5	1
3	0	2	1	10	3	5	3	2	1	4	1	9	1	4	1
1	2	4	2	2	2	6	4	0	1	3	2	6	3	5	0
4	2	2	1	1	2	5	10	3	13	17	3	0	5	5	1
4	0	6	2	2	11	3	3	1	7	12	2	1	3	7	3
3	2	3	6	3	2	6	0	6	6	11	2	2	1	0	3
3	1	2	4	1	7	3	2	10	7	10	1	1	6	2	3
6	2	2	4	2	6	2	2	6	1	4	0	10	7	2	2

Total samples 144
Total nymphs 499
Mean number of nymphs 3.4653

This permitted a comparison of the catch under identical conditions.

The results of this work showed means of 3.125 ± .2190 and 3.465± .2536. In testing for significance the "t"test was used(Snedecor 1940). In this experiment t = .340/.3350 = 1.01, hence the difference was not significant.

A comparison of the catch at different times of the day:

On July 24, 1947 three sets of samples were made during the day. Climatic conditions and counts are given in table III. The results of this work showed an average of 4.5667, 4.4167 and 4.7000 for the morning, mid-day and evening collections. The analysis of variance is given in table IV.

There was no significant difference between the three collecting periods. The relatively high mean square for error indicates a heterogeneous, rather than a homogeneous population. This was the same condition as found by Gray and Treloar (1933) and Beall (1935) working with other insects.

A similar experiment was undertaken on July 31, 1947. However only two samplings were made as a light rain prevented taking the evening collections. Data on these samplings are given in table V.

An analysis of variance for this work gave a mean square of 1.21 for time of day and 11.17 for error. As in the previous experiment there was no significant difference between sampling periods. The high mean square for error further substantiates the theory of the heterogeneous character of the population.

A comparison of the catches of adult grasshoppers with the square foot sampler at different times of day:

TABLE III

Grasshopper nymphs per square foot taken by the cage method at three different times of the day on July 24, 1947

Time	6:30-8:30 A.M.			12:30-2:30 P.M.			5:45-7:45 P.M.		
Temperature	68-82°F.			95-95°F.			90-80°F.		
Wind	0-1 mph			0-1 mph			1-3 mph		
Atmosphere	clear			clear			mostly cloudy		
	Number of grasshoppers per sample								
	1	0	3	3	1	6	3	3	3
	1	6	1	3	6	2	8	8	0
	8	2	10	6	2	6	3	6	2
	6	9	0	3	2	8	4	3	7
	3	1	2	3	3	5	1	4	5
	5	5	5	2	2	6	14	0	7
	4	0	1	5	1	11	1	1	19
	0	6	0	3	2	7	8	3	3
	19	0	27	4	1	6	4	2	9
	2	4	0	5	2	6	2	1	8
	13	11	1	1	3	22	9	2	12
	2	2	10	5	1	7	3	0	0
	12	1	6	2	7	9	1	0	1
	0	7	1	2	2	17	5	3	15
	7	1	3	2	3	5	5	3	0
	1	4	0	4	1	2	6	3	6
	4	4	8	5	3	2	0	0	6
	3	7	2	11	3	3	2	3	11
	0	2	9	1	3	7	9	6	3
	4	15	3	3	6	1	1	23	2
Total samples	60			60			60		
Total nymphs	274			265			282		
Mean	4.5667			4.4167			4.7000		

TABLE IV

Analysis of variance of the data recorded in table III

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	179	3,666.3278	
Time of day	2	2.4111	1.2055
Error	177	3,663.2167	20.6961

TABLE V

Grasshopper nymphs per square foot taken by the cage method at two different times of the day on July 31, 1947

Time	6:45-8:30 A.M.				12:45-2:30 P.M.			
Temperature	71°-85°F.				101°-93°F.			
Wind	5-10 mph				1-3 mph			
Atmosphere	light scattered clouds				high clouds			
	4	6	3	0	3	4	0	1
	5	3	1	0	6	1	4	0
	5	1	10	1	0	3	0	2
	1	0	0	1	1	3	3	0
	7	3	3	3	11	11	3	2
	2	1	3	4	11	0	7	1
	3	3	7	6	2	1	0	5
	1	0	7	1	6	0	3	6
	12	3	10	5	1	1	4	9
	2	10	7	13	4	2	6	12
	0	0	2		0	3	4	
	0	0	0		5	4	2	
	10	2	1		7	3	1	
	1	4	0		4	4	5	
	1	4	2		10	2	4	
	7	11	8		1	1	1	
	7	4	1		5	1	7	
	2	11	0		9	2	6	
	0	1	0		1	0	1	
	10	1	2		2	1	1	
Total samples	70				70			
Total nymphs	249				236			
Mean	3.5571				3.3714			

On August 25, 1947 from 1:15 P.M. to 3:25 P.M., 100 square foot samples were taken of the adult population in idle land. The temperature was from 90°F. to 89°F. For comparison, an equal number of samples were taken from 7:10 P.M. to 9:35 P.M. with a temperature of 76°F. to 70°F. It was fairly dark by the time the second collections were started and all samples had to be made with the aid of a flashlight. The counts from this work are given in table VI.

The afternoon collections gave a mean of 2.44± .1251 and the night collections 3.55± .1777. There was a highly significant difference between these counts.

Another set of collections was made on September 9, 1947, but in this instance the collections were made in the morning and afternoon. Data on this experiment are given in table VII. In this case the morning collection had a mean of 1.49± .0978 as compared to 1.47± .0963 for the afternoon. There was no significant difference between the collections.

A comparison of adult grasshopper populations determined by cage and PCH "knock-down" methods:

This work was conducted October 2 and 3, 1947. Due to a rather low population, a wooden framed cage with an inside area of three square feet was constructed to increase the catch per sample. This cage was 30 inches high, and was covered with sheeting the same as the round metal sampler.

The first sampling was done on wheat stubble on October 2, 1947. Five separate areas of the field were sampled, four near the four

TABLE VI

Adult grasshoppers per square foot taken by the cage method at two different times of the day on August 25, 1947

Time	1:15-3:25 P.M.				7:10-9:35 P.M.			
Temperature	90°-89°F.				76°-70°F.			
	number of grasshoppers per sample							
	2	1	2	1	0	2	1	3
	2	1	4	1	0	1	0	1
	0	3	2	0	3	3	2	1
	1	0	7	2	0	2	9	1
	3	0	10	0	0	3	3	0
	1	7	3	0	0	1	8	0
	3	0	1	2	3	21	4	0
	5	11	4	0	3	20	0	1
	0	1	5	0	2	2	3	1
	1	1	4	0	1	36	0	0
	1	4	0	0	1	4	1	1
	1	10	3	0	2	4	1	2
	0	4	1	1	0	6	0	4
	1	3	3	0	3	1	0	0
	0	2	0	3	2	5	1	4
	3	8	0	4	0	26	6	2
	1	17	3	2	0	33	4	1
	3	15	2	4	1	3	0	2
	1	8	1	3	0	17	3	3
	1	7	0	1	1	5	1	1
	1	2	2	4	12	1	1	0
	2	0	3	5	12	9	0	1
	2	0	2	1	5	7	1	0
	0	3	2	0	2	4	2	0
	1	1	0	1	6	0	0	0
Total samples	100				100			
Total adults	244				355			
Mean	2.44				3.55			

TABLE VII

Adult grasshoppers per square foot taken by the cage method at two different times of the day on September 9, 1947

Time	8:35-10:30 A.M.				3:00-4:45 P.M.			
Temperature	67°-72°F.				78°-75°F.			
Wind	3-5 mph				5-8 mph			
Atmosphere	cloudy				clear			
	number of grasshoppers per sample							
	1	4	4	3	2	2	1	0
	0	2	0	3	0	2	0	1
	0	0	2	7	1	1	4	1
	1	0	0	3	3	1	1	2
	1	3	0	3	2	1	0	3
	1	2	3	2	1	3	3	0
	1	2	2	2	0	3	6	2
	0	1	1	1	0	5	2	0
	0	1	2	1	0	5	1	1
	4	3	2	1	1	3	2	1
	0	0	1	1	1	2	1	2
	1	1	0	0	0	2	1	4
	4	2	0	0	1	1	1	1
	1	0	2	1	1	0	4	5
	0	2	3	1	2	0	1	0
	0	2	0	2	0	3	0	0
	0	3	5	1	2	2	2	0
	2	1	1	2	1	1	2	1
	3	0	0	2	0	0	0	1
	0	1	3	0	0	4	2	0
	0	2	6	2	0	3	0	4
	2	2	0	5	3	3	0	3
	2	2	3	0	1	0	1	0
	0	0	0	0	1	1	1	1
	1	1	3	1	4	0	3	0
Total samples	100				100			
Total adults	149				147			
Mean	1.49				1.47			

corners, and the fifth in the center. Twenty samples were taken in each area. Counts of the live grasshoppers were made on each area by the cage sampling method. These areas were then dusted and another series of counts taken. Data obtained from this experiment are given in table VIII. There was a mean of .49 grasshoppers per sample before dusting and .59 from the samples taken after dusting. The analysis of variance showed no significant difference between sampling methods or areas within the field.

A similar experiment was conducted on the idle land where there was a somewhat higher population. In this experiment the mean catch of live grasshoppers by the cage sampling method was 1.17 as compared to .99 for samples from the dusted areas. In this as in the previous experiment there was no significant difference between sampling methods or areas in the field. Data from this experiment are given in table IX.

A comparison of visual estimates of adult grasshopper populations by two observers:

Several estimates of adult grasshopper populations were made on plots in an alfalfa field by two observers. By making these estimates simultaneously a comparison was permitted between the results obtained by the two observers. Results of this work are given in table X.

It may be noted that on August 5, 1947 observer B recorded counts higher than observer A in all but 5 cases, and in 4 of these the counts were the same. Using Miles (1935) method of testing, there was a highly significant difference between the observers, and odds of 15.9 to 1 that the difference would be two or more grasshoppers per square yard.

TABLE VIII

Counts of adult grasshoppers in grain stubble per 3 square feet taken by the cage method alive and after "knock-down" treatment with PCH dust on October 2, 1947

Method of collecting	alive					PCH "knock-down"				
Area of field	NE	NW	SW	SE	Center	NE	NW	SW	SE	Center
	number of grasshoppers per sample									
	0	2	1	4	0	0	0	1	2	1
	0	0	0	2	0	0	0	2	1	0
	1	0	1	0	0	2	2	0	2	0
	0	2	2	0	0	0	1	1	0	2
	1	0	0	0	1	1	0	0	1	0
	0	1	0	1	1	0	0	0	0	2
	0	0	0	0	0	0	1	2	0	0
	0	2	2	0	0	0	0	1	2	0
	1	0	2	0	0	0	0	1	1	1
	0	0	1	0	0	2	1	0	0	1
	2	0	0	0	1	0	0	1	0	0
	0	1	1	0	0	0	0	0	0	0
	0	0	1	0	0	0	0	1	0	0
	0	0	0	0	1	0	1	1	0	0
	0	1	2	0	0	0	0	0	0	2
	0	1	0	0	0	0	0	1	3	1
	0	1	1	1	0	0	2	0	1	2
	1	0	0	1	0	0	0	0	2	1
	0	1	1	1	2	0	0	0	1	1
	0	0	0	0	0	2	1	1	0	0
Area totals	6	12	15	10	6	7	9	13	16	14
Method totals			49					59		

TABLE IX

Counts of adult grasshoppers in idle land per 3 square feet taken by the cage method alive and after "knock-down" treatment with PCH dust on October 3, 1947

Method of collecting	alive					PCH "knock-down"				
Area of field	SE	NE	Center	NW	SW	SE	NE	Center	NW	SW
	number of grasshoppers per sample									
	2	2	1	1	0	1	1	2	1	1
	3	1	2	1	0	1	0	1	1	1
	0	2	2	0	2	1	0	1	0	0
	1	0	1	0	0	1	3	2	3	0
	1	1	1	0	2	2	0	2	0	0
	0	1	1	1	0	0	1	1	0	1
	0	0	1	2	0	1	0	0	0	2
	4	0	1	1	1	0	0	1	0	0
	2	0	2	1	3	1	0	1	2	0
	1	3	1	1	0	0	1	0	1	2
	2	0	0	2	0	2	0	1	2	2
	1	2	0	2	5	2	1	2	2	0
	2	0	0	2	0	4	1	2	0	0
	1	1	2	2	1	2	0	0	3	2
	0	2	1	3	2	0	1	1	1	1
	0	3	0	0	0	1	1	2	0	0
	1	0	3	3	1	4	3	1	3	0
	0	2	3	0	3	1	1	2	0	1
	1	3	0	0	2	1	0	1	0	1
	2	0	1	3	0	1	1	1	0	1
Area totals	24	23	23	25	22	26	15	24	19	15
Method totals			117					99		

TABLE X

Visual estimates made by two observers of adult grasshopper populations on plots in an alfalfa field

Plot number	Estimated number of grasshoppers per square yard by observers A and B on various dates							
	August 5		August 19		August 22		August 25	
	A	B	A	B	A	B	A	B
1C	12	18	20	16	10	10	5	5
2C	10	16	25	18	12	12	6	4
3C	10	16	10	7	3	5	4	1
4C	15	25	10	5	3	5	4	4
5C	12	18	7	5	3	4	3	4
6C	7	10	5	4	3	3	2	1
7C	8	8	5	3	2	4	3	3
8C	8	8	8	7	3	3	2	3
9C	11	15	12	9	3	5	4	4
10C	4	3	10	5	2	3	2	1
Total of C plots	97	137	112	79	44	54	35	30
1S	2	6	20	18	12	12	2	2
2S	3	5	20	18	12	12	1	0.5
3S	5	8	15	8	5	5	0.5	0.5
4S	3	4	8	8	2	4	0.5	0.2
5S	2	3	7	4	2	3	0.5	0.25
6S	4	5	7	5	3	4	0.5	0.2
7S	4	5	6	5	2	3	1	0.5
8S	4	4	10	8	4	5	1	1
9S	5	7	10	8	3	5	0.5	0.67
10S	2	2	8	6	1	3	0.5	0.25
Total of S plots	34	49	111	88	46	56	8.0	6.07
Grand total	131	186	223	167	90	110	43	36.07

However on August 19, 1947 the reverse situation occurred. Observer A was higher than observer B on all counts except one, and in this instance it was the same. By the same test there was a highly significant difference between observers, and odds of 451 to 1 that the difference would be two or more grasshoppers per square yard. On August 22, 1947 observer B exceeded observer A in 13 of the 20 plots, and on the remaining 7 they were even. On August 25, 1947 they were again even on 7 of the plots while observer A was ahead on 10 plots and observer B ahead on only 3 plots.

Sweep net and cage collections for determining percentages of various instars of grasshoppers:

As sweep net collections are used for determining the development of grasshopper nymphs during the spring and early summer, several comparable collections were made with the sweep net and cage to determine if any differences in the percentages of the various instars resulted from the use of the two methods. Data collected for this study are given numerically in table XI, and converted to percentages in table XII.

The work was not designed to test the total catch of one method with another but was to compare the percentages of the different instars obtained by use of the two different methods. Thus samples were taken that were deemed adequate to give reliable percentage figures. In all cases over 200 specimens were collected. The method for testing these differences was that given by Hoel (1947). The formula used was $\sigma p_1 - p_2 = \sqrt{\frac{p_1 q_1}{n_1} + \frac{p_2 q_2}{n_2}}$, and the test for significance was $p_1 - p_2 \pm 2(\sigma p_1 - p_2)$, where p_1 and p_2 were the percentages, q_1 and q_2 the remainder of the percentages, and n_1 and n_2 the number of trials. An example of the use of this method

TABLE XI

Collections of nymphs by sweep net and cage methods for determining the numbers of the various instars

Date and time	Method	Number collected in the different instars and adult stage						
		1st	2nd	3rd	4th	5th	adult	Total
July 14	Cage	96	153	138	36	14	0	437
	Sweep	40	137	224	50	21	1	473
July 16	Cage	58	121	151	75	15	2	422
	Sweep	35	90	184	68	18	1	396
July 24, 6:30-8:30 A.M.	Cage	1	25	148	56	36	4	270
	Sweep	5	73	165	71	23	3	340
July 24, 12:30-2:30 P.M.	Cage	6	34	94	73	42	6	255
	Sweep	0	37	149	94	32	8	320
July 24, 5:45-7:45 P.M.	Cage	6	39	118	69	41	4	277
	Sweep	5	20	127	98	49	6	305
July 31, 6:45-8:30 A.M.	Cage	0	2	32	99	90	34	257
	Sweep	0	2	47	94	124	60	327
July 31, 12:45-2:30 P.M.	Cage	0	3	27	70	78	42	220
	Sweep	0	0	32	101	106	69	308

TABLE XII

Percentages of the various instars of grasshoppers from the data of table XI

Date and time	Method	Percentages of grasshoppers in the different stages					
		1st	2nd	3rd	4th	5th	adult
July 14	Cage	22.0	35.0	31.6	8.2	3.2	0
	Sweep	8.5	29.0	47.3	10.6	4.4	0.2
July 16	Cage	13.7	28.7	35.8	17.8	3.5	0.5
	Sweep	8.8	22.7	46.5	17.1	4.6	0.3
July 24, 6:30-8:30 A.M.	Cage	.4	9.3	54.8	20.7	13.3	1.5
	Sweep	1.5	21.5	48.5	20.9	6.7	.9
July 24, 12:30-2:30 P.M.	Cage	2.3	13.3	36.9	28.6	16.5	2.4
	Sweep	0	11.5	46.6	29.4	10.0	2.5
July 24, 5:45-7:45 P.M.	Cage	2.2	14.1	42.6	24.9	14.8	1.4
	Sweep	1.6	6.6	41.6	32.1	16.1	2.0
July 31, 6:45-8:30 A.M.	Cage	0	.8	12.5	38.5	35.0	13.2
	Sweep	0	.6	14.4	28.8	37.9	18.3
July 31, 12:45-2:30 P.M.	Cage	0	1.4	12.3	31.8	35.4	19.1
	Sweep	0	0	10.4	32.8	34.4	22.4

is taken from the data of July 24, 1947 (table XII). In the morning collections there was 1.5 per cent in the first instar by the cage method, and 0.4 per cent by the sweep net method. Hence $p_1 - p_2 = 1.5 - 0.4$ or 1.1, and $\sigma_{p_1 - p_2} = \sqrt{\frac{(1.5)(98.5)}{270} + \frac{(0.4)(99.6)}{340}} = \sqrt{.0000581} = .0076$. Then $2(\sigma_{p_1 - p_2}) = 2(.0076) = .0132$, and the difference of 1.1 per cent converted to hundredths is .011 which is less than the .0132 required for significance. The difference between percentages by the two methods was not significant. Similar calculations were made for the other instars and are as follows:

Instar	Difference in per cent	$2(\sigma_{p_1 - p_2})$
2nd	12.2	.0568
3rd	6.3	.0812
4th	.2	.0662
5th	6.6	.0494
adult	.6	.0180

This indicates significant differences between the methods for the second and fifth instars. However an examination of table XII shows that there was just as great a difference between the different times of day by the same method as there was between the two methods. Such differences could be due to a selective type of sampling under different climatic conditions, but it seems more logical to assume that the samples were too small to give reliable results.

DISCUSSION AND CONCLUSIONS

As mentioned earlier, the two methods of sampling which were expected to give accurate estimates of the population were not satisfactory. These methods were the use of an insecticidal dust to give a rapid and complete "knock-down" of all the grasshopper nymphs followed by counts of the population on the ground, and the use of cages placed over the vegetation at night thus enclosing all insects so that the numbers present could be recorded. Without one or both of these methods as a measure of the actual population present, the data derived from cage sampling of live nymphs is subject to criticism as to its accuracy. However, certain features of the work, particularly under the conditions encountered, tend to verify the accuracy of the cage method. For instance, the fact that a heterogeneous population was recorded while sampling in rows of alfalfa indicates that the method was securing accurate samples. In other words, the grasshoppers were concentrated on the alfalfa and the sampling was accurate enough to detect this. Such a method might well be used for sampling micro-habitats for the determination of populations in special ecological studies such as exposure or host plant preference.

The fact that collections from a given habitat, at various times of the day showed no significant difference in numbers is a further verification of the accuracy of the method. Even if the method is not absolutely accurate as to the total number present, its accuracy is not altered by climatic conditions; hence it could be used in following population trends in a habitat or comparing populations in similar habitats.

Counts taken at night by the cage method would seem to offer the

best means of getting an accurate count of the adult population present. Unfortunately this was not thought of during the time sampling of nymphs was being conducted. With adults there was little or no disturbance of the population when the operator was walking about thru the field. Samples could be taken on any type of micro-habitat. The fact that the sampling of adult populations which was conducted at night showed a highly significant difference from the daytime sampling does not exclude the use of the cage as an accurate method for determining the grasshopper populations. Night sampling by the cage method has several advantages over other methods. It requires only one cage, thus reducing equipment to a minimum. Fumigation is not necessary, in fact is undesirable, as some activity on the part of the insects makes them easier to find. A stimulant such as a light application of pyrethrum dust might be desirable in finishing off each sample to be sure all grasshoppers are counted. By using the killing flask as an aid in counting, the speed and accuracy of the cage method are greatly improved.

The use of PCH dust to stupify or kill adult grasshoppers so they could be counted on the ground gave results similar to those obtained be counting live adult grasshoppers by the cage method. However, this work was done rather late in the season when temperatures were around 70 F. and the grasshoppers were not as active as during warmer periods earlier in the season. Whether or not the daytime application of the cage method would be accurate for determining adult populations under all conditions needs further study. The fact that uniform results were obtained at different times of the day on September 9, 1947 would indicat accurate

sampling, but on this date the temperature was in the seventies so that activity was again restricted.

Limitations of both the cage and dusting method should be pointed out. The cage is not satisfactory for vegetation over 24 to 30 inches high. Thirty inches is the maximum height of a cage that will allow the operator to reach to the ground and pick up any specimens that fail to jump onto the side. A cage with a top has been used by beet leafhopper workers, and such a method might be applicable for grasshopper sampling in higher vegetation without increasing the height of the cage, but this was not tried in these experimants.

A certain amount of speed is required in the manipulation of the cage. However, no more is required than for any other type of sampling of an active insect population. By comparing samples taken by any operator at different times of the day it should soon be possible to determine if an accurate sample is being obtained.

A restriction in the use of the dust method has already been pointed out, namely that small nymphs cannot be detected due to their faculty of crawling under debris or falling into cracks in the soil. The use of a "knock-down" dust for sampling adult populations in high vegetation would seem a legitimate practice for obtaining samples of such a population. The work is simplified if the samples are taken while there is still some movement of the grasshoppers as even the adults are difficult to find if they are motionless, when there is litter on the ground.

The data gathered on percentages of the various instars collected by the sweep net and the cage methods were too meager to draw any

conclusions on the relative value of the two methods. However the work did show the present method of collecting about 100 or even 200 specimens, was not a sufficient sample for statistically sound comparative work.

In sampling work there often arises the question of the number of samples required for certain levels of accuracy. Numerous methods are available for determining this number. The one used in this study is that given by Dr. F. M. Wadley (U.S.D.A. statistician) in correspondence with the Bozeman laboratory, which is as follows: $n=\left(\frac{\sigma}{Sx}\right)^2$, where n number of samples; σ standard deviation; and Sx standard error. This is simply a transposition of the formula $Sx=\frac{\sigma}{\sqrt{n}}$. Thus, by taking a few samples and determining the standard deviation, the number of samples necessary to secure any specified accuracy may be obtained. The above statistician recommends using one half the standard error for greater accuracy. This is equivalent to saying that with twice the standard error, 95 per cent of the samples will fall within the desired range of means. Waugh (1943) uses the same formula, but in a somewhat different manner, for checking the standard error after the number of samples has been secured by another method. He uses one third the standard error for odds of 1000 to 1; in other words, with 3 times the standard error the odds are 1000 to 1 that similar sample means will fall within this range.

In the present analysis the desired standard error was used rather than one half or one third of the value. Data were taken from the July 24, 1947 sampling given in table III. Starting with the first 10 samples,

a mean of 4.9 was obtained with a standard deviation of 5.5467 and standard error of 1.7540. The standard error was 36 per cent of the mean. If a standard error of 25 per cent of the mean is desired, take 25 per cent of 4.9, which equals 1.225, and substitute it in the formula $n = \left(\frac{\sigma}{Sx}\right)^2$ Hence $n = \left(\frac{5.5467}{1.225}\right)^2 = 20.5$. The next 10 samples were added to the first which gave a mean of 4.75± 1.1165. The standard error was 23.5 per cent of the mean which was less than the amount desired. Similar computations were made using the standard error as 20, 15, and 10 per cent of the mean. These figures were derived by using a new mean and standard deviation by adding the required number of samples to the last calculation. Similar calculations were made using the original 10 samples and figuring each percentage from it. The number of samples required by the two different methods as well as the numbers used and results obtained are as follows;

Sx/mean desired	n by continuous calculations	n from original 10 samples	n used	Sx/mean obtained
.25	20.5	20.5	20	.235
.20	27.6	32.0	30	.188
.15	46.8	56.9	50	.159
.10	133.0	128.1	130	.086

It may be noted that the number of samples obtained by the two methods was fairly close. Also the ratio of Sx/mean was below that desired in 3 out of the 4 cases. This is about what one would expect if the data is from a uniform source. Of course if one desires odds of 20 to 1 that the standard error will fall at or below the desired level then one half the figure used in these calculations should be employed.

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