II.2 Evaluation of Rangeland Grasshopper Controls: A General Protocol for Efficacy Studies of Insecticides Applied From the Air

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NOTE: Acephate is no longer approved by EPA for rangeland grasshopper control.

Introduction

Many chemical compounds are registered for use against grasshoppers, but only a few are used in the large-scale cooperative private–State–Federal rangeland grasshopper management programs directed by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (USDA/APHIS). APHIS chooses and approves compounds based on (1) effective performance against grasshoppers on rangeland, and (2) minimal or negligible impact on the environment and nontarget species. On rangeland, APHIS normally uses these compounds at the lowest active ingredient (AI) level listed on the label.

To be approved for use by APHIS, chemical insecticides must be evaluated for effectiveness, or efficacy. Efficacy testing determines the levels of performance for a specific compound formulation at different doses of active ingredient and in different application volumes of diluent (a diluting liquid or solid) per unit of surface area. Candidate treatments may be newly developed compounds, new formulations of currently used compounds, or registered compounds proposed for rangeland use for the first time. Based on 15 years of development, the following describes the protocol (procedure) used to evaluate candidate treatments for use on rangeland grasshoppers in APHIS-managed programs.

Geographic Location

The first step in an efficacy test is selecting a location for the study. The test is only as good as the location where it is conducted. The location should be typical of areas commonly treated in cooperative large-scale management programs. Also, the location should have a typical population mix of rangeland grasshoppers or a majority of species commonly considered as potentially damaging to rangeland. Average population levels should be at least 10–25 grasshoppers/yd^2. Lower populations may limit the level and type of statistical analysis performed on the data.

Test locations commonly are selected from areas experiencing a significant outbreak of grasshoppers and near where control programs are planned. These locations have two major advantages. First, such locations allow researchers to experience firsthand some of the local problems that may exist in controlling grasshoppers. Second, the proximity to a major control program activity allows a control program manager a firsthand view of the potential tool.

While there are distinct advantages in locating research and program activities near each other, doing so may cause problems. First, the large-scale program and the researcher may be competing for the same infested land. The program manager is interested in improving the control plot by simplifying boundaries or protecting its integrity from migration of grasshoppers from untreated plots in the research design. The researcher looks for desirable population and topographic features typical of a program. For the private party, a cost share will be required if the land is included in the control program, but charges are generally not assessed for land used in research. Close communication with the program manager is the only solution to these potential conflicts.

Sometimes, the test area may be located adjacent to the program area. In such cases, researchers must take extra precautions to ensure that no contamination from the control block will compromise the integrity of the test area. In many cases, it is easier to choose a test area separated from a nearby control block. With appropriate approval, both public and private lands can be used. Permission to use public lands usually requires additional procedures compared to private lands. Because of the brief period of time between locating a test area and beginning the test (occasionally as few as 3–4 days), researchers most often choose private land with approval of landowners, lessees, or others who may be involved. Tests on rangeland usually require the use of trail bikes and the temporary positioning of other equipment. Researchers discuss use of these items with and get approval from the landowner as one of the first steps in site selection.

Once general permission for use of the land is obtained, a preliminary survey on the parcel of land proposed for the test is conducted. The preliminary survey generally consists of conducting population estimates every $^1/_4$ mi and a cursory examination of terrain and vegetation types. This survey ensures adequate uniformity in the general vegetation types and grasshopper population levels for the study proposed. The absence of livestock during the study period is not a requirement but simplifies counting and eliminates the need to build temporary fences for protection of any required specialized equipment.

Close proximity of the test area to a landing strip or airport is extremely important. Many experiments require several changes in equipment and formulations, and since only 1–2 hours of application time may be available each day, ferrying distances should be kept to a minimum. Lodging close to the test area also is worth consideration. Daily travel will be needed during setup and application and usually for 2–4 weeks after the final application.

Types and Sizes of Experiments

Several general types and sizes of experiments take place when APHIS evaluates a candidate treatment for potential program use. The evaluation usually begins with replicated (repeated) small rangeland plots and eventually progresses to larger blocks. Each type of experiment is important in producing a complete evaluation and recommendation that both industry and the user communities will accept. Later, for treatments used in cooperative programs, APHIS evaluates each program to document the performance of the compound and the success of the program in which it was used.

Small-Scale Replicated Plot Studies.—After a compound has shown a potential for producing mortality to the target pest either in the laboratory or on small (less than 10 acres) field plots, the evaluation process graduates to replicated field plots of substantial size. At this stage in the development of a treatment, testing for the first time incorporates the aerial application aspect. APHIS typically designs the experiments to determine the (1) lowest effective dose of active ingredient, (2) minimum volume of application, and (3) optimal type of diluent (water, oil, or solid bait carrier). These experiments also serve to determine if proposed formulations are compatible with existing commercial aerial application equipment. Experiments also may be designed to determine the optimal nozzle type and size to be used with the final formulation.

Plots are typically square and 40 acres in size ($^1/_4$ mi by $^1/_4$ mi). This size allows for a buffer zone on all sides of the centrally located evaluation site. The buffer area protects the evaluation site from grasshoppers that have been exposed to different treatments and may migrate from adjacent plots. Additionally, buffer areas ensure that any drift contamination near the edges of plots will not jeopardize the integrity of the evaluation site. In studies of aerially applied insecticide on rangeland, smaller plots are simply inadequate for evaluating treatment impact on grasshopper populations. Plots larger than 40 acres may be used. Larger plots increase the protection of the evaluation area but rapidly use up valuable rangeland test acreage. In small-scale studies, using four replications of each aerially applied treatment is typical and is considered minimal.

An example of a typical small-scale study follows. Grasshopper mortalities resulting from three dosages of a candidate formulation at a fixed volume of application are compared with each other. Mortalities are also compared to those produced by a treatment currently used for controlling grasshoppers, called a standard. Mortalities resulting from all four treatments are compared with mortalities in untreated plots. These untreated plots will show the mortality rate that naturally occurs during the experiment. In this experiment, there are five different kinds of plots called "treatments" with each replicated four times. The entire experiment takes 20 plots and uses 800 acres. The untreated control plots are the most important in the experiment. All other treatments are judged against the controls. Control plots are part of the experimental design and must be included in the process of assigning treatments to specific plots. Other actual examples can be seen in Foster et al. (1983 unpubl.) and Jech et al. (1993).

Because densities of grasshoppers may vary considerably over the study area, it is important to ensure that any one treatment is not assigned exclusively to high or low grasshopper population levels. In small-scale experiments, the population-level values of the plots are typically arranged in descending order of density. In the case of the above example, each of the five treatments are randomly assigned to plots within the top five densities, five treatments to the next five densities, and so on until the desired number of replications have been performed. This ensures that all treatments are tested against similar population densities. Typically, one or more treatments of those tested in small replicated plot studies will be suitable for large-scale testing. (See table II.2–1 and fig. II.2–1.)

Large-Scale Simulated Program Studies.—After successful small-scale testing, the next step is to evaluate the candidate formulations under simulated program conditions. Doing this ensures that the level of performance seen in tightly controlled small-scale experiments can be expected when much larger acreages are treated. These tests challenge the formulation (1) under environmental and meteorological conditions expected during a program, and (2) for compatibility with commercial spraying equipment for extended periods of time. Successful performance in these studies may result in recommendations for program use.

In these experiments, application flights of at least 1 mi in length are desirable. Plot size typically ranges from 640 acres (a section) to 1,000 acres. With a plot of this size and a single aircraft such as an Ag Truck, researchers can use much or all of acceptable early morning application time in a single plot. The changing meteorological conditions that occur over this time period allow for assessment over the varying conditions that occur during a typical control program application day. Aircraft altitude (application height) in these studies will be similar to those APHIS uses during programs.

A typical large-scale study may consist of one or two different formulations of a candidate compound, a standard treatment, and an untreated control plot, each on a minimum of 640 acres. Because of the size of acreage involved in these tests, true statistical replication, in the general vicinity, is usually impossible. However, it is common to conduct the same test in other areas or States. Typically, the candidate and standard treatments, as well as the untreated control, are randomly assigned to one of several (in this case, three) adjoining plots. Before treatment, these plots are assessed to make sure they are suitable for the experiment. Unfortunately, in many cases, enough grasshopper-infested acreage is not available. In such cases, the untreated check sites are established outside of the treated plots and at a distance to ensure that there is no contamination from treatment.

A large-scale experiment usually relies on 9–10 evaluation sites per treatment plot. Without prior knowledge of plant communities, soil characteristics, or species composition of grasshoppers, the researchers determine the location of each evaluation site using topographic and county maps. These sites generally are distributed evenly over the entire plot (see fig. II.2–2). With this technique, each type of habitat is represented proportionately in the evaluation of each plot. An actual example can be found in Foster et al. (1993 unpubl.).

Efficacy Evaluation of Control Programs

Evaluation of performance continues even after treatments have been recommended for cooperative programs. APHIS evaluates each program to determine the performance of the treatment and to document the level of success of the program in which it was used.
Cooperative programs may vary greatly in size, from 10,000 acres to 100,000-plus acres, and may rely on several aircraft flying in formation for application. Evaluation of a program treatment is similar to that which occurs for program-simulated experiments. Evaluation sites are evenly distributed within the treatment area, while allowing for access by roads or trails. Sites are selected at 1 per 1,000 acres for the first 100,000 acres, and 1 per each 10,000 acres above 100,000 acres. Where programs are less than 10,000 acres, we recommend using a minimum of 10 treatment evaluation sites. We identify the evaluation sites before application. Evaluation of those sites is in addition to the more cursory visual mortality checks, commonly conducted on all cooperative control programs.

APHIS also establishes an equal number of untreated check sites that can be used for comparison in the evaluation. The untreated sites are mandatory. However, because a program goal is usually to treat all land infested with grasshoppers that cause damage at economic levels in a given area, untreated control sites within the treated block are not possible. Consequently, untreated control sites are situated outside, but near to, the boundary of the program block and surround the entire perimeter of the area tested.

Plot and Evaluation Site Setup

In both small- and large-scale simulated program studies, corner boundaries of all plots have flexible poles to which streamers of flagging tape are attached. We use two colors, usually orange and white, to increase visibility. Corners also are marked with a wooden stake labeled to identify the plots.

We mark evaluation sites with flexible poles and wooden stakes. In replicated small-plot studies, only a single color of tape is attached to the site markers to prevent confusion with corners. At each evaluation site, we use 0.1-m^2 aluminum rings (Onsager and Henry 1977) to delimit 40 areas for counting grasshoppers. Starting at the wooden stake, we arrange the rings about 5 yd apart in a large circle about 64 yd in diameter. Placement of individual rings is simply a random drop at the end of each 5-yd interval.

The circle arrangement provides for a curved transect of 200 yd which allows the sample counter to finish at the initial stake. Compared to techniques where counting areas are concentrated and uniform habitat is desired, this arrangement of sample rings allows for sampling a more diversified habitat. The circular arrangement also ensures that counting at all sites will be affected by wind and sun angles from all directions. Ring spacing of 5 yd between rings ensures that there is no disturbance to the next area to be counted during an ongoing count. In some programs, we may base pesticide effectiveness on estimates of grasshoppers in 18 visualized 1-ft^2 areas at evaluation sites rather than counts from rings. While not as accurate as counting from rings, the resulting data generally yield good estimates of the level of control achieved by the treatment.

Application

Calibration of the aircraft delivery system (spreader for baits and spraying systems for liquids) is the most important aspect of application. The accuracy of application in experiments and programs depends on repeatable precision obtained through the use of proven calibration procedures. Details of some of these procedures are in the chapters on “Calibration of Aerially Applied Sprays” (II.8) and “Equipment Modification, Swath Width Determination, and Calibration for Aerial Application of Bran Bait with Single-Engine Fixed-Wing Aircraft” (II.18) in this section of the User Handbook.

In small-scale replicated plot experiments, we consider the order of treatments. Similarly based formulations are grouped together in the sequence of application to minimize equipment cleanup and changeover time between treatments. We arrange the dosages tested in increasing or decreasing order depending on the complexity of mixing required for test formulations.

Conventional replication in an experiment requires all treatments to be applied once before repeating. Then all treatments are applied a second time before a third treatment is applied, and so forth. The arguments against this type of sequencing are numerous and usually win out to preserve time and money and to maintain a uniform grasshopper age structure against which the treatments are applied. Typically, we apply each treatment to all of its assigned plots before changing over equipment for the next formulation in the sequence of application.

Table II.2–1—Pretreatment grasshopper densities per square meter, arranged in descending order with randomly assigned treatments for each density group

Grasshopper density per m^2	Plot number	Assigned treatment
41	17	Treatment 2
41	16	Treatment 1
36	13	Treatment 3
36	1	Untreated
29	11	Standard
29	3	Treatment 1
25	18	Treatment 2
23	12	Treatment 3
22	6	Untreated
19	20	Standard
18	19	Treatment 1
18	2	Standard
14	7	Untreated
13	15	Treatment 3
13	4	Treatment 2
11	10	Untreated
9	5	Standard
9	9	Treatment 3
9	14	Treatment 2
6	8	Treatment 1

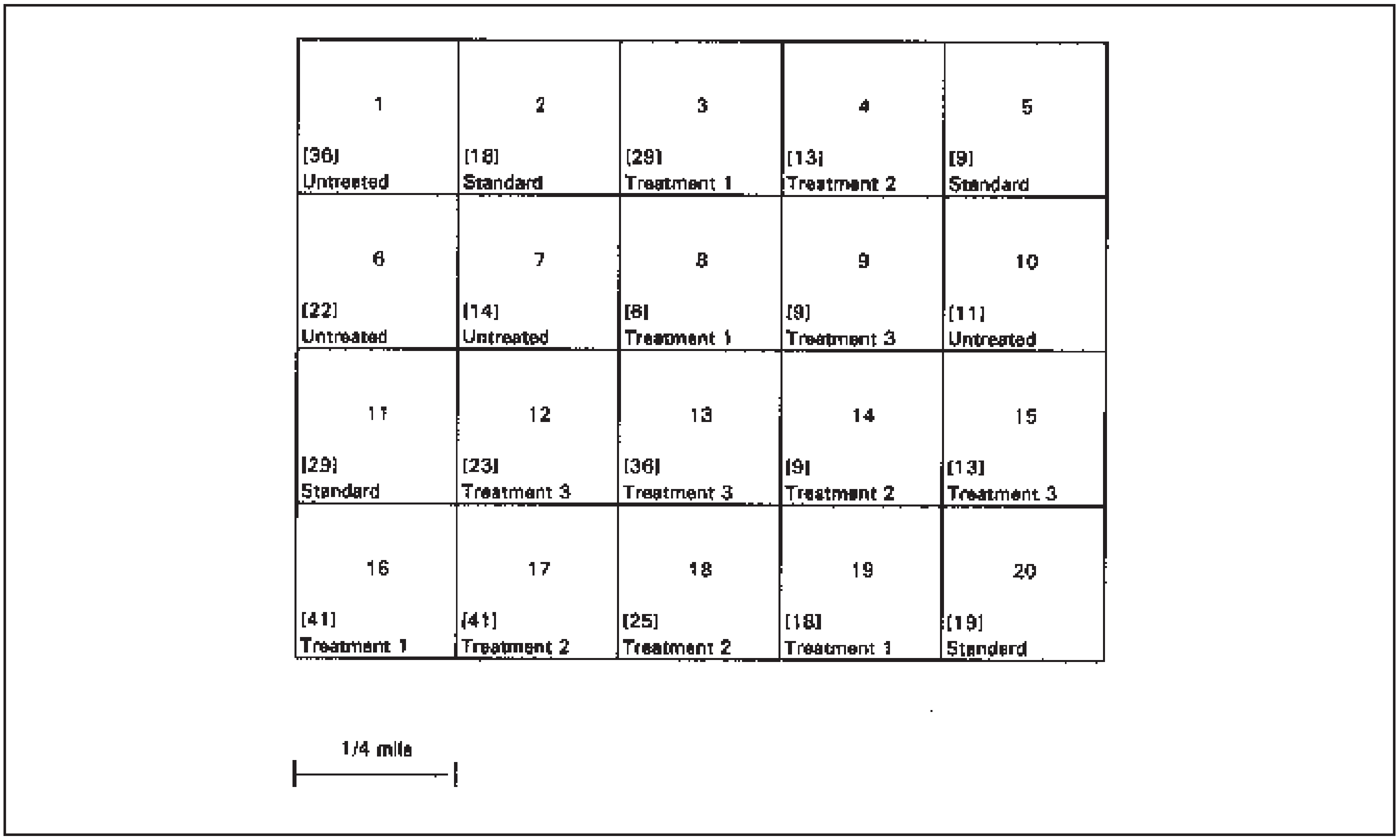

Figure II.2–1—Plot map showing pretreatment mean density of grasshoppers per square meter, in parentheses, and assigned treatments from table II.2–1.

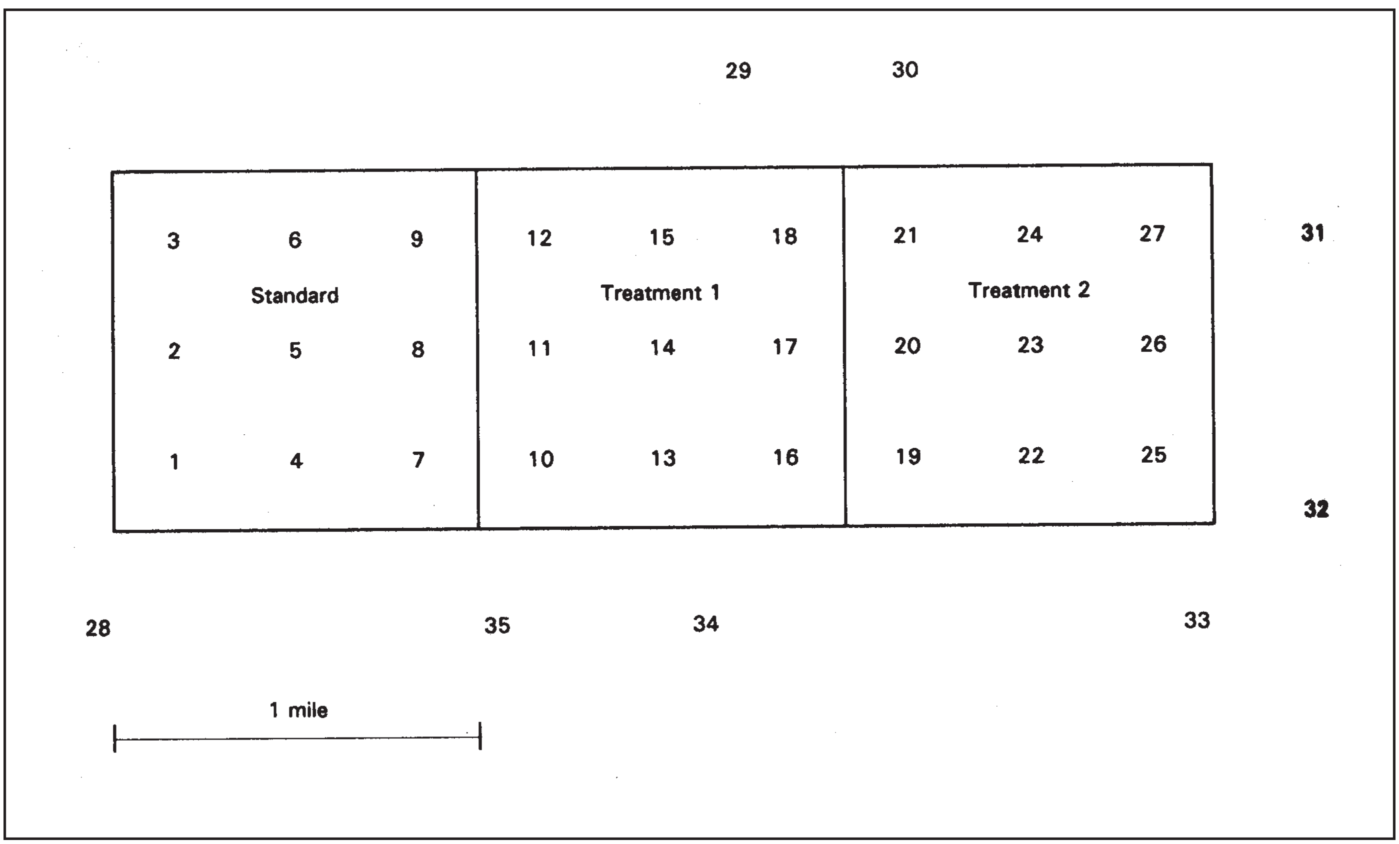

Figure II.2–2—Map showing 640-acre (1-section) plots showing evaluation sites numbered within the plots and numbered untreated evaluation sites located around the perimeter of the treated plots.

Deciding when to start and stop application is not only a decision made daily, but one made on each pass or run of an applying aircraft. Decisionmaking requires consideration of windspeed, ground and air temperatures, amount of moisture on vegetation, and the possibility of precipitation.

In some States, laws define some of the guidelines under which applications are made. Generally the smaller the plot size, the more restricted the guidelines for application become. Typically, with 40-acre replicated plots, application is stopped when winds exceed 3–4 miles per hour or ground temperatures exceed air temperatures. Monitoring spray-sensitive cards in adjacent plots or designated no-spray areas during application is important to determine unacceptable pesticide drift.

Aircraft Guidance

Guidance of aircraft during application varies from sophisticated electronic guidance systems used in many programs to simple but effective flag-waving provided by ground personnel in small plots. However, all guidance depends on the specific swath width assigned to a particular type of aircraft and equipment and the material being applied. Ground crews can determine the location of each swath by using measuring tapes or calibrated wheels or by accurately pacing a known distance equal to the desired swath width. Also, ground crews can make and mark these measurements ahead of time or as application is occurring.

The width of a swath is determined through extensive testing prior to small-plot or program application. Swath widths of 75 ft for most water-based formulations and 100 ft for most oil-based formulations are typical for small-plot work with a Cessna Ag Truck aircraft, for example. Swath width assignments for other types of aircraft are found in the USDA-APHIS-Aerial Application Prospectus. APHIS generally conducts applications at a height equal to 1 $^1/_2$ times the wingspan of the aircraft.

Recordkeeping

Recordkeeping is essential in assessing any treatment in both test work and program use. At the airport, it is important to maintain a record of the final calibration for comparison with the actual acreage covered and material used for each flight. In the field, it is important to measure and record numerous parameters: (1) beginning and ending time of actual application, (2) windspeeds during application, (3) ground and air temperatures during application, and (4) passes that the aircraft makes when applying material. In experimental work, these parameters should be measured and recorded at the beginning and ending of treatment for each plot treated. In programs where multiple aircraft are used, the number and location of partially or completely inoperable spray tips on each aircraft should immediately be reported to the official in charge. In test work, seeing such occurrences requires landing the aircraft to correct the problem.

Evaluation Site Data

The basic types of data collected are grasshopper species composition and density. The conditions, including weather, present during data collection are recorded. Depending on the specific study, we may collect other types of data for association with population estimates, such as vegetation composition and quality or spray droplet size and frequency.

We estimate the grasshopper population by counting the number of grasshoppers found in 40 0.1-m^2 rings at each site. We count and record each ring separately. In our evaluations, the order of counting is always the same, counterclockwise from the site stake. A more detailed description and discussion of procedures for counting grasshoppers is in the chapter on survey in the Decision Support Tools section of the User Handbook.

A typical square mile of infested rangeland will contain 15 to 40 different grasshopper species, some of which may not be causing damage. Estimating the relative abundance of each species is important in order to determine the need for control and the effectiveness of treatments on target species. Base estimates on samples taken from the population with a sweep net. Such sampling is done by taking equal numbers of low–slow (ground level) and high–fast (canopy level) sweeps uniformly along the margin of the circle of rings. Low–slow sweeps ensure the capture of early instar and slow-moving species, while high–fast sweeps ensure the capture of older instars and more-active species. Try to get a collection of at least 100 grasshoppers at each site. Do this by conducting 100–200 low–slow and high–fast sweeps each. Determine the density of the individual species by multiplying the frequency of occurrence, from the sweep sample, by the total density of grasshoppers at the site (counts from rings). Except in some program evaluations, take sweep samples whenever a grasshopper count is conducted.

Make pretreatment counts to determine the population levels against which posttreatment levels are compared. In small replicated plot studies, use the initial pretreatment count to assign treatments appropriately. These studies require additional pretreatment counts conducted closer to the date of treatment for comparison with posttreatment counts. If at all possible, take pretreatment counts 0–48 prior to treatment.

Counts from untreated and treated sites taken on the same day will allow for converting reduction calculations (posttreatment count divided by pretreatment count) to a percentage control value (Conin and Kuitert 1952). This formula is discussed in the chapter "Bait Acceptance by Different Grasshopper Species and Instars." Using the untreated control-plot data in this fashion allows for adjustment for any natural mortality that occurs and will provide a value of the actual mortality that can be attributed to the treatment. Just as important, if not more so, this procedure will provide an adjusted value that accommodates the day-to-day meteorological changes (such as wind, temperatures, and precipitation) that affect the actual grasshopper counts.

The interval between treatment and the posttreatment count depends on the purpose of the evaluation and the treatment(s) used. With solid baits or fast-acting, short residual sprays, posttreatment intervals of 2, 4, and 7 days are typical. For slower acting or longer residual treatments, weekly intervals at 1, 2, 3, and 4 weeks posttreatment are typical. If two or more treatments that work at different speeds are to be compared, collect the data at similar posttreatment intervals for all treatments. In such cases, an end-of-study or season comparison is helpful in addition to evaluation at specific intervals.

Conclusion

The above protocol is not a detailed standard operating procedure but is intended to serve as a general guideline for several types of treatment evaluations on rangeland grasshoppers. The kinds of data and methods of collection discussed here will allow researchers and program evaluators to use numerous kinds and strategies of analysis.

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