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Evaluation of the Fungus *Beauveria bassiana* in Canola Oil and Paraffinic Oil and Identifying the Low Dose of a Bacteria Derived Toxin, Spinosad, for Use Against Rangeland Grasshopper

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Evaluation of the fungus *Beauveria bassiana* in canola oil and paraffinic oil and identifying the low dose of a bacteria derived toxin, spinosad, for use against rangeland grasshoppers.

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INTRODUCTION:

There are no generally accepted non-traditional (non-chemical insecticide) treatments available for use against grasshoppers and Mormon crickets in the United States. Sensitive areas commonly encountered within the western states in potential program areas preclude traditional insecticide treatments. The number of sensitive situations is increasing and in areas of grasshopper infestations their presence at best complicate and more often prevent much needed local or area-wide treatments of grasshoppers and/or Mormon crickets on rangeland.

In the early 1990's, extensive work on the development of the fungus *Beauveria bassiana* by Mycotech Corporation and USDA-APHIS "Methods" (Foster et al. 1996-1999) led to its registration in the US even though final development for wide scale acceptance and use was never achieved. From 1995 until 2002, USDA-CPHST efforts in its development were redirected. However, recent renewed interest in the original stressor concept developed by "Methods" (Foster et al. 1996; Reuter et al. 1996) and the recent combination of the registered

isolate formulated with potential attractants has indicated significant promise. Collaborative work conducted by the University of Wyoming (Lockwood, 2003) and the USDA- ARS (Jaronski et al. 2003) has indicated that an effective formulation may result by simply applying the spores in canola oil rather than paraffinic oil. While non-replicated, that work has indicated substantial differences between *Beauveria bassiana* treatments applied in canola oil compared to untreated control populations.

Spinosad, a bacteria derived toxin, has recently been approved for use on organic crops and as such should be accepted in many if not all sensitive areas. Though currently not registered for grasshoppers, Spinosad has shown activity at evaluated doses against grasshoppers in the laboratory (Foster et al. 1996) and on rangeland (Foster et al. 2002). However, the lowest effective dose has not been determined.

Both of the aforementioned non–traditional potential treatments require further development, but appear realistically and extremely promising. Additionally, significant controversy exists regarding the attractancy and resulting impact on grasshopper treatment efficacy of some diluents. All of these areas require further detailed and extensive evaluation. The following studies were conducted to reinitiate strong involvement in developing non-traditional tools that could be used in traditional as well as sensitive areas and were specifically designed to evaluate the diluents used with *Beauveria bassiana* and to identify the lowest active dose of Spinosad against rangeland grasshoppers.

OBJECTIVES:

Determine if the Emerald BioAgricultutal Corporation registered isolate and formulation of *Beauveria bassiana* is effective against grasshoppers on rangeland.

Compare the registered isolate of *Beauveria bassiana* applied in Orchex oil (paraffinic) or canola oil (vegetable oil) for effectiveness against grasshoppers on rangeland.

Compare the effectiveness of uniform coverage and reduced agent area treatment (RAATs) with 50% of the area treated using *Beauveria bassiana* against grasshoppers on rangeland.

Evaluate the droplet density and distribution in uniform and RAATS applications with *Beauveria bassiana.*

Evaluate canola oil for stimulating major grasshopper movement between untreated and treated rangeland.

Evaluate several doses of spinosad applied at less than 3 fluid oz/acre to determine the lowest effective dose against grasshoppers on rangeland.

Determine the length of residual activity of low doses of spinosad in field cages on rangeland.

MATERIALS AND METHODS:

The center of the study areas was located in Fall River County of southwestern South Dakota approximately 11 miles west and 8 miles north of the town of Edgemont on the Mark Tubbs ranch. The general location was selected because of the density of grasshoppers, history of grasshoppers and the abundance of contiguous grasshopper infested rangeland.

All treatments were applied with an USDA, APHIS aircraft (Cessna Ag-Truck equipped with a standard commercial spraying system) which was operated by an APHIS pilot who is highly experienced with precision work for research. The aircraft was also equipped with differentially corrected guidance and recording systems. However, primary guidance was provided by ground personnel that measured each swath and gathered meteorological data during application. The aircraft was additionally equipped with winglets (DBA-Ag Tips; Clark Oberholtzer, Alberta Canada). Prior to application, the aircraft spray system was calibrated to operate under parameters which resulted in delivery of spray within one percent of the desired rate per acre for each of the treatments applied. Calibration for each of the treatments was accomplished by collecting and measuring the amount of material sprayed through each nozzle for each treatment set up, for a predetermined amount of time and making adjustments in pressure until the desired output was achieved. During calibration for *Beauveria bassiana*, temperatures of the formulated material ranged from 80° F to 96.2^{o} F in the hopper of the aircraft. The aircraft was calibrated for a 100 feet wide swath for treatments containing oil (*Beauveria bassiana*) and a 75 feet wide swath for water based treatments (Spinosad).

Beauveria bassiana Field Studies.

The Emerald BioAgricultural Corporation GHA (128924) strain of *Beauveria bassiana,* was applied to 40 acre rangeland plots in the following treatments: (1) 1×10^{13} spores/acre in 32 fluid oz/acre of formulation containing Orchex oil to 100% of the plot. (2) 1×10^{13} spores/acre in 32 fluid oz/acre of formulation containing canola oil to 100% of the plot and (3) 1×10^{13} spores/acre of formulation containing canola oil to 50% of the plot. Treating 50% of the plot was achieved by calibrating the aircraft for a 100 foot wide swath and spacing the aircraft during treatment at 200 feet. All treatments were replicated four times. Untreated control plots were included in the experimental design.

All treatments were applied with 9 CP nozzles using the 0.062 inch orifice and a 90 degree deflection setting (Fig. 1). These were the same type of nozzles and settings (except for pressure) previously used in the collaborative studies by the University of Wyoming (Lockwood, 2003) and the USDA-ARS (Jaronski et al. 2002). The treatments in canola oil were applied at 53 psi on July 5, 2003. All of the treatments in Orchex were applied at 39 psi, one plot on July 5 and three plots on July 6, 2003. All treatments were applied from an altitude of 30 to 70 feet. Winds during application ranged from 0.5 to 5.3 mph and averaged 2.0 mph. Winds for specific plots and other meteorological conditions recorded during application of the treatments are summarized in Table 1. During the study, minimum temperatures ranged from 45° to 76° and averaged 63° F while maximum temperatures ranged from 76° to 104° and averaged 92° F. Detailed temperatures and precipitation recorded during the study can be seen in Figs. 2 and 3.

Generally grasshopper density and species composition sampling followed protocols established by Foster and Reuter, 1996. Grasshopper populations in treated and untreated plots were counted and sampled 1 to 3 days before treatment and at 7, 14 and 21 days after treatment. Untreated control plots were counted and sampled on any day a treated plot was monitored. Grasshopper densities were determined by counting grasshoppers in (40) 0.1 m^2 rings arranged in a circle near the center of each plot. Rings were separated from adjacent rings by ca. 5 yards. One person conducted all of the counts in this study.

The abundance of each species was determined from uniform sweep samples taken at each site (Foster and Reuter, 1996). Each sample consisted of 20 high and fast sweeps and 20 low and slow sweeps. Low and slow sweeps performed at ground level insured capture of very young instars and less active grasshopper species while high and fast sweeps performed at the canopy of the vegetation insured capture of older instars and the more active species. Sweep samples were always collected immediately after grasshopper densities had been determined at each site on each visitation. Densities of individual species can be determined by multiplying the frequency of occurrence times the total density of grasshoppers at the same site. After collection, samples were cold stored until they could be sorted and identified in the lab.

Near the center of one plot of each of the different treatments, oil sensitive spray cards were placed ca. 18 inches above the ground in a 220 yard long line located perpendicular to the line of flight to characterize spray deposition. Cards were separated from adjacent cards by ca. 5 yards and placed immediately prior to application and were collected shortly after application and returned to the laboratory for analysis. Using a template, five, one square centimeter areas on each card, were counted to determine the density of spray droplets deposited. In addition, six 100-mm plastic Petri plates were placed open on the ground in one plot each from the *Beauveria* in Orchex and in canola oil treatments (both 100% coverage). Plates were placed more or less at equal distances in a transect approx. 36 feet long in the center of one plot, on a diagonal to the path of the aerial applicator. Plates were immediately covered after application of materials. Spores from each plate were suspended in 15 ml of dilute Stepan DD7®. This suspension was then counted directly in a hemocytometer using 400X phase contrast microscopy. Conidial concentrations were converted to conidia per cm^2 of exposed Petri dish and then conidia per acre.

Beauveria bassiana Laboratory Evaluation Studies.
Grasshoppers were collected by sweeping in one plot from each treatment, beginning with untreated control, using disinfected nets; grasshoppers were chilled and sorted for the genera *Aulocara* and *Ageneotettix*, and transferred to 60-cm long, 10-cm wide, cellulose acetate tubes with screened ends, and shipped cold to Sidney MT. There, hoppers were transferred to clean tubes (15-20 insects of a species per tube), and incubated at 25-28° C. for 14 days. All hoppers were fed washed Romaine lettuce ad libitum during this observation period. Mortality was recorded daily. All cadavers were surface decontaminated (immersion in 0.5% NaOCl for 30 sec, followed by two deionized water rinses) and incubated 3-4 days at >95% relative humidity to elicit *Beauveria* mycoses, if present. The data used for analyses represent mortalities after 7 days of incubation (at which time mortalities had reached an asymptotic maximum).

Potential grasshopper body temperatures resulting from basking during the trial were recorded by means of thermal surrogates (Lactin and Johnson, 1989). These consisted of 1.5 ml plastic

Eppendorff tubes filled with soy sauce. A copper-constantin wire thermocouple (No. 5SC-TT-T-30-36, Omega Corp.), connected to a Cole-Parmer Digisense® data logger, was embedded in each tube. One tube was mounted on a bamboo skewer in grass canopy 10 cm above the ground. This tube was oriented perpendicular to the ground and with southern exposure mimicking a grasshopper perched on a grass stem. The other tube was placed on bare ground on an east-west axis to mimic a grasshopper actively basking and thermoregulating. Temperatures were recorded at 15 minute intervals for the 10 days following treatment of the plots. These observations were designed to identify the maximum possible thermoregulatory temperatures attained by grasshoppers during the post-application observation period, as well as the amount of time during which grasshopper body temperatures were within the optimal range for *Beauveria* growth. We recognized that at body temperatures greater than 45°C. grasshoppers would alter their behavior to avoid exceeding tolerable temperatures (Lactin and Johnson, 1989).

Beauveria bassiana Persistence Studies.

Immediately after applications, approximately 1 quart of representative vegetation was collected from treated swaths of one plot each from the 100% coverage with *Beauveria* in Orchex and 100% coverage with Beauveria/Canola oil treatments, as well as one untreated plot. Vegetation was cut with scissors, which were disinfected between samples. Each set of vegetation samples was placed in a clean Ziploc® bag, chilled and shipped within one day to Sidney MT by overnight express. In Sidney, standardized, replicate samples of the vegetation were placed in 10-cm wide, 30-cm long, cellulose acetate cylinders with screened ends. Adults from a nondiapause *Melanoplus sanguinipes* colony were randomly assigned to each samples, for a total of 15-20 insects per sample and added to the cylinders. All steps were performed as aseptically as possible to prevent cross contamination of samples. Grasshoppers were exposed to vegetation samples for 48 hr at 26-28°C., then transferred to clean tubes. Grasshoppers were subsequently incubated at 26-28°C. for 14 days with decontaminated Romaine lettuce (washed in 0.5% NaOCl) provided ad libitum. Mortality was recorded daily. Infections were verified by incubation of surface decontaminated cadavers for 3-4 days at high humidity. Grasshopper mortalities on Day 10 post-exposure were plotted against the number of days of weathering (days post-treatment) and best-fit decay regressions calculated using Excel®.

Spinosad Field Studies.

Four 10 acre plots each were treated on June 29, 2003 with the following selected doses of the Dow AgroSciences Tracer formulation of spinosad: (1) 3 fluid oz/acre, 42.5 g AI/ac. (2) 1.5 fluid oz/acre, 21.25 g AI/ac. (3) 0.75 fluid oz/acre, 10.625 g AI/ac. and (4) 0.375 fluid oz/ace, 5.3125 g AI/ac. Four untreated control plots were included in the study. All treatments were diluted in water and applied at a total volume of 32 oz/acre. Treatments were applied through (15) 8003 size flat fan Tee Jet stainless steel nozzle tips oriented straight down and equipped with 50 mesh strainers. Applications occurred at 117 mph and 30 psi. The 3 fluid oz/acre rate was achieved by flying the plot twice with the 1.5 fluid oz/acre rate and the 0.75 fluid oz rate was achieved by flying the plot twice with the 0.375 fluid oz/acre rate. All treatments were applied from an altitude of 30 to 70 feet. Winds during application ranged from 0.5 to 2.0 mph. Winds for specific plots and other meteorological conditions recorded during application of the treatments are summarized in Table 1. During the study, minimum temperatures ranged from 45° to 66° and averaged 57° F while maximum temperatures ranged from 76° to 97° and averaged 88° F. Detailed temperatures and precipitation recorded during the study can be seen in Figs. 2 and 3.

Grasshopper density and species composition sampling was conducted similarly to that described for the *Beauveria bassiana* study. Except, because of the small size of plots, 0.1m rings were arranged near the center of each plot in 4 parallel rows of 10 for the 1.5 fluid oz/acre treatment. The other three treatments were evaluated using 8 parallel rows of 10 rings. Rings were separated from adjacent rings by ca. 5 yards and rows were separated from adjacent rows by ca. 10 yards. The untreated control plots described in the previous study were also utilized in this study.

Spinosad Cage Studies

To determine the length of residual activity of selected doses of Spinosad, small bucket cages (two gallon bottomless buckets modified with screen sides and top) were established on additional plots treated with each dose. Ten cages (replicates) were established in each of the plots and in a nearby untreated plot on 0, 3, and 7 days after treatments occurred. Cages were stocked at the rate of 5 *Ageneotettix deorum* (fourth instar – adult stage) per cage. Grasshoppers for cage studies were collected from a nearby untreated area. Grasshopper mortality within cages was monitored daily.

Diluents/Attractant Study.

A single 4800 feet long by 100 feet wide swath of canola oil was applied on July 9, 2003 to rangeland to evaluate the gross attractiveness of canola oil in terms of stimulating major grasshopper movement from an untreated area into a treated area (Figs. 4a). Pure canola oil was applied at a volume of 32 fluid ozs/acre, using the same spraying configuration that was used in the *Beauveria bassiana* study. In this study, to insure less movement of the spray out of the designated treated area, application occurred from an altitude of 10 feet.

Along a line within the center of the sprayed swath, four separate sets of (40) 0.1 m^2 rings were established to determine grasshopper density. Each set was separated from adjacent sets by 200 yards and rings within each set were separated from adjacent rings by ca. 5 yards. Two additional parallel transects of rings were established on both sides of the treated swath at 150 and 250 feet from the center on the treated swath. This allowed for estimating grasshopper densities at 100 and 200 feet from either edge of the treated swath. An untreated area with 5 parallel transects of rings was similarly established for comparison (Fig. 4b) The two areas were separated by ¼ of a mile. Oil sensitive spray cards were placed at 15 foot intervals on a line perpendicular to the application flight line on both ends of the applied swath. One pre (July 8) and four (6 hours after application on July 9, and July 10, 16 and 24) post-treatment grasshopper density estimates were conducted for each of the treated swaths (plots) and untreated control plots. Each of four different people counted one set of treated and untreated rings for the first two post counts. After that, each of two people continued to count one set of treated and untreated rings for the one and two week post-treatment density estimates. Grasshopper species composition and age structure data was also collected for each plot through sweep net collected samples. Density and species composition data was generally obtained as described by Foster and Reuter, 1996.

ANALYSIS:

Field data were expressed as percent survival based on pretreatment counts in the same plot or were adjusted for the natural population change by the method of Connin and Kuitert (1952) by using the mean values of the untreated plots on the appropriate day. This allowed for converting data from percentage mortality to percentage control and accommodated the natural population change to insure against natural mortality and other environmental factors that affect grasshopper counts, which can confound real differences between treatments.

The adjusted percentage control of the treatment (which takes into account natural changes in the untreated population) was calculated by the formula 100 (1 – Ta x Cb/Tb x Ca).Where Tb equals the total population of grasshoppers counted before the plot was treated, Ta equals the total counted after treatment, Cb equals the total counted for the check sites before treatment, and Ca equals the total counted for the check sites after treatment.

Field data was analyzed using an analysis of variance with the Tukey multiple comparison test to separate means. All field analyses were performed with SYSTAT for Windows (SPSS Inc. 1997).

Treatment mortalities of post-treatment grasshopper collections and bioassays of vegetation samples were compared using Fisher's Exact Test or Chi Square Analysis performed with Statistix 7 (Analytical Software, Inc., 2002). Day 10 mortality data from the replicate bioassays of vegetation samples taken immediately after application were subjected to angular transformation before being subjected analysis of variance with Statistix® 7.

RESULTS AND DISCUSSION:

Beauveria bassiana Field Studies.

Pretreatment densities from individual sites ranged from 9.5 to 24 and averaged 18 grasshoppers/m^2 in the treated plots and from 12 to 29 and averaged 22 grasshoppers/m^2 in the untreated plots. At the time of treatment, the population was composed of <1% first instars, 4% second instars, 6% third instars, 18% fourth instars, 50% fifth instars and 21% adults. The total mean age was 4.78. The five most dominant species were *Ageneotettix deorum* 42.9%, *Cordaillacris occipitalis* 15.8%, *Melanoplus sanguinipes* 15.4%, *Amphitornus coloradus* 7.8% and *Aulocara elliotti* 7.5%. The relative abundance of all species in pretreatment samples is shown in Table 2.

At one, two and three weeks after application, none of the *Beauveria bassiana* treatments showed a significant difference in population reduction compared to the untreated populations (Table 3 a, b and Fig. 5). There was no statistical difference between treatments with canola oil compared to treatments with Orchex oil. Additionally, there was no statistical difference between treatments applied to 100% of the area and 50% of the area. While these results are inconsistent with those reported by Lockwood (2003) and Jaronski et al. (2002) for a collaborative study conducted in Wyoming they are consistent with data from studies in South and North Dakota reported by Foster et al. (1991, 1992, and 1993). In those studies no statistical difference in

reductions of grasshopper populations could be detected between unconfined populations treated with *Beauveria bassiana* at 1 x 10^{13} spores per acre when compared to untreated populations, except when a sub-lethal stressor was added to the formulation in a Nebraska study (Foster et al. 1996).

One of the most unexpected results of the study was the extremely large droplets produced with canola oil (Fig. 6) (Quartarone, 2003) compared to smaller droplets produced with Orchex oil. However, this appears consistent with the higher viscosity of canola oil which was observed during initial calibration trials where 27% less canola oil flowed through the nozzles at 39 psi compared to the Orchex oil. As stated earlier, for this study, canola oil treatments required application at 53 psi to achieve the desired rate per acre that was produced with 39 psi with Orchex oil. Examination of spray cards revealed that a mean number of 2.6 and 1.5 droplets /cm^2 were deposited in the 100% and 50% coverage canola treated plots respectively (Figs. 7 and 8). These values seem reasonable since the plot treated with 50% coverage was expected to demonstrate about half as many droplets as in the 100% coverage plots. Additionally, and as expected, 50% coverage treatments, with spaces of 100 feet between swaths demonstrated sufficient lateral displacement of the spray even with winds as low as <1 to 3 mph, to achieve some level of deposition in all of the area not directly treated. Droplets were recorded on all but one of the cards in the 50% treatment coverage plot, including those cards that occurred in the 50% of the acreage not directly treated. However, while this single card demonstrated no droplets in the five 1 cm^2 areas counted, it was noted that numerous droplets had been deposited on the card outside of the areas predetermined for sampling. Surprisingly, a mean number of 1.4 droplets /cm^2 were recorded on spray cards in the monitored plot treated with Orchex oil (Fig. 9). Because of the increased viscosity and large droplet size produced with the canola oil treatments it was anticipated that Orchex oil treatments would result in higher densities of droplets compared to canola oil treatments. However, it was noted during examination that these droplets were fading and difficult to see on the oil sensitive cards, an occurrence not uncommon to paraffinic oils on this type of spray card. Therefore, these values are considered by the authors to be severely underestimated.

The droplet densities in this study, resulting from the CP nozzles, were remarkably low in comparison to other studies where similar total volumes per acre have been applied. When flat fan nozzle tips were used to apply 32 oz/acre of a water based Sevin XLR Plus formulation, 7.4 droplets /cm^2 resulted (Foster et al. 2000). Additionally, when flat fan nozzle tips were used to apply a 31 oz/acre paraffinic oil and water based Dimilin formulation, 7.6 droplets/cm^2 resulted. (Foster et al. 2002.)

The amount of *Beauveria* delivered to the plots, based on the deposition plates, were only 16-24% of the target level (1x10^{13} conidia/acre), but we have some concern that these plates gave us an underestimate despite prior validation of the technique. Quality assurance checks of spore concentrations of test materials, before and after aerial application, and records of the amounts sprayed aerially indicated that the target amounts of materials were applied to the plots.

Beauveria bassiana Laboratory Evaluation Studies.
The grasshoppers collected from plots of the 100% coverage treatments with *Beauveria* in Orchex and in canola oil suffered 100% mortality within 6 days of collection from the treated

plots (approx. two days at cool, in transit temperatures, followed by 4 days at constant 28° C.) (Figure 10). This progression of cumulative mortality with time is typical for *Beauveria* infections. Mortality in untreated populations remained low until the fifth day of incubation when it rose to 34%. Prevalence of *Beauveria* among the cadavers was 72-100% (Table 4). These observations indicate that grasshoppers received fully infectious doses of fungus. A highly lethal dose for *Aulocara elliotti* was applied throughout the sprayed and unsprayed swaths in the 50% coverage plots (Table 4). Day 7 mortalities from some RAATs *Beauveria* were equal to those in the 100% coverage plots (Figure 10), based on controlled incubations of grasshoppers collected immediately after the *Beauveria* applications. There was, however, a lower rate of cumulative mortality among grasshoppers from the 50% coverage plot (Figure 10). Observations with *A. deorum* were similar (data not shown).

Aulocara collected from a narrow transect along the center of an untreated swath in one 50% coverage (RAATS) plot within 30 minutes of application suffered 100% mortality by 6 days post-collection (2 days in cool transit, then 4 days incubation at 28°C.) vs. 89% among *Aulocara* collected along the center of a sprayed swath (Table 4). These two mortalities were not significantly different (Fisher's Exact Test, p=.06). *Ageneotettix* presented a different result: individuals of this species from the unsprayed swath had only 60% mortality 6 days post-collection vs. 100% among insects from the sprayed swath. This difference was significant (Fisher Exact Test, $p<0.001$). *Beauveria* mycoses ranged from 88-100% among the killed insects. The presence of a low prevalence of *Beauveria* in untreated grasshoppers (17-55%) is troublesome, but these prevalence data are still much lower than among treated insects.

The absence of significant differences in mortality and fungal prevalence rates in *A. elliotti* from sprayed (89% with 100% prevalence of *Beauveria* in cadavers) and unsprayed (100% mortality, 100% *Beauveria* prevalence) swaths in RAATS plot (Table 4) indicates that the RAATS system did not result in absolute sprayed/unsprayed swaths. If it had, then one would expect significantly lower levels of infectious residues in samples from the unsprayed swath. There was a statistically significant difference between the samples for *A. deorum* from sprayed and unsprayed swaths (Table 4), but there was still a substantial amount of *Beauveria* in *A. deorum* from the unsprayed swath

Based on observations of collected grasshoppers, the rate of $5x10^{12}$ spores/acre in the RAATS plots gave infection rates equivalent to that in the 100% coverage plots with the effective rate of $1x10^{13}$ spores/acre. This observation is also supported by the vegetation clip bioassays. Vegetation collected from the unsprayed swath immediately after application of *Beauveria* had enough conidia to cause a mean mortality of 34% among teneral *M. sanguinipes* exposed to it for 48 hr vs. 50% mortality seen for the vegetation from the sprayed swath (Table 5). These two mortalities were not significantly different from each other nor from the corresponding mortalities in bioassays of vegetation samples from the 100% Orchex and 100% canola plots (73% and 91%, resp.) (Table 5). It should be noted, however, that there was considerable variability among replicate assays of several of the samples. This variability may well have contributed to the nonsignificance of the differences.

Prevalence of *Beauveria* remained high in grasshoppers collected 3 and even 10 days post-treatment (Table 4). These prevalence data indicate that even though grasshopper mortality was

not very evident in the population counts, the insects were infected with the fungus. This phenomenon was described by Inglis et al., (1996) and is a frequent occurrence when post treatment conditions are sunny and warm. Under these conditions infected grasshoppers will increase their basking and raise their body temperatures to levels, that, while not fatal to *Beauveria* infections, greatly prolong the prepatent period of disease and delay onset of mortality ("behavioral fever"). That behavioral fever was possible is indicated by the temperatures recorded by the grasshopper thermal surrogates. Generally body temperatures were in excess of the upper threshold for growth by *Beauveria* (32° C.) for as much as 10-12 hr each day on bare ground (6-15 in the grass canopy). In addition, night temperatures were below the optimum range (<18° C.) for as many as 9 hr each night (Figure 11). The end result was that temperatures in these surrogates were permissive for *Beauveria* growth for only 6-15 hr each day, for a total of 83 hr for the 10 days following application of *Beauveria* to the plots (Table 6). Grasshoppers collected post-treatment and incubated at constant 28° C., died of *Beauveria* infection within 72-96 hr.

Beauveria bassiana Persistence Studies.
There was a rapid degradation of *Beauveria* residues on vegetation as evidenced by the mortalities among *M. sanguinipes* exposed to samples collected at intervals post-treatment. Mortalities fell to almost 0% by 5 days post application; half-life of the fungus residues, as measured by infectivity, was about 2 days (Figure 12). Direct measurement of spore viabilities after variable exposure to sunlight was not possible due to procedural problems. Our observations are similar to those of Inglis et al. (1993).

All of these results indicate that development of *Beauveria bassiana* formulations and evaluations of nozzle and diluent types warrant significant further investigation.

Spinosad Studies.
Pretreatment densities from individual sites ranged from 8 to 15 and averaged 10 grasshoppers/m^2 in the treated plots and from 12 to 29 and averaged 22 grasshoppers/m^2 in the untreated plots. At the time of treatment, the population was composed of 5% first instars, 11% second instars, 21% third instars, 41% fourth instars, 19% fifth instars and 3% adults. The total mean age was 3.68. The five most dominant species were *Ageneotettix deorum* 54.3%, *Melanoplus sanguinipes* 11.7%, *Cordaillacris occipitalis* 10.1%, *Amphitornus coloradus* 4.6%, and *Aulocara elliotti* 4.1%. The relative abundance of all species in pretreatment samples is shown in Table 7.

All four doses of Spinosad (Tracer) tested, produced substantial reductions in the population at 1, 3, 6, and 9 days after treatment (Fig. 13, Tables 8 and 9). The highest dose in this study, 3 fluid oz/acre, produced mortality similar to that seen in previous studies where 3 fluid oz/acre was the lowest dose evaluated (Foster at al. 2002). All doses generally performed in dose rank order except the 0.375 dose at 1 and 3 days after treatment which produced slightly higher mortality than the 0.75 dose (Table 8). Treatments generally reached maximum mortality by 3 days after treatment. Populations increased slightly during the study in the untreated plots indicating some additional hatch or some movement into the plots. Data adjusted for the change in untreated populations (Table 9) showed spinosad at 3, 1.5, 0.75, and 0.375 fluid ozs/acre producing 98%, 84%, 78% and 78% mortality respectively.

In the spinosad cage studies, except for the 0.75 fluid oz/ace dose at 0, 3 and 7 days after treatment and the 0.375 fluid oz/acre treatment at 7 days after treatment, all treatments produced residual activity in dose rank order (Fig.14). Inexplicably, the 0.75 fluid oz/acre dose produced higher mortality than was expected for the entire study. The highest dose in this study, 3 fluid oz/acre, produced residual activity similar to that seen in a previous study (Foster et al. 2002). In that study, 6 fluid oz/acre showed significant residual activity compared to an untreated populations at 3 days after treatment, while the 3 fluid oz/acre treatment showed no significant mortality resulting from 3 day old residuals compared to untreated populations. Three day old residuals of the three highest doses in this study showed significant activity compared to untreated populations. None of the treatments showed activity with 7 day old residuals except for the 0.75 fluid oz/acre dose. Data from a previous study had indicated that no significant activity would be detected with 7 day old residuals of doses applied at 3 or fewer fluid oz/acre.

These studies indicate that doses in the area of 0.75 to 1.5 fluid oz/acre could produce acceptable reduction in grasshoppers while leaving little residual activity at 3 days after treatment. However, efficacy studies with replication should be conducted if spinosad becomes a viable candidate for registration for grasshopper on rangeland. Additional studies will also be required to determine exactly where significant activity against grasshoppers ceases between 0 and 3 days after treatment.

Diluents/Attractants Study.

Pretreatment densities from individual sites ranged from 9 to 25 and averaged 15 grasshoppers/m^2 in the treated plots and from 5 to 23 and averaged 12 grasshoppers/m^2 in the untreated plots. Determination of species composition and the associated age structure is incomplete at the time of this report. However, the species composition will be similar to that reported for the other studies in this report and the age structure will be slightly older than that reported for the *Beauveria bassiana* study.

Strong winds in excess of 10 mph from the north occurred the morning of treatment (Fig. 4). However, a brief lull occurred which allowed for application of the pure canola oil at 5:37 A.M. from an altitude of 10 feet. Spray cards on the west and east end of the swath treated indicated spray was deposited within the desired designated 100 foot wide swath. However, because of the wind blowing almost perpendicular to the flight line, some of the canola spray moved significantly to the south, 150 feet from the south edge of the desired swath on the west end and 180 feet from the south edge of the desired swath on the east end of the swath (Fig. 4a). While unplanned, this occurrence resulted in a more clearly defined boundary between treated and untreated rangeland on the north side of the treated swath. As can be seen in Fig 4a, no spray was deposited in sets of rings designated D and E on the north side of the desired treated swath. Spray was deposited in the center sets of rings (C), all of the sets of rings immediately south of the designated treated swath (B) and some of the rings in the southern most (A) sets of rings.

Populations in the C sets of rings within the treated swath were expected to increase due to attraction from the untreated area to the north into the treated swath. However, populations in the treated swath did not increase at any time during the study (6 and 24 hours after treatment and 7 and 14 days after treatment) (Fig. 15). Additionally, it was anticipated that of the movement of

spray into the B sets of rings would result in population increases in the B sets of rings, immediately downwind of the sprayed swath. However, populations not only failed to increase in any of the treated swath but also in any of the downwind monitoring sites. The only population increases detected were in upwind locations. Those increases were generally less than 20%. Major decreases in populations in all sets of rings associated with the treated swath occurred at 2 weeks after treatment, an indication of movement out of the general area and or mortality.

Unfortunately, population decreases were also occurring in most of the untreated sites (Fig.16), indicating some wide scale movement out of the area and or mortality thus confounding the results. Results from this study were unable to demonstrate major grasshopper movement from untreated rangeland into treated rangeland. Any subtle population changes resulting from grasshoppers being attracted toward the canola oil treated rangeland may have been masked by general movement which was occurring in the area. The short, sparse grass and high temperatures which can trigger migration and an aging population may have contributed to general declines in these populations. Additional studies should be conducted to confirm these conclusions and to determine if short distance movement or increased feeding without significant movement may be elicited with canola oil.

CONCLUSIONS:

In this study there was no statistical difference in population reductions among the *Beauveria bassiana* treatments or between those treatments and untreated control populations, even though laboratory evaluations showed there was sufficient grasshopper exposure to conidia to cause death. In terms of efficacy, neither canola oil nor Orchex oil showed an advantage. Spray droplets were recorded on all spray cards in both 100% and 50% coverage applications indicating that no area is actually escaping some deposition of spray when reduced treatment areas such as 50% coverage are treated. Canola oil was substantially more viscous than Orchex oil and required significantly higher spray pressures compared to canola oil to deliver similar volumes. CP nozzles and/or highly viscous canola oil produced substantially larger spray droplets compared to flat fan nozzle tips. All of these results indicate that development of *Beauveria bassiana* formulations and evaluations of nozzle and diluent types warrant further significant investigation.

The spinosad studies indicate that doses in the range of 0.75 to 1.5 fluid oz/acre could produce acceptable reduction in grasshoppers while leaving little residual activity at 3 days after treatment. However, replicated efficacy studies should be conducted if spinosad becomes a viable candidate for registration for grasshopper on rangeland. Additional studies will also be required to determine exactly when significant activity against grasshoppers ceases between 0 and 3 days after treatment.

In this study, no conclusive evidence was detected to indicate that canola oil stimulates major grasshopper movement from an untreated area into an area treated with canola oil. However, additional studies should be conducted to confirm these conclusions and to determine if short distance movement or increased feeding without significant movement may be elicited with canola oil.

ACKNOWLEDGEMENTS:

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Figure 1. CP nozzle set up to deliver material through a 0.062 orifice size with a 90° deflection setting.

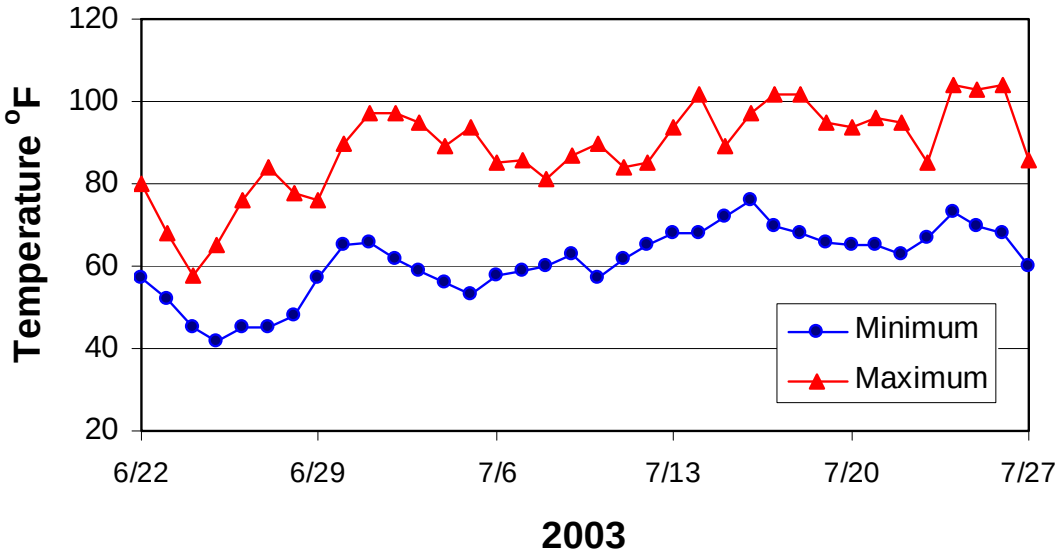

Figure 2. Daily minimum and maximum temperatures recorded in the study area near Edgemont, South Dakota, 2003.

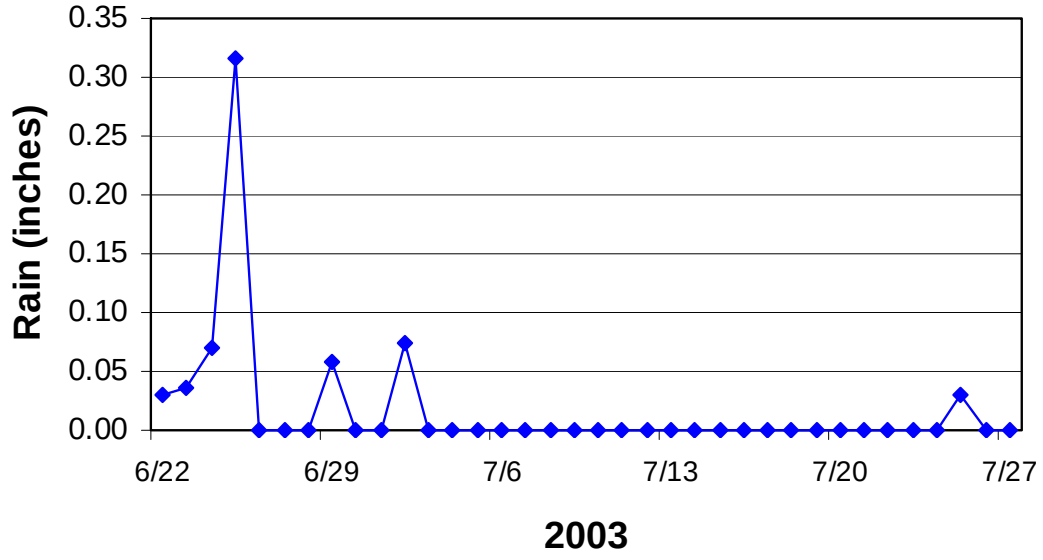

Figure 3. Daily precipitation in the study area near Edgemont, South Dakota, 2003.

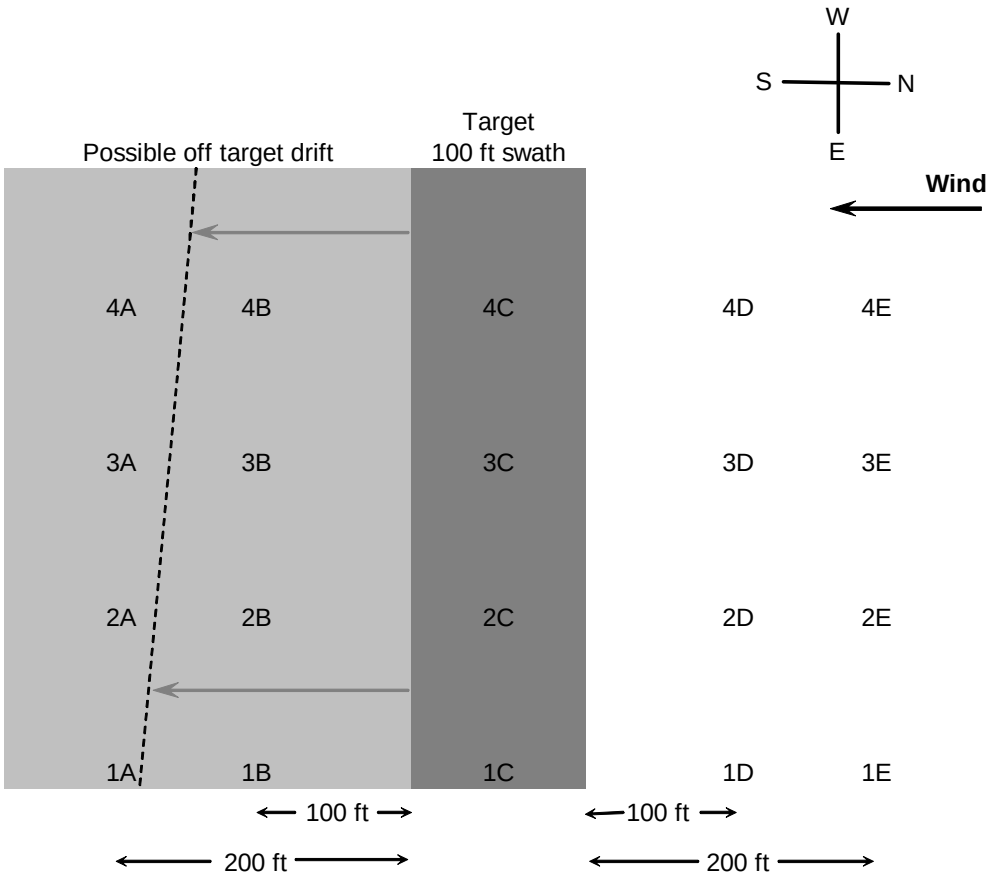

Figure 4a. General schematic of the attractant study (treated area) showing locations of treatment and evaluation sites.

Figure 4b. General schematic of the attractant study (untreated area) showing locations of evaluation sites.

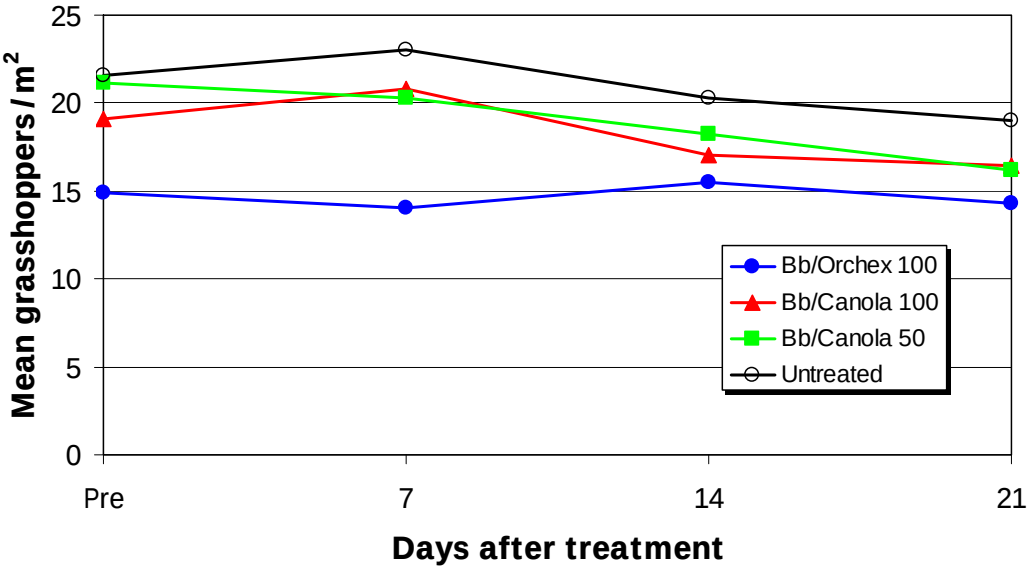

Figure 5. Density of grasshoppers on replicated 40 acre rangeland plots aerially treated with *Beauveria bassiana*. Edgemont, South Dakota, 2003.

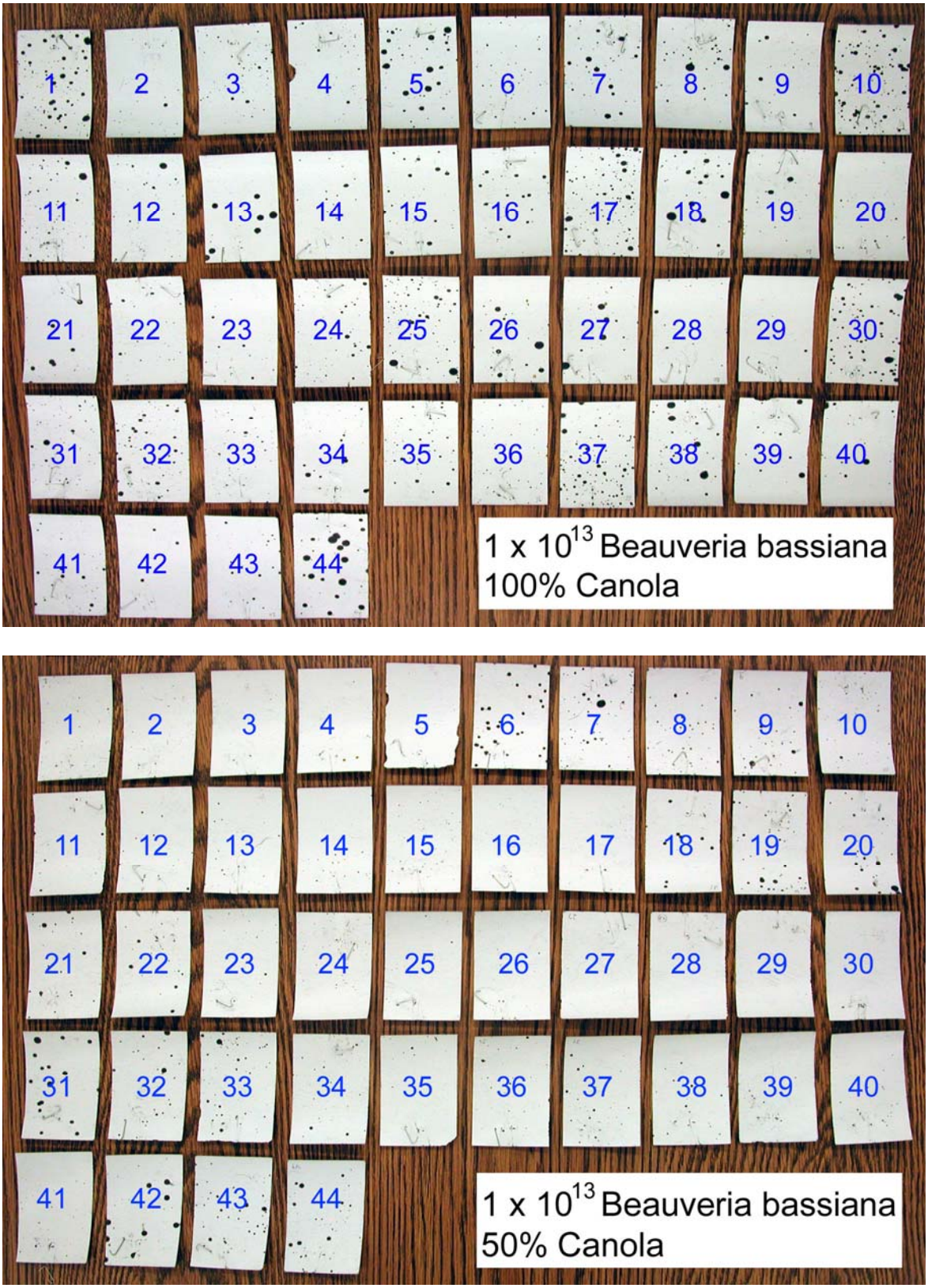

Figure 6. Spray droplets of canola oil formulations on oil sensitive spray cards.

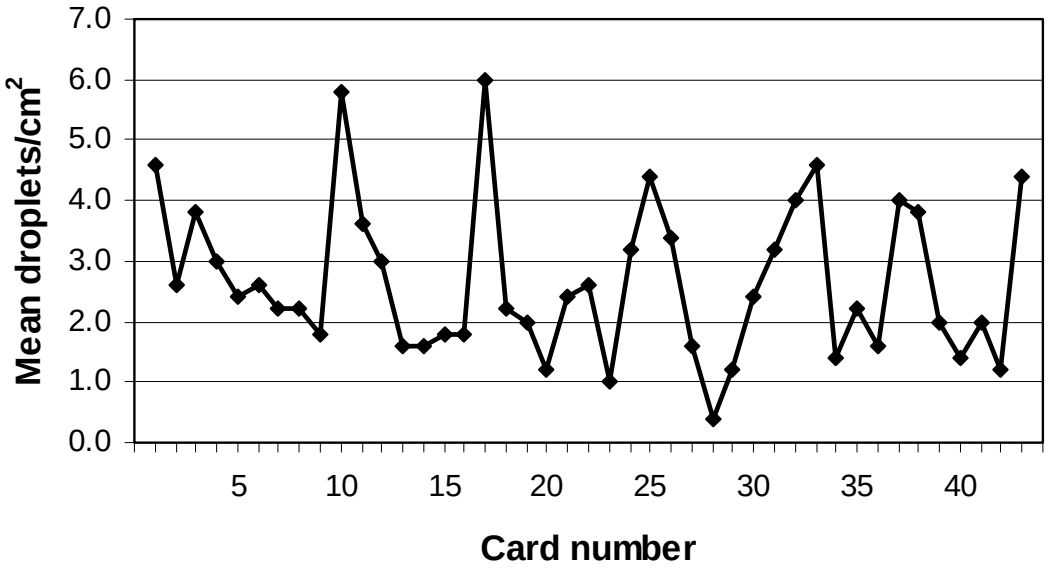

Figure 7. Mean spray droplet deposition/cm^2 in a plot treated with *Beauveria bassiana* and canola oil at 100% coverage.

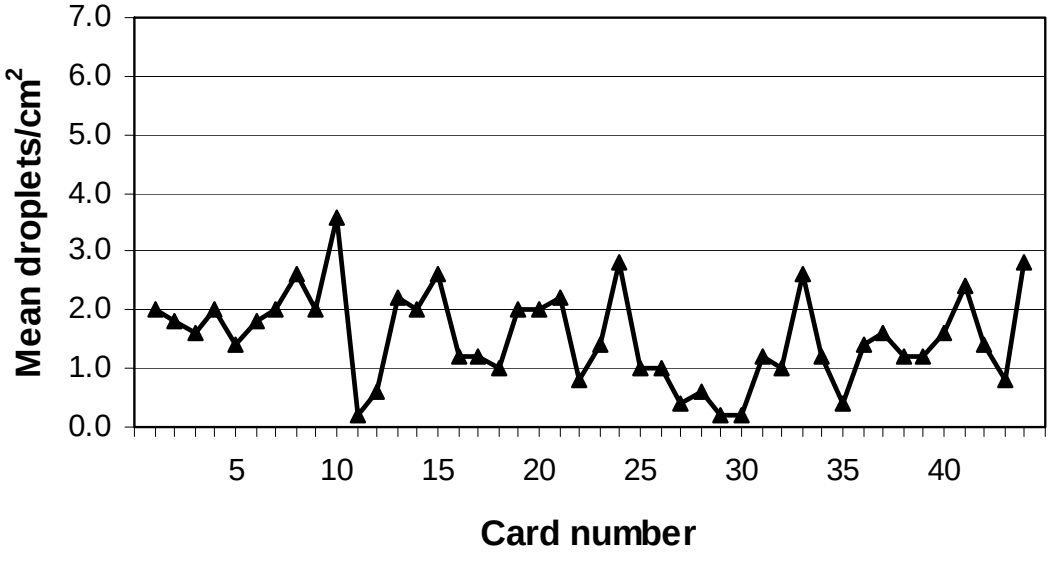

Figure 8. Mean spray droplet deposition/cm^2 in a plot treated with *Beauveria bassiana* and canola oil at 50% coverage.

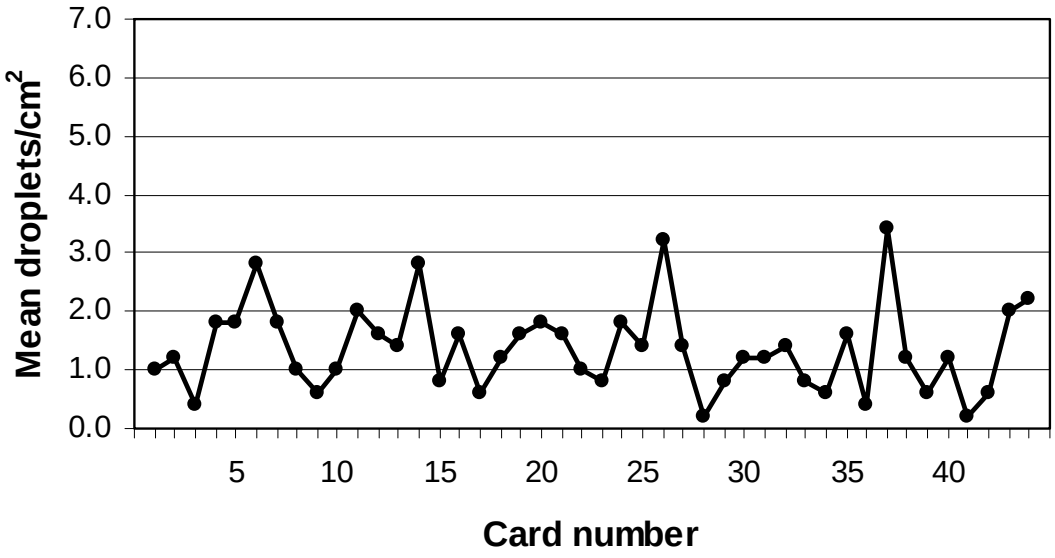

Figure 9. Mean spray droplet deposition/cm^2 in a plot treated with *Beauveria bassiana* and Orchex oil at 100% coverage.

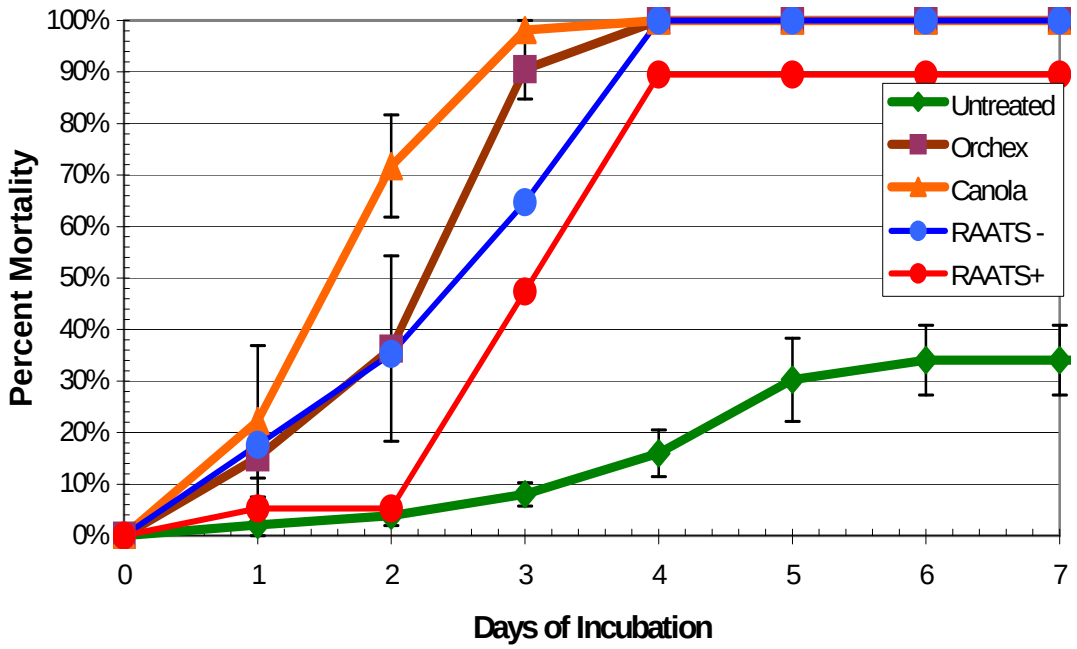

Figure 10. Time course of mortality of *Aulocara elliotti* collected from the 2003 Edgemont field plots immediately after materials were applied, shipped cold to Sidney MT and incubated at constant 28° C. Untreated = untreated control plots; Orchex = $1x10^{13}$ conidia/acre in Orchex oil at 100% coverage; Canola = $1x10^{13}$ conidia/acre in canola oil at 100% coverage; RAATS- = center of an unsprayed swath in the 50% coverage with *Beauveria* conidia in canola oil; RAATS+ = center of sprayed swath. N = 45 insects per sample. Error bars represent standard error of the mean.

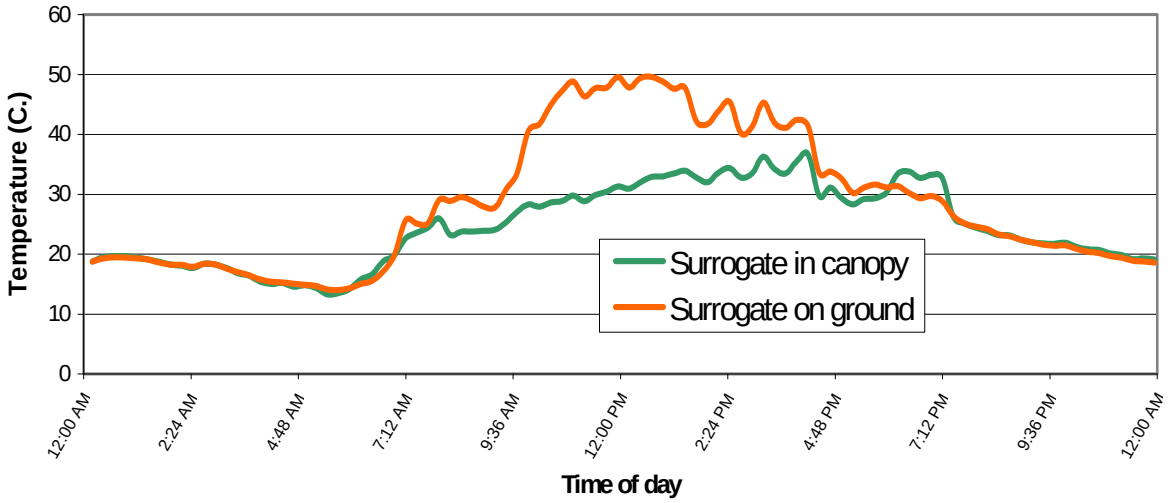

Figure 11. Body temperatures of surrogate grasshoppers on July 7, 2003as an example of typical trends during the trial. Surrogates were positioned either in the grass canopy, approximately 4 inches above the ground or on bare ground. Temperatures 18-32° C. are permissive for *Beauveria* growth; below 18° C., growth is very slow; between 32-34° C. growth is very slow; and above 34° C., growth is virtually stopped. Living grasshoppers would compensate when their body temperatures exceeded 45° C. Temperatures were recorded using copper-constantin thermocouples embedded in 1.5 ml plastic Eppendorff tubes filled with soy sauce, following the method of Lacklin and Johnson, 1989 .

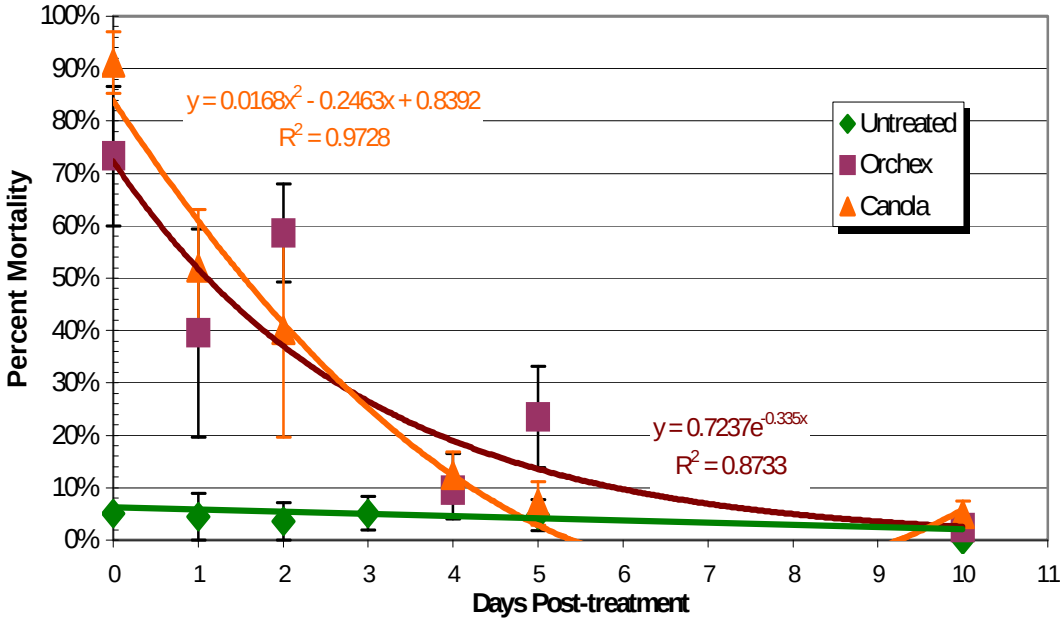

Figure 12. Degradation of *Beauveria bassiana* residues on sprayed vegetation as assessed by mortality of *Melanoplus sanguinipes* teneral adults exposed to vegetation clips for 48 hr then incubated 14 days. Regressions illustrated are best fit equations for each formulation. Error bars represent standard deviations of each point.

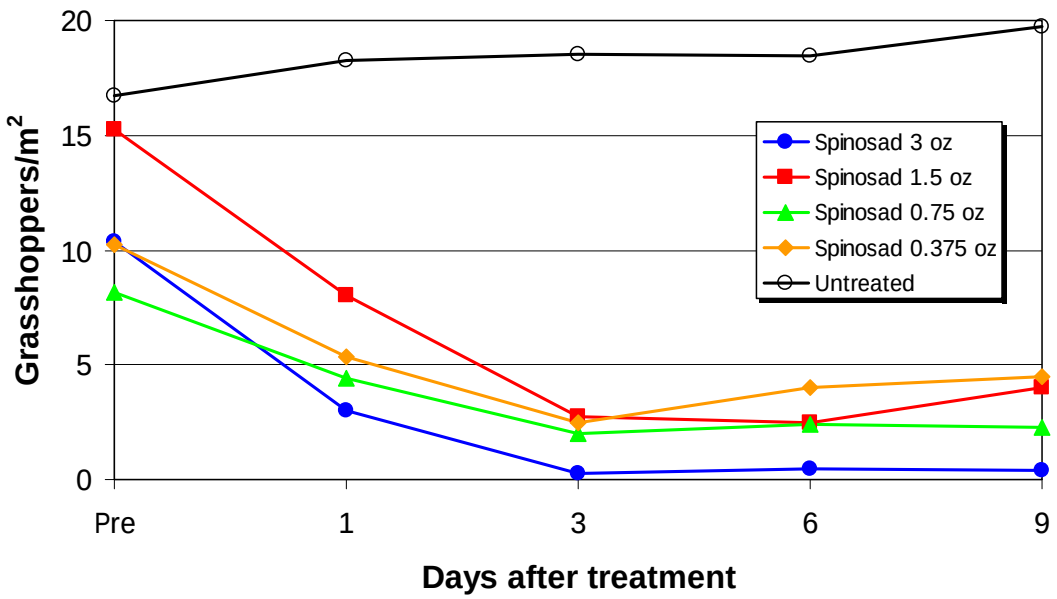

Figure 13. Density of grasshoppers on 10 acre plots treated with four doses of spinosad near Edgemont, South Dakota, 2003.

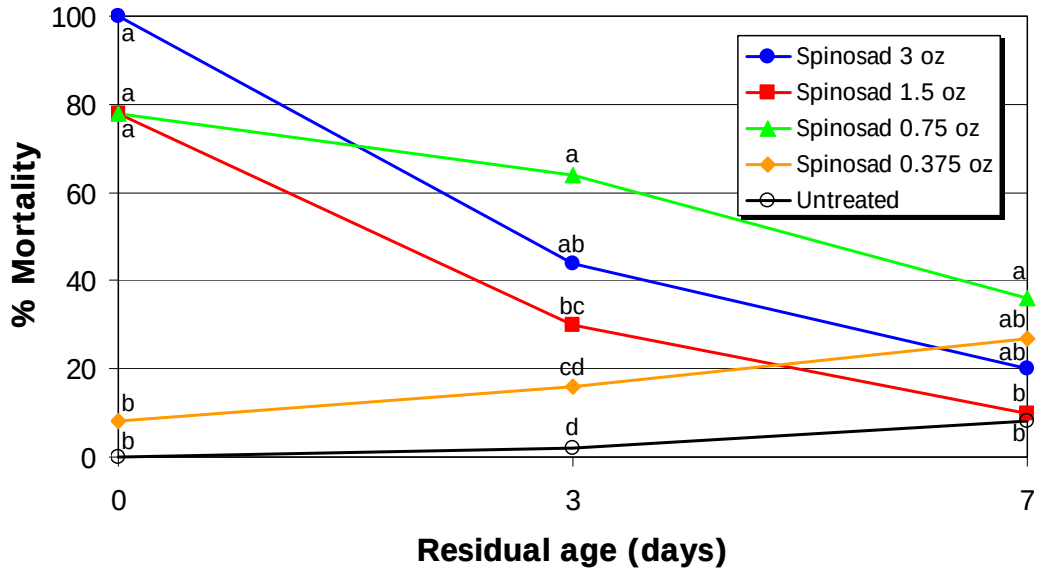

Figure 14. Mean percentage mortality of grasshoppers exposed to selected residual ages of four doses of spinosad in field cages near Edgemont, South Dakota, 2003.

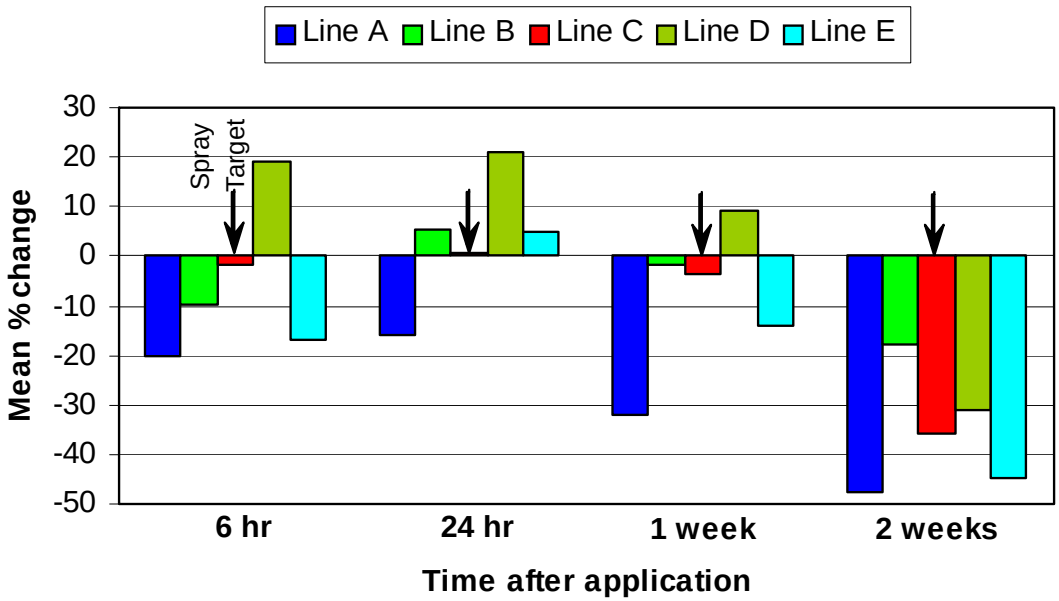

Figure 15. Changes in grasshopper populations within and adjacent to canola oil treated swath.

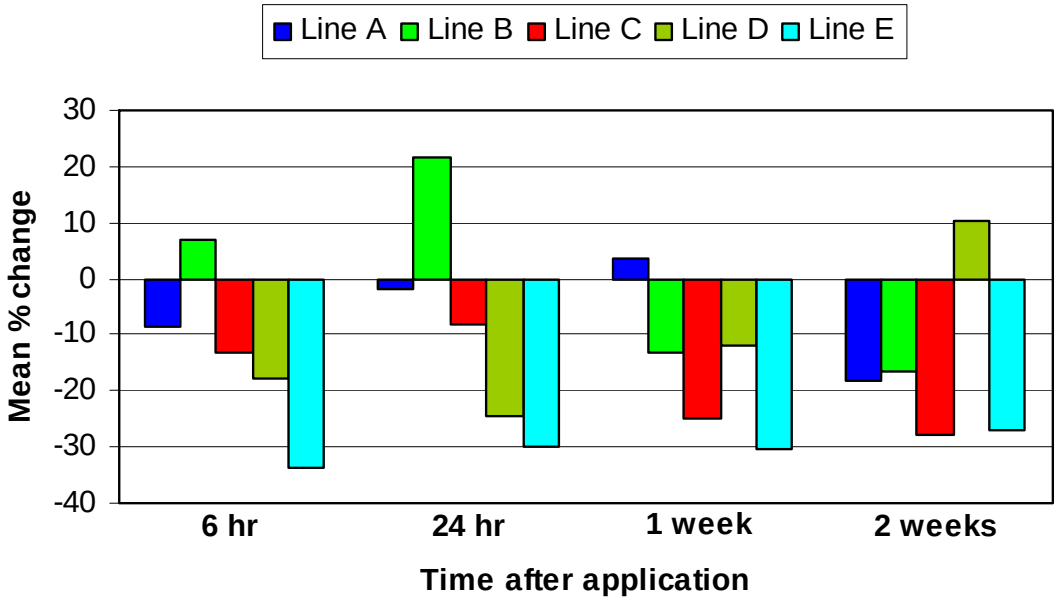

Figure 16. Changes in grasshopper populations in the untreated area of the canola attractant study.

Table 1. Meteorological conditions recorded during aerial application of treatments in the grasshopper study near Edgemont, SD, 2003.

					Temperatures ° F.						
			Time (AM)		Ground		Air		Pilot	Wind (mph)	
Treatment	Plot	Date	Start	End	Start	End	Start	End		Start	End
Tracer 3	D	6/29	5:27	5:37	59	59	55	55	?	1.5 E	1.0 E
Tracer 1.5	B	6/29	5:10	5:17	57	57	54	53	?	1.5 E	2.0 E
Tracer 0.75	A	6/29	6:44	6:50	62	63	57	58	?	1.0 E	< 1 E
Tracer 0.375	C	6/29	6:33	6:37	61	62	58	59	?	0.5 E	1.0 E
Bb/Orch 100	3,7	7/6	5:28	5:42	52	54	53	55	56	1.6-2 W	4-5.3 W
Bb/Orch 100	18	7/6	5:15	5:25	55	54	53	54	53	2-2.5 W	2-3.0 W
Bb/Orch 100	21	7/5	5:15	5:25	58	58	54	55	55	< 1 W	1.5-2 W
Bb/Can 100	8	7/5	7:13	7:23	?	?	66	67	60	2.5 W	1.5-2 W
Bb/Can 100	14	7/5	6:12	6:24	59	59	58	64	58	0.5 W	0.5-1 W
Bb/Can 100	19	7/5	7:00	7:10	?	65	63	66	60	3.0 NW	2.3-4 NW
Bb/Can 100	31	7/5	?	7:35	?	?	70	71	65	2.9 NW	2.5-4 NW
BB/Can 50	16	7/5	6:25	6:33	?	?	62	?	60	< 1 W	< 1 W
BB/Can 50	23,27	7/5	6:35	6:48	60	?	64	65	60	1.0 NW	3.0 NW
BB/Can 50	24	7/5	6:50	6:57	?	?	65	?	60	1-2.0 NW	1-1.5 NW

Table 2. Grasshopper species composition prior to treatment applications in the *Beauveria bassiana* 40 acre plots (incomplete summary – 12/16 plots).

	Instar							
Species	1	2	3	4	5	Adult	Total	%
Subfamily Gomphocerinae								
Ageneotettix deorum		14	28	193	559	21	815	42.94
Amphitornus coloradus			1	19	108	19	147	7.74
Aulocara elliotti		5	1	8	68	60	142	7.48
Aulocara femoratum		4		3	1		8	0.42
Cordillacris occipitalis				1	51	247	299	15.75
Eritettix simplex						3	3	0.16
Mermiria bivittata			3		3		6	0.32
Opeia obscura			1	4			5	0.26
Phlibostroma quadrimaculatum	7	8	3	6	6		30	1.58
Psoloessa delicatula						6	6	0.32
Subfamily Melanoplinae								
Melanoplus angustipennis			1		1		2	0.11
Melanoplus confusus							11	0.58
Melanoplus infantilis		3	13	2	2		20	1.05
Melanoplus occidentalis						3	3	0.16
Melanoplus packardii		3	1	7	10	2	23	1.21
Melanoplus sanguinipes		27	59	69	113	24	292	15.38
Melanoplus "species'		4		1			5	0.26
Phoetaliotes nebrascensis		1		2			3	0.16
Subfamily Oedipodinae								
Hadrotettix trifasciatus		1		4	3		8	0.42
Hippiscus ocelote		2	6	5			13	0.68
Metator pardalinus					3		3	0.16
Pardalophora haldemani						3	3	0.16
Spharagemon collare		1	1	13	23	1	39	2.05
Spharagemon equale				2			2	0.11
Trachyrhachys kiowa				5	5		10	0.53
Total grasshoppers	7	73	118	344	956	400	1898	
%	0.37	3.85	6.22	18.12	50.37	21.07		

Table 3a. Efficacy of *Beauveria bassiana* with selected diluents utilizing conventional and RAATs aerial application spacing on replicated 40 acre plots.

Treatment[1]	Mean % change at indicated days after treatment[2]		
	7	14	21
B. bassiana/Orchex 100%	-7 a	+9 a	0 a
B. bassiana/Canola 100%	+10 a	-9 a	-13 a
B. bassiana/Canola 50%	-3 a	-13 a	-23 a
Untreated	+3 a	-12 a	-14 a

[1] The aircraft was calibrated to deliver spores at the rate of $1x10^{13}$ per acre
[2] ANOVA with Tukey pairwise multiple comparisons, $P \leq 0.05$

Table 3b. Efficacy of *Beauveria bassiana* with selected diluents utilizing conventional and RAATs aerial application spacing on replicated 40 acre plots (adjusted for untreated control mortality).

Treatment[1]	Mean adjusted % mortality at indicated days after treatment[2]		
	7	14	21
B. bassiana/Orchex 100%	7 a	-6 a	-2 a
B. bassiana/Canola 100%	-3 a	4 a	1 a
B. bassiana/Canola 50%	9 a	8 a	12 a

[1] The aircraft was calibrated to deliver spores at the rate of $1x10^{13}$ per acre
[2] ANOVA with Tukey pairwise multiple comparisons, $P \leq 0.05$

Table 4. Mortality and prevalence of *Beauveria bassiana* in cadavers among grasshoppers collected from the 2003 Edgemont field plots. Untreated = untreated control plots; Orchex = $1x10^{13}$ conidia/acre in Orchex oil at 100% coverage; Canola = $1x10^{13}$ conidia/acre in canola oil at 100% coverage; RAATS- = center of an unsprayed swath in the 50% coverage with *Beauveria* conidia in canola oil; RAATS+ = center of sprayed swath. Grasshoper were collected, immediately post application of materials (0 Days), 3 and 10 days after treatment. n = 30-45 insects per sample.

	Days post treatment		***Untreated***	**Orchex**	**Canola**	**RAATS, Spray -**	**RAATS, Spray+**
Aulocara	0	Mortality[1]	**34%**	**100%**	**100%**	**100%**	**89%**
		Bb[2]	*55%*	*88%*	*92%*	*100%*	*100%*
	3	Mortality[1]	**32%**	**95%**	**98%**	**100%**	**67%**
		Bb[2]	*32%*	*72%*	*88%*	*100%*	*71%*
	10	Mortality[1]	**33%**	**80%**	**76%**	**-**	**-**
		Bb[2]	*17%*	*98%*	*92%*		
Ageneotettix	0	Mortality[1]	**25%**	**100%**	**100%**	**60%**	**100%**
		Bb[2]	*36%*	*91%*	*98%*	*100%*	*100%*
	3	Mortality[1]	**21%**	**100%**	**97%**	**-**	**-**
		Bb[2]	*32%*	*72%*	*88%*		
	10	Mortality[1]	**12%**	**78%**	**57%**	**-**	**-**
		Bb[2]	*22%*	*83%*	*83%*		

1 Mortality after two days in transit and 4 days of incubation at 28° C.
2 Prevalence of *Beauveria* among cadavers decontaminated then incubated at 100% relative humidity and 25° C. for 3-4 days
* significantly different from the sprayed swath (Fisher's Exact Test, p<.001)

Table 5. Day 10 mortalities of teneral *Melanoplus sanguinipes* adults exposed for 48 hr to vegetation samples from plots subjected to the various treatments (vegetation samples were collected within 1 hour of application) and incubated a further 7 days at 28° C. Data represent means and standard errors of three replicate bioassays for each vegetation sample.

Treatment	Mean Day 7 Mortality (± S.E.M., n=3)
Untreated Control	5% (0%)
Beauveria in Orchex oil (100% coverage)	73% (13.3%)
Beauveria in canola oil (100% coverage)	91% (5.9%)
Beauveria in canola oil (50% coverage) Unsprayed swath	34% (9.5%)
Beauveria in canola oil (50% coverage) Sprayed swath	50% (25.4%)

Means are not significantly different, ANOVA F 1.29, 3 df, p=0.36

Table 6. Temperature profiles of thermal surrogate grasshoppers during 10 days after application of *Beauveria* to the Edgemont SD site. Temperatures 18-32° C. are permissive for *Beauveria* growth; below 18° C., growth is very slow; between 32-34° C. growth is very slow; and above 34° C., growth is virtually stopped. Temperatures were recorded using copper-constantin thermocouples embedded in 1.5 ml plastic Eppendorff tubes filled with soy sauce, following the method of Lacklin and Johnson, 19 .

	Hours per day					
	canopy			ground		
	<18° C	**18-32° C**	>32° C	<18° C	**18-32° C**	>32° C
4-Jul						
5-Jul	9.00	**5.25**	9.75	7.25	**6.75**	10.00
6-Jul	7.50	**8.25**	8.25	6.75	**8.25**	9.00
7-Jul	3.75	**15.50**	4.75	4.00	**12.50**	7.50
8-Jul	6.00	**9.00**	9.00	5.25	**8.25**	10.50
9-Jul	9.00	**6.00**	9.00	8.00	**5.75**	10.25
10-Jul	8.00	**6.25**	9.75	7.50	**6.50**	10.00
11-Jul	8.00	**7.25**	8.75	6.75	**7.25**	10.00
12-Jul	6.50	**7.75**	9.75	6.50	**6.50**	11.00
13-Jul	7.00	**5.75**	11.25	5.25	**6.75**	12.00
14-Jul	3.00	**11.75**	9.25	2.50	**10.25**	11.25
Total	67.5	**83**	89.5	59.75	**78.75**	101.5

Table 7. Grasshopper species composition prior to treatment applications in the Tracer (spinosad) 10 acre plots.

	Instar							
Species	1	2	3	4	5	Adult	Total	%
Subfamily Gomphocerinae								
Acrolophitus hirtipes					1		1	0.07
Ageneotettix deorum	12	46	187	468	94		807	54.31
Amphitornus coloradus			8	42	19		69	4.64
Aulocara elliotti			3	18	39	1	61	4.10
Aulocara femoratum		4	1	7			12	0.81
Cordillacris occipitalis			1	19	111	19	150	10.09
Eritettix simplex						3	3	0.20
Mermiria bivittata			2	1			3	0.20
Opeia obscura	2	11	4	1			18	1.21
Phlibostroma quadrimaculatum		1					1	0.07
Psoloessa delicatula						10	10	0.67
Subfamily Melanoplinae								
Melanoplus angustipennis			2	1			3	0.20
Melanoplus bowditchi		1	2				3	0.20
Melanoplus confusus						10	10	0.67
Melanoplus infantilis	9	17	13				39	2.62
Melanoplus occidentalis						2	2	0.13
Melanoplus packardii		7	3	3	8		21	1.41
Melanoplus sanguinipes	21	60	58	34	1		174	11.71
Melanoplus "species'	20	15					35	2.36
Subfamily Oedipodinae								
Chortophaga viridifasciata	1						1	0.07
Hadrotettix trifasciatus			2	7			9	0.61
Hippiscus ocelote	2	1	3				6	0.40
Pardalophora haldemani						1	1	0.07
Spharagemon collare	1	1	9	10	1		22	1.48
Spharagemon equale		1	3	1			5	0.34
Trachyrhachys kiowa		4	10	1	1		16	1.08
Xanthippus corallipes						3	3	0.20
Banded-wing "species"			1				1	0.07
Total grasshoppers	68	169	312	613	275	49	1486	
%	4.58	11.37	21.00	41.25	18.51	3.30		

Table 8. Mean percentage mortality of grasshoppers on 10 acre plots treated with four doses of spinosad near Edgemont, South Dakota, 2003.

Treatment [1]	% change in population – days after treatment			
	1	3	6	9
Spinosad 3 oz	-71	-98	-95	-96
Spinosad 1.5 oz	-48	-82	-84	-74
Spinosad 0.75 oz	-46	-75	-71	-72
Spinosad 0.375 oz	-48	-76	-61	-56
Untreated	+9	+11	+10	+18

Table 9. Mean percentage mortality of grasshoppers on 10 acre plots treated with four doses of spinosad near Edgemont, South Dakota, 2003 (adjusted for untreated control mortality).

Treatment [1]	% change in population – days after treatment			
	1	3	6	9
Spinosad 3 oz	73	98	96	97
Spinosad 1.5 oz	52	84	85	78
Spinosad 0.75 oz	51	78	73	76
Spinosad 0.375 oz	52	78	65	63

TITLE: November 2003

Comparison of flakey wheat bran and apple pumice baits for controlling Mormon cricket: Evaluating initial and secondary mortality with baits containing 2% and 5% carbaryl

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INTRODUCTION:

The Mormon cricket is a pest of historic record and is currently demonstrating another cycle of notable population increase to outbreak proportions. In 2002 the combined federal, state and private infested acreage in the western states was estimated to be ca. 3.4 million acres. Although 310,517 acres were protected from Mormon crickets with spray and bait treatments in 2003, slight increases in populations of Mormon cricket are predicted in California, Colorado, Montana and Washington, and major increases are predicted in Idaho, Nevada and Utah for the 2004 treatment season. Historically, treatments have been carbaryl wheat bran or steam rolled oat baits applied by ground equipment. However, in the last two years, significant aerial application has occurred with baits and the recently approved diflubenzuron (Dimilin 2L) spray. Even though the Dimilin spray has proved extremely effective (Foster et al. 2002) and popular the last two years, baits applied by ground or air are an important tool and accounted for 39% of the APHIS sponsored treatments applied to protect lands from Mormon crickets in 2003.

Flaky wheat bran bait (2% carbaryl) is used in most states and steam-rolled wheat and/or apple pumice bait (5% carbaryl) is used in Idaho. Apple pumice bait is available in two forms, pelletized and crumbled and was used for the first time in APHIS sponsored Mormon cricket programs in Idaho in 2002. While its use appeared effective, data has not been generated comparing it with the wheat bran standard used in most states. Studies in

Idaho during 1976-1977 (Foster et al. 1979) and Nevada in 1995 (Foster et al. 1996), have shown no advantage with 5% versus 2% carbaryl on wheat bran baits. However, users in Idaho indicate an advantage with the 5% bait. Development of additional data in Idaho will help to standardize the use of carbaryl concentrations on Mormon cricket bait and may lead to a new bait option or standard for Mormon cricket control. Lower use rates of carbaryl will have a reduced impact on the environment and should reduce the cost of the formulated bait. The following studies were conducted to compare the effectiveness of wheat bran and apple pumice baits containing 2% and 5% carbaryl for initial and secondary kill against immature Mormon crickets in Idaho.

OBJECTIVES:

Evaluate and compare in field cages the efficacy of apple pumice bait (crumbled) and wheat bran bait.

Evaluate and compare in field cages the efficacy of 2% and 5% concentrations of carbaryl in baits.

Determine in field cages the level of secondary kill that can be attributed to selected baits when Mormon crickets are exposed to potential meals of single bait-induced cadavers (Mormon cricket cadavers resulting from mortality produced by poison baits).

Determine in field cages the level of secondary kill that can be attributed to selected baits when Mormon crickets are exposed to potential meals of multiple bait-induced cadavers.

Additionally, explore alternative methods of evaluating the impact of treatments on Mormon cricket field populations.

METHODS AND MATERIALS

The study area was located ca. 15 miles east of Boise, Idaho on rangeland provided by Lori Atkins. The general location was selected because of the presence of Mormon crickets, history of Mormon crickets in the area and availability of rangeland devoid of livestock.

Field Cage Initial Kill Studies.

To determine the primary mortality of Mormon crickets exposed to 10 lbs/acre of 2% and 5% carbaryl in apple pumice and wheat bran baits, small bucket cages (two gallon bottomless buckets modified with screen sides and top) were established on untreated improved rangeland (Figs. 1 and 2). Ten cages were established for each of the five treatments including the untreated control. Each of the treatments including the untreated control, were replicated 4 times. Therefore, the study utilized a total of 200 cages. The appropriate amount of bait was pre-weighed for each cage in the laboratory and introduced into the cages prior to cricket introductions. Cages were stocked at the rate of

one third or fourth instar cricket per cage to prevent cannibalism, a common occurrence when more than one cricket is confined in a cage. Cricket mortality within cages was monitored daily for 8 days. The study was conducted from April 26 through May 3, 2003.

Field Cage Secondary Kill Studies – single cadaver.

Similarly, field cages were established to determine the secondary mortality of Mormon crickets resulting from consumption of poisoned cadavers. In this study unexposed Mormon crickets were confined in untreated field cages with crickets freshly killed using the previously described treatments. The freshly killed crickets were obtained by confining single unexposed crickets to appropriate amounts of bait in 4.5 inch diameter x 3 inch high squat paper cups with screen tops in a makeshift laboratory established in a motel room. Again, all treatments were replicated 4 times with 10 cages per replicate utilizing a total of 200 squat cups and 200 bucket cages. An artificially killed (a non toxic death produced by blunt force trauma) cricket was included in the cages designated as untreated controls. Cricket mortality within cages was monitored daily for 8 days. The study was conducted from April 29 through May 7, 2003.

Field Cage Secondary Kill Studies – multiple cadavers.

Additionally, 10 bucket cages were established to determine the level of secondary kill that can be attributed to 10 lbs/acre of 5% carbaryl wheat bran bait when Mormon crickets are exposed to potential meals of multiple bait-induced cadavers. Each cage was again stocked with one unexposed cricket. In this study, crickets were killed nightly in the makeshift laboratory in squat cups with the appropriate amount of bait. A freshly killed cricket was then introduced into each cage daily for 8 days. Therefore, each healthy cricket was exposed to a total of 8 freshly killed crickets introduced daily without removing previously introduced cadavers. The untreated controls from the previously described study also served as controls in this study. Cricket mortality within cages was monitored daily for 8 days. The study was conducted from April 29 through May 7, 2003.

Exploring Alterantive Mormon Cricket Treatment Evaluations.

During the period of the studies and in the general vicinity of the studies, on three separate days the two senior authors slowly traveled over an 11 mile stretch of dirt road observing and recording the frequency of occurrence of bands and density of Mormon cricket within the bands encountered. A GPS unit was used to record the beginning and end of each band of crickets as it was encountered.

Analysis.

Analysis of all cage study data relied on a one-way analysis of variance and an all pair wise multiple comparison procedure (Tukey Test). Analyses were conducted on both unranked and ranked data.

RESULTS AND DISCUSSION

Field Cage Initial Kill Studies.

Numerically, both wheat bran and crumbled apple pumice baits generally performed in dose rank order except at one and two days after treatment where the 2% crumbled produced higher mortality than the 5% crumbled (Fig. 3, Table 1 and 2). However, these differences were not significant. All four bait treatments demonstrated statistically equivalent performance in terms of mortality and produced mortality significantly greater than seen in the untreated controls. Mortality increased for all four treatments from one through three days after treatment. However, mortalities increased little if any from day 4 through the remainder of the study at 7 days after treatment. Mortality in the untreated control populations ranged from 2.5% to 5% during the study. These results with both baits were similar to those seen with wheat bran bait on immature crickets in Idaho in 1977 (Foster et al. 1979).

Field Cage Secondary Kill Studies – single cadaver.

Because great variability was seen in this study between replicates, generally, no significant secondary mortality was detected among the treatments or between the treatments and the untreated controls (Fig. 4, Table 3 and 4). However, numerical values do indicate that crumbled baits trended toward higher secondary mortality compared to wheat bran baits. Additionally, at 8 days after treatment, while there was not statistical difference among any of the baits in producing secondary mortality, there was a significant difference in mortality between crickets in the untreated controls and those exposed to cadavers resulting from the 5% crumbled bait. This significant difference was also seen in the analysis of unranked data at 2 days after treatment. No secondary mortality was indicated with the wheat bran baits in this study which was consistent with the results from unpublished secondary kill studies conducted in Nevada in 1995 by the two senior authors (Foster et. al 1996).

Field Cage Secondary Kill Studies – multiple cadavers.

In this study each of 10 separate and unexposed caged Mormon crickets were presented daily with a recently produced cadaver that resulted from ingestion of 5% carbaryl wheat bran. These potential meals accumulated over the 8 days of the study in each cage so that each Mormon cricket in the study had an opportunity to feed on a total of 8 bait induced cricket cadavers. Compared to the untreated control, little mortality was recorded during the first three days and only slightly more was seen through 6 days after beginning of exposure to cadavers (Fig. 5). At 8 days, a 45% difference in mortality was demonstrated between the untreated controls and the crickets exposed to treatment induced cadavers. This difference appears more similar to that seen for crumbled apple pumice bait than wheat bran bait in the previous study. However, in this study each unexposed cricket was ultimately exposed to a greater level of toxicant in the accumulated cadaver meals compared to the previous study. It is important to note that this was essentially an unreplicated study. Never the less, the data indicate a potential for secondary kill to occur if an unexposed Mormon cricket had an opportunity to feed on several freshly bait induced cadavers over several days. In a realistic field situation both uneaten bait and cadavers resulting from ingestion of bait would be present as a potential meal for a

Mormon cricket thus confounding any determination as to exact cause of mortality. Additional studies will be required to fully reveal potential levels of secondary mortality that may be realized with baits on Mormon cricket.

Exploring Alterantive Mormon Cricket Treatment Evaluations.

The following outline was devised and is suggested for future trials to evaluate the effectiveness of Mormon cricket treatments over large areas.

Mormon cricket presence may be evaluated by counting and recording numbers of bands, width of bands (linear distance encountered), and if possible, density of bands along a known distance either on roads and trails or on established transects within the area to be protected by treatment.

A minimum number of miles of roads, trails or transects should be predetermined. The method of travel on roads, trails or transects to be determined as appropriate.

The distance surveyed on the road, trail or transect is extremely important. It must be measured. If an odometer is available it should be used to measure and record the distance traveled during the survey and the length of each band of crickets encountered. When a band is encountered it should be recorded as a band. A GPS reading should be recorded at the beginning and end of each band encountered. If possible the density of the band should be determined and recorded (method to be determined). Eventually the measured distance of bands encountered per distance of survey conducted will be compared between pre and post-treatment counts for both treated and untreated blocks. Additionally, the GPS locations of the bands will allow for accurate plotting of the bands and will facilitate constructing replication (pseudo replication) (electronically dividing each plot into 4 separate zones) in the data set for analysis if needed.

Pre-treatment data collection should occur in both the treated plot and the untreated plot 1-2 times before treatment occurs. The closer to the treatment date the better. Preferably 1-2 days before treatment. In case inclement weather occurs the day before treatment, an earlier pre-treatment count in both plots should have been taken. Counts in both plots should be occurring at the same time. This insures that weather conditions impact the same on both groups of cricket populations and survey data. Good pre-treatment data is required for post-treatment reductions to be calculated.

Post treatment data collection (in both plots) should be conducted similarly to pre treatment counts and should occur at least two times a week after treatment occurs (duration in weeks to be determined).

Weather data for the duration of the study (pre through post treatment) should be collected and is most important during the actual time that data is collected.

In Summary:

In each Plot:
Measure distance of route surveyed during each survey.
Measure linear distance of each band encountered
Maintain a running total of the number of bands encountered during each survey run.
Take a GPS reading at the beginning and each of each band encountered.
If possible categorize the density of the band.
Record all data as encountered including meteorological conditions.

At present there is no method used to quantify the success of a Mormon cricket control or suppression program other than by recording satisfaction or dissatisfaction voiced by the landowners or managers. Implementing the above in a trial situation may for the first time provide some definitive numerical evaluation of the efforts undertaken to control Mormon cricket.

CONCLUSIONS:

Wheat bran and apple pumice baits resulted in equally effective primary mortality to third and fourth instar stage Mormon crickets confined in field cages. The 5% concentration of carbaryl showed no advantage over the 2% concentration in terms of mortality when presented to immature Mormon crickets. In single cadaver presentation studies, little if any significant secondary mortality was seen with either type of bait. An additional study with multiple cadaver presentations only indicated any substantial increase in secondary mortality after 6 days and the potential for feeding on 6 cadavers. While advantages of the higher concentration were not evident in the secondary mortality studies with wheat bran bait, some indications (although not statistically significant) of increased secondary mortality were seen with the higher concentration of carbaryl in the apple pumice bait against immature Mormon crickets. All of these studies were conducted against immature Mormon crickets. Additional studies should be conducted with both concentrations and types of baits against adult Mormon crickets to fully reveal any advantages that the higher concentration of carbaryl may provide.

With Mormon cricket outbreaks increasing in importance and scope of impact, a quantitative method of evaluating the treatment efforts to mitigate these pests should be developed. Basing an evaluation on the linear amount of Mormon cricket bands encountered per linear ground surveyed in pre and post-treatment surveys may provide for the first time, some definitive numerical evaluation of the efforts undertaken to control Mormon crickets and should be evaluated as a trial in an operational Mormon cricket control program.

ACKNOWLEDGEMENTS:

The authors thank Lori and Donna Atkins for providing land used in the study, facilities for storage of equipment and shelter for the researchers during inclement weather. Thanks are also extended to Neal Wagner, Peacock Industries for providing wheat bran bait and to Joe Millinax, Wilber Ellis for facilitating the obtaining of apple pumice bait.

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Figure 1. Field bucket cage studies near Boise, Idaho 2003.

Figure 2. Evaluating a field cage for Mormon cricket mortality.

Figure 3. Primary mortality of immature Mormon crickets exposed to selected carbaryl baits in field cages – Boise, Idaho 2003.

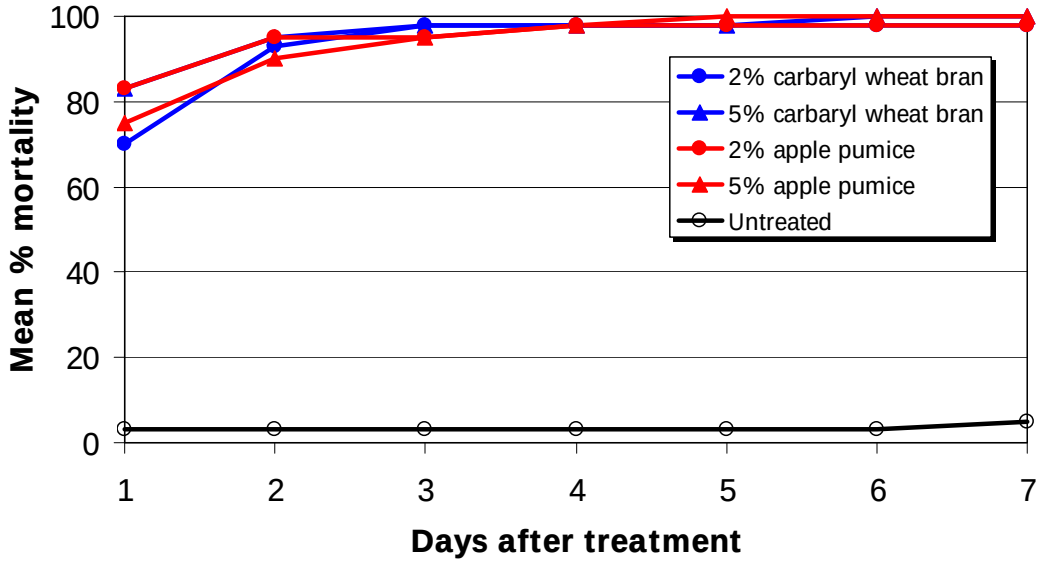

Figure 4. Secondary mortality of immature Mormon crickets exposed to single selected bait induced cadavers in field cages – Boise, Idaho 2003.

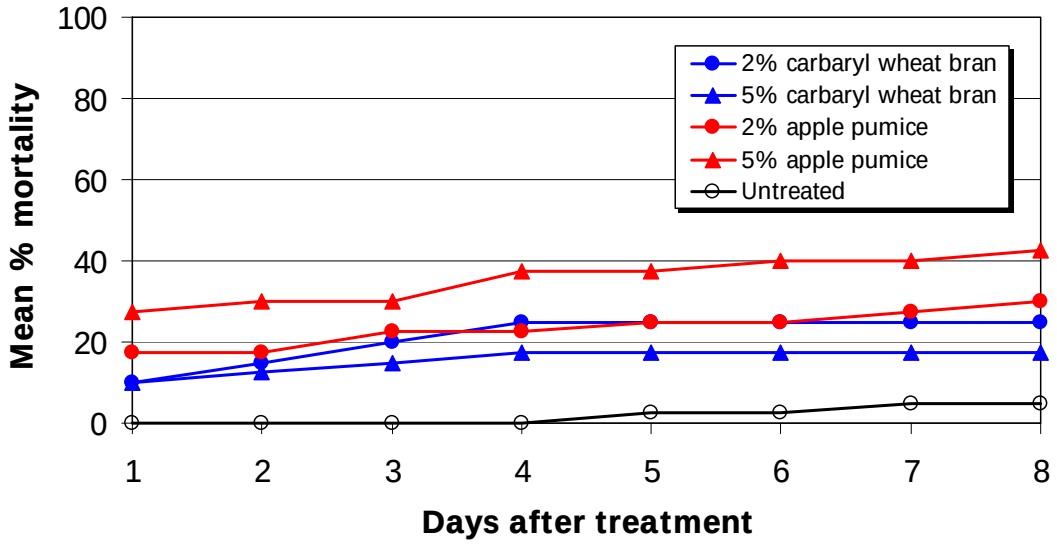

Figure 5. Secondary mortality of immature Mormon crickets exposed to multiple cadavers in field cages – Boise, Idaho 2003.

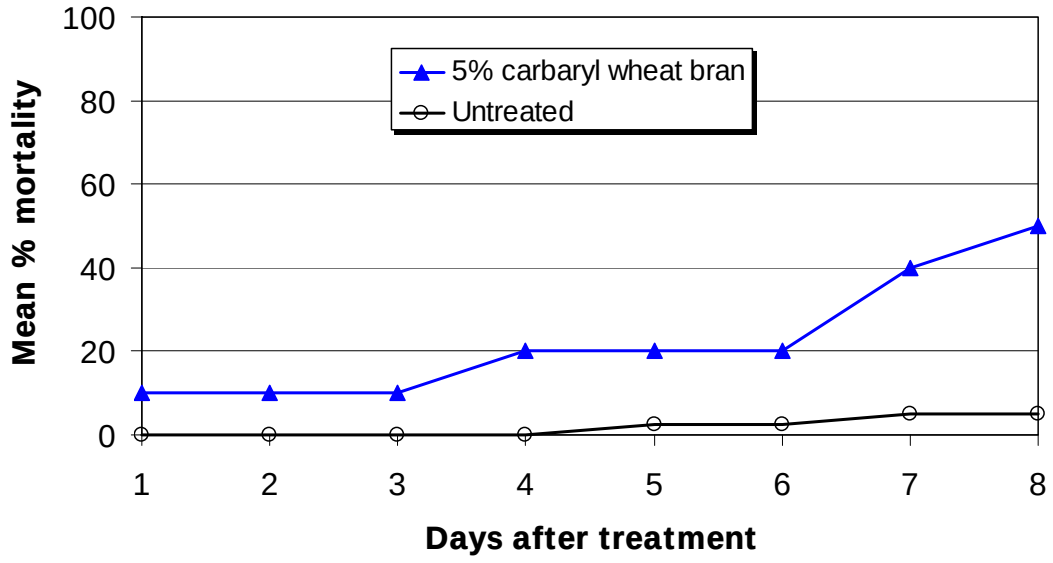

Table 1. Primary mortality of immature Mormon crickets exposed to selected baits in field cages (unranked data).

	Mean % mortality at indicated days after treatment [2]						
Treatment [1]	1	2	3	4	5	6	7
2% cwb	70 a	93 a	98 a	98 a	98 a	98 a	98 a
5% cwb	83 a	95 a	98 a	98 a	98 a	100 a	100 a
2% cap	83 a	95 a	95 a	98 a	98 a	98 a	98 a
5% cap	75 a	90 a	95 a	98 a	100 a	100 a	100 a
Untreated	3 b	3 b	3 b	3 b	3 b	3 b	5 b

[1] **cwb** = carbaryl wheat bran bait and **cap** = carbaryl apple pumice bait
[2] ANOVA with Tukey pairwise multiple comparisons, $P \leq 0.05$

Table 2. Primary mortality of immature Mormon crickets exposed to selected baits in field cages (ranked data).

Treatment [1]	Mean % mortality at indicated days after treatment [2]						
	1	2	3	4	5	6	7
2% cwb	70 ab	93 a	98 a	98 a	98 a	98 a	98 a
5% cwb	83 a	95 a	98 a	98 a	98 a	100 a	100 a
2% cap	83 a	95 a	95 a	98 a	98 a	98 a	98 a
5% cap	75 a	90 ab	95 a	98 a	100 a	100 a	100 a
Untreated	3 b	3 b	3 b	3 b	3 b	3 b	5 b

[1] **cwb** = carbaryl wheat bran bait and **cap** = carbaryl apple pumice bait
[2] ANOVA with Tukey pairwise multiple comparisons, $P \leq 0.05$

Table 3. Secondary mortality of immature Mormon crickets exposed to selected bait induced cadavers in field cages (unranked data).

Treatment [1]	Mean % mortality at indicated days after treatment [2]							
	1	2	3	4	5	6	7	8
2% cwb	10 a	15 ab	20 a	25 a	25 a	25 a	25 a	25 ab
5% cwb	10 a	13 ab	15 a	18 a	18 a	18 a	18 a	18 ab
2% cap	18 a	18 ab	23 a	23 a	25 a	25 a	28 a	30 ab
5% cap	28 a	30 a	30 a	38 a	38 a	40 a	40 a	43 a
Untreated	0 a	0 b	0 a	0 a	3 a	3 a	5 a	5 b

[1] **cwb** = carbaryl wheat bran bait and **cap** = carbaryl apple pumice bait
[2] ANOVA with Tukey pairwise multiple comparisons, $P \leq 0.05$

Table 4. Secondary mortality of immature Mormon crickets exposed to selected bait induced cadavers in field cages (ranked data).

Treatment [1]	Mean % mortality at indicated days after treatment [2]							
	1	2	3	4	5	6	7	8
2% cwb	10 a	15 a	20 a	25 a	25 a	25 a	25 a	25 ab
5% cwb	10 a	13 a	15 a	18 a	18 a	18 a	18 a	18 ab
2% cap	18 a	18 a	23 a	23 a	25 a	25 a	28 a	30 ab
5% cap	28 a	30 a	30 a	38 a	38 a	40 a	40 a	43 a
Untreated	0 a	0 a	0 a	0 a	3 a	3 a	5 a	5 b

[1] **cwb** = carbaryl wheat bran bait and **cap** = carbaryl apple pumice bait
[2] ANOVA with Tukey pairwise multiple comparisons, $P \leq 0.05$

Dec. 2003

Field Cage Evaluation of Tast-E Bait Against the Rangeland Grasshopper, *Ageneotettix deorum.*

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Advanced Organic Inc., David I. B. Vander Hooven, Maumee. OH.

Abstract

A preliminary study conducted in southwestern South Dakota with two concentrations of carbaryl incorporated into the Advanced Organic Inc. Tast-E-Bait bait formulations demonstrated activity against caged populations of the rangeland grasshopper *Ageneotettix deorum*. In these studies the formulations produced mortality ranging from 14% to 68% and 18% to 76% for 2% and 5% concentrations of carbaryl respectively when the bait was applied at 10 lbs/acre. This compared with 0% to 8% mortality seen in the untreated controls over the same period of time.

Introduction

Baits containing carbaryl have been used for many years to suppress grasshopper or Mormon cricket populations on western rangelands. Currently carbaryl is used in wheat bran and apple pumice based baits at both 2% and 5% concentrations against Mormon crickets and is commonly applied at a rate of 10 lbs/acre with ground or aerial application. Grasshoppers are currently treated with 2% carbaryl bran bait applied at 2 lbs/acre by ground and 1.5 lbs/acre by air. A recent increase in both grasshopper and Mormon cricket populations in the western states has renewed interest in developing new options for controlling these rangeland pests. A new product, Tast-E-Bait, produced by Advanced Organic Inc. has recently become available for evaluation. While extensive evaluation was planned with this product against Mormon crickets in Idaho earlier in the year (Fig. 1), logistics in delivering the product precluded its inclusion in the earlier studies. In conjunction with several other studies occurring in South Dakota during the summer of 2003, the following study was designed as a cursory evaluation of activity against a common rangeland grasshopper to develop some preliminary data with this product.

Methods and Materials

The study was conducted in Fall River County of southwestern South Dakota ca. 12 miles west and 9 miles north of the town of Edgemont on the Mark Tubbs ranch during the period of June 28 through July 5, 2003. The location was selected because of the density of grasshoppers, history of grasshoppers in the area, availability of a pasture of un-stocked rangeland for the period of time required in the study and the closeness to other studies occurring in the area.

The Advanced Organic Inc. Tast-E-Bait formulations containing 2% and 5% carbaryl and bakers waste product, were applied to rangeland in cages. Both formulations were applied at the rate of 10 lbs/acre. Ten cages (two gallon bottomless buckets modified with screen sides and top) were established for each formulation. Ten additional cages were established on nearby untreated vegetation as controls for comparison. Each cage was stocked with five, fourth instar stage *Ageneotettix deorum* grasshoppers collected from an adjacent untreated area. This species was selected because it was the most prevalent rangeland grasshopper species in the area. The appropriate amount of bait was pre-weighed in the laboratory and introduced into each cage separately in the field. Grasshoppers were introduced into cages after bait was applied. Both treated and untreated cages were monitored daily for 7 days after exposure to treatments occurred (Fig. 2). Unfortunately, time constraints due to previous commitments prevented a direct comparison with standard wheat bran or apple pumice carbaryl bait in this study.

Results

In this study, at one day after treatment, no statistical difference in population reductions occurred with either formulation compared to the untreated control populations (unranked data, Table 1). However, with ranked data at one day after treatment, the highest concentration of Tast-E-bait, 5%, produced significantly greater reduction than the untreated control but was statistically equivalent to the lower dose (Table 2). From two days after treatment until the end of the study at seven days, both formulations performed statistically equivalent to each other and produced mortality significantly greater than seen in the untreated controls. Both formulations demonstrated steady increases in mortality during the study (Fig. 3). Surprisingly, the two formulations did not consistently perform numerically in dose rank order. This study indicates activity against rangeland grasshoppers and warrants further comparative studies with other currently used baits against several rangeland grasshopper species and Mormon cricket.

Acknowledgements

The authors thank David I. B. Vander Hooven, Advanced Organic Inc. for providing the material for testing and Neil Wagner, Peacock Industries, for formulating the material. Thanks are extended to Mark Tubbs for providing the rangeland for the study and continued cooperation since 1989.

Figure 1. Evaluating Mormon cricket bait in cages in Idaho.

Figure 2. Monitoring bucket cages for grasshopper mortality in South Dakota.

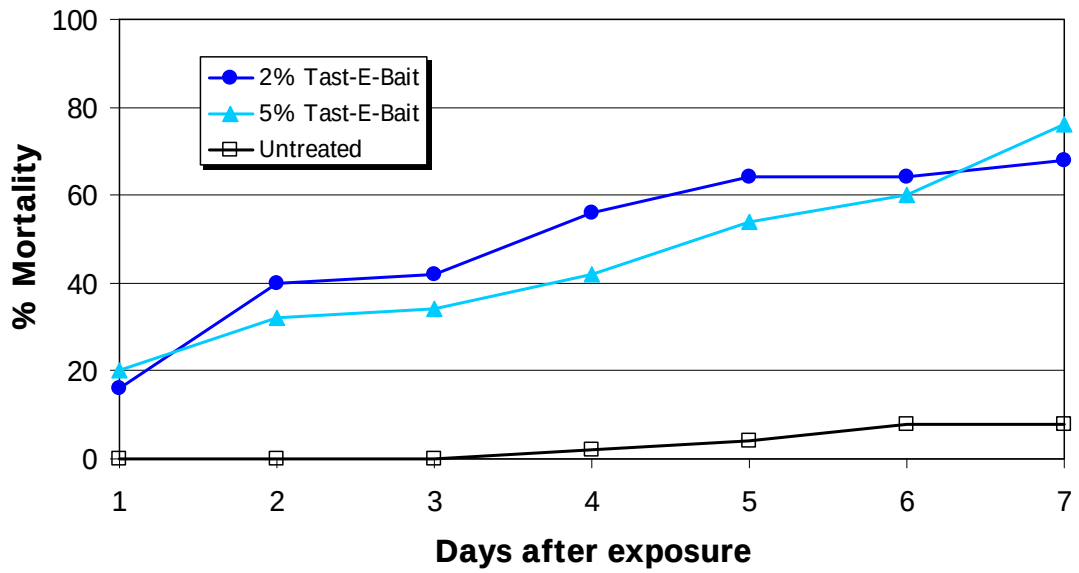

Figure 3. Mortality of caged *Ageneotettix deorum* grasshoppers treated with Tast-E-Bait.

Table 1. Efficacy of Tast-E-Bait® (carbaryl) against 4th instar *Ageneotettix deorum* grasshoppers in field cages using **unranked** data in the analysis.

	Mean % reduction at indicated days after treatment						
Treatment [1]	1	2	3	4	5	6	7
2% Tast-E-Bait	14 a	36 a	42 a	54 a	62 a	64 a	68 a
5% Tast-E-Bait	18 a	30 a	32 a	42 a	54 a	60 a	76 a
Untreated	0 a	0 b	0 b	2 b	4 b	8 b	8 b

[1] Bait composed of bakers waste product treated with carbaryl.
[2] ANOVA with Tukey pairwise multiple comparisons, $P \leq 0.05$

Table 2. Efficacy of Tast-E-Bait® (carbaryl) against 4th instar *Ageneotettix deorum* grasshoppers in field cages using **ranked** data in the analysis.

	Mean % reduction at indicated days after treatment						
Treatment [1]	1	2	3	4	5	6	7
2% Tast-E-Bait	14 ab	36 a	42 a	54 a	62 a	64 a	68 a
5% Tast-E-Bait	18 a	30 a	32 a	42 a	54 a	60 a	76 a
Untreated	0 b	0 b	0 b	2 b	4 b	8 b	8 b

[1] Bait composed of bakers waste product treated with carbaryl.
[2] ANOVA with Tukey pairwise multiple comparisons, $P \leq 0.05$

December, 2002 rev.

TITLE:

Field Evaluation of Aerially Applied Dimilin and Field Cage Evaluation of Selected Doses of Dimilin and Fipronil Bran Baits Against Mormon Cricket, *Anabrus simplex* Haldeman, on Rangeland in Utah.

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INTRODUCTION:

The Mormon cricket is a pest of historic record. In 2002 the combined federal, state and private infested acreage in Utah alone was estimated at ca 3.2 million acres. However, these lands are not uniformly infested. Damaging populations of crickets occur in bands, which are highly mobile and therefore may require immediate response, spot treatments resembling old fashion warfare (3 to 4 months of labor and equipment intensive efforts) or uniform blanket aerial treatments (uneconomical) of the entire area. The traditional treatment is carbaryl bran bait, usually applied by ground equipment (pick-up truck or ATV mounted spreader). While very effective against Mormon crickets, the field life of bran bait is short lived (only a few days). Previous experience (1988-1999) in the development of Dimilin, an insect growth regulator, for range grasshoppers (Foster et al. 1991, 1993, 1996-99; Jech et al. 1993) and in the development (1997-1999) of reduced agent – area treatment (RAATs) technologies (Foster et al. 2000) suggest that this combination could substantially improve the overall economics and quality of Mormon cricket control efforts. Dimilin provides the residual activity needed for control of highly

mobile populations over an extended period of time. RAATs technology offers ease in application coupled with reduced acreage treated and improved economics compared to uniform coverage aerial spray treatments or ground applied treatments which occur over a period of weeks or months. Dimilin may offer the opportunity to treat the block of concern one time rather than continuing treatments over the entire season. Early season use should result in substantial reduction in personnel requirements, which will free up personnel for other unrelated or similar activities over larger areas. Additionally, new baits may offer additional opportunities for dealing with Mormon crickets in an integrated pest management manner.

Several state and federal land managing agencies, private land owners, Uniroyal Chemical and USDA-APHIS are extremely interested in determining the effectiveness of Dimilin against Mormon cricket. Both Uniroyal and Aventis Crop Science are interested in evaluating additional baits for use against Mormon cricket. The following study was conducted to evaluate the usefulness of Dimilin spray and Dimilin and Fipronil baits for control or suppression of Mormon cricket on rangeland.

OBJECTIVES:

Determine if intensive field density estimates of Mormon cricket populations are usable for comparison of selected treatments.

Determine the level of population reduction that Dimilin RAATs provides compared to the standard carbaryl bran bait treatment.

Determine the economics associated with Dimilin RAATs compared to the standard carbaryl bran bait treatment.

Evaluate oil and water sensitive spray cards for use in droplet deposition studies.

Determine in field cages, the length of effective residual activity of Dimilin against Mormon cricket.

Evaluate in field cages, three doses of Dimilin in a bait formulation for effectiveness against Mormon cricket.

Evaluate in field cages (in terms of significantly increasing mortality), the effectiveness of a coating viewed as a potential feeding attractant added during the formulation of Dimilin bait.

Evaluate in field cages, three doses of Fipronil for initial and secondary kill effectiveness against Mormon cricket.

MATERIALS AND METHODS:

Field Studies.

The center of the study area was located in eastern Millard County, Utah ca. 14 miles SE of Delta (10 miles NE of Holden). The study utilized land provided by Greg Kesler, Wesley Stephenson and the US Forest Service. The general location was selected because of the presence of Mormon cricket, history of Mormon cricket in the area and abundance of contiguous rangeland.

Two adjacent areas, 640 acres (1 mile x 1 mile) each, with similar Mormon cricket populations were scheduled for treatment; one by air with Dimilin and the RAATs strategy, the other with ground applied bran bait in the standard program manner for Utah. A third adjacent similarly infested 640 acre plot (1 mile x 1 mile) was used as an untreated control for comparison.

The Dimilin 2L formulation of diflubenzuron was applied with a Cessna Ag Truck owned by the USDA, Animal and Plant Health Inspection Service (APHIS) and equipped with winglets (DBA – Ag Tips: Clack Oberholtzer, Alberta, Canada). Winglets are added to spray aircraft to reduce the production of fine droplets and and to improve handling characteristics. The aircraft was operated by a USDA – APHIS pilot. The aircraft was equipped with a standard commercial spraying system and differentially corrected guidance and recording system. Ground personnel also provided guidance and ensured acceptable operating parameters during application. The Dimilin was applied at 0.0117 lb AI/ acre = 5.3 grams/acre (0.75 fluid ozs) in a total mix volume of 31 fluid oz/acre to 50% of the plot. Specifically the treatment was formulated to contain 0.75 fluid oz of Dimilin 2L, 10 fluid oz of Herbimax Oil-Surfactant Adjuvant (Loveland Industries Inc. Greely, CO) and 20.25 fluid oz of water/acre. A spray dye, Hi-Light Blue Spray Indicator, was also added to the mix at the rate of 0.3 oz per gallon to facilitate droplet analysis on spray cards. Treating 50% of the plot was achieved by calibrating the aircraft for a 100 foot wide swath and spacing the aircraft during treatment at 200 feet. Prior to application the aircraft spray system was calibrated to operate under parameters which resulted in delivery of spray within 1% of the desired rate per acre. Calibration was accomplished by collecting and measuring the amount of material sprayed through each nozzle for a predetermined amount of time, and making adjustments in pressure until the desired output was achieved.

The treatment was applied from an altitude of ca 50 feet at 120 mph and 36 psi through 20 (8003) size flat fan Tee Jet stainless steel nozzle tips oriented straight down. Treatment occurred between 7:00 am and 7:30 am on April 23, 2002. Nozzles were equipped with 50 mesh strainers. Winds during application ranged from 2 to 5 mph. At the beginning of treatment ground and air temperatures were 36.8^{o} F and 45° F respectively. At completion of application, ground and air temperatures were 42° F and 59° F degrees respectively.

Ground applied bait treatments were to be applied by APHIS program personnel as usual in Utah. However, conflicting schedules prevented ground applications and estimates of

the amount of bait that would have been applied, based on detailed weekly field population counts, were provided by Greg Abbott, USDA-APHIS-PPQ Richfield, Utah.

Estimating Mormon cricket populations over large areas is difficult at best and no generally accepted precise method for estimating reductions in populations has been developed. In this study, pre and post treatment estimates of population densities at weekly intervals were recorded by conducting visual counts in areas delimited by round hoops encompassing one square yard at 10 yard intervals along diamond shaped transects 400 yards long at 16 evenly distributed sites within each plot (Fig. 1). Sites centers were separated from adjacent site centers by 0.2 mile within each block.

Additionally, water and oil sensitive spray cards were spaced alternately at 5 yard intervals ca 18 inches above the ground along a line transecting the center most one half mile of the plot and situated perpendicular to the flight direction. Cards were placed immediately prior to application and were collected immediately after application and returned to the laboratory for analysis. Using a template, five, one square centimeter areas on each card, were counted under a microscope at 8x magnification to determine the density of spray droplets.

Cage Studies.

To determine the length of residual activity of Dimilin, small bucket cages (two gallon bottomless buckets modified with screen sides and top) were established on Dimilin treated improved rangeland and stocked with untreated Mormon crickets at post treatment intervals of 0, 7, 14, 21, 28, 32, and 45 days. Ten cages were used for each evaluation interval. Cages were stocked at the rate of one cricket per cage to prevent cannibalism, a common occurrence when more than one cricket is confined in a cage. Cricket mortality within cages was monitored daily. An untreated check area with cages and crickets was similarly established for comparison at each post treatment interval. This study utilized a total of 140 cages. Initiation of this study occurred after all aerial application of Dimilin had been completed.

Similarly, field cages were also established with single Mormon crickets to evaluate 3 doses (1.0%, 0.5% and 0.0025% AI) of Dimilin bait applied at the rate of 5 lbs/acre (22.7 g AI/acre, 11.35 g AI/acre, and 0.05675 g AI/acre respectively) on improved rangeland. The 0.0025% dose was further evaluated with and without the potential feeding attractant. This study included an untreated check for comparison. All treatments were replicated 4 times. (10 cages/replicate).

Additionally, laboratory and field cages were established with Mormon crickets to evaluate three doses (8 g AI, 0.32 g AI and 0.02 g AI) of Fipronil bait applied at the rate of 5 lbs/ acre (40 g AI/acre, 1.6 g AI/acre, and 0.1 g AI/ acre respectively). These studies were conducted to determine initial kill produced by each of the three doses and subsequent mortality (secondary kill) when new unexposed crickets were exposed to crickets freshly killed with the original bait. This study was conducted first in the laboratory and then repeated in the field in bucket cages. An untreated check was included in all four of threes studies. In secondary kill studies an artificially killed cricket

was included in the cages with the untreated cricket controls. All treatments were replicated 4 times (10 cages/replicate).

Laboratory cage studies were conducted in 4.5 inch diameter x 3 inch high squat paper cups with screen tops in a makeshift laboratory established in a motel room. All field cage studies were conducted on rangeland improved with seeded rye grass on the Greg Kessler Ranch ca. 5 miles NE of Holden, Utah. Each cage contained one live Mormon cricket and the appropriate dose of bait/dead cricket or no bait (control). All treatments were replicated 4 times (10 cages/ replicate).

Analysis.

Field populations were analyzed by totaling the number of square yards surveyed with at least one cricket for each of the 16 sites in each plot, calculating percentages of reduction based on pre (conducted within 3 days of treatment) and post counts and converting to ranks for analysis with the Mann-Whitney Rank Sum Test. Analyses of cage study data relied on a one-way analysis of variance and an all pair wise multiple comparison procedure (Tukey Test).

RESULTS AND DISCUSSION:

Dimilin field efficacy.

Treatment was originally scheduled for April 15, however extraordinary winds which reached 45 mph in the area prevented application. Two inches of snow during the night prevented treatment the following day. Snow, rain and severe winds occurred through April 21. Meteorological conditions did not allow for treatment until April 23. Prior to the storm, mean Mormon cricket densities were 1.56/yd^2, 2.22/yd^2, 2.58/yd^2 for the untreated, carbaryl bait and Dimilin plots respectively (Fig. 2). Generally it is thought that snowfall may have only little effect if any on Mormon cricket populations. However, when counts were again conducted on April 24-26, population levels had dropped substantially. In this case several days of inclement weather appeared to have an impact on the overall populations in the area or a major movement out of the general area during extremely inclement weather had occurred.

Post treatment changes in the populations, excluding pre storm levels, are shown in Figure 3. Numerical, reductions were greater in the Dimilin plot compared to the untreated plot at one week after treatment. However, these reductions were not statistically different. At ca two weeks after treatment, population reductions were significantly greater in the Dimilin treated plot compared to the untreated plot (Table 1) and remained significantly different at ca 5 weeks after treatment. Unfortunately, data collected at ca 3 and 4 weeks after treatment were unusable for analysis. At ca 6 and 7 weeks after treatment, movement of Mormon crickets into the plots resulted in no significant differences in reductions being detected in the analyses. Several additional analyses provided no improvement in demonstrating significant differences between population changes in the Dimilin treated plot and the untreated plot. The detailed density counts and their locations within each of all three plots for the duration of the study

(except for 3 and 4 weeks post treatment) can be seen in the density color coded Figures 4 – 10.

While populations are shown for the carbaryl bait plot, no analyses comparing this plot to the other plots were conducted because ground applied treatments of bait did not occur. In lieu of actual bait treatments, estimates of the amount of bait that would normally have been applied were supplied by Greg Abbott. These estimates were provided for each of the 8 weeks after Dimilin treatments occurred and were based on the detailed population density and location data collected for all three plots during the study. At each of the 16 separate transects within the bait plot, treatments were triggered by a mean count of 0.5 Mormon rickets/yd^2. The total estimates of bait applied on a weekly basis are shown in Table 2. Treatments were estimated to occur on 7 of the 8 weeks. The largest estimated application was 555 lbs on May 7. The average weekly application was estimated to be 257 lbs. The total amount estimated for the entire 8 weeks was 2055 lbs. However, it is unlikely that once the population received treatment, it would have required re-treatment every week. Unfortunately, we do not know how each treatment would have affected the succeeding weekly population count. However, Greg Abbott has indicated that in an area requiring ground treatments, it is not unusual for the area to require retreating ca every 2 weeks. With this scenario, treatments occurring on April 25, May 7, May 22, and June 5, would total 1180 lbs and treatments occurring on April 30, May 16, May 29, and June 11 would total 875 lbs. The mean of these two scenarios is 1027.5 lbs of bait and was considered a non conservative and worst case scenario for bait treatment use estimates for the plot, because none of the population was removed in the data which was used to estimate needed bait treatments. At the risk of confusing the issue, the following is also provided. The detailed calculations contained in the estimates provided by Greg Abbott, show that a total of 203.3 acres would have been directly treated. Because treatments would have been applied in patterns ranging from every other swath up to one treated swath in 5, and the areas treated in many cases would have been retreated, a total of 920 acres would have been subjected to incomplete pattern treatment of some kind.

Economics.

The cost of Dimilin spray applied at 0.75 fluid oz /acre and applied to 50% of the acreage has been estimated at $ 1.57/ protected acre (Foster et al. 2000). Based on previous bait programs conducted in Utah, 2% carbaryl bran bait applied at 10 lbs/acre cost $25/acre. At these costs, 40 acres treated with bait (40 x $25 = $1000) would cost as much as 640 acres treated with Dimilin in a RAATS strategy ($1.57 x 640 = $1004.80)

In this study the cost of the Dimilin treatment was ca. $1005. There is less confidence in determining the cost of what would have occurred in the bait plot. However, if for simplicity we assume that for each 10 lbs of bait estimated to be applied, the cost will be $25, then the cost of treating infested areas within a section (640 acres) with carbaryl bran bait would have been: Worst case scenario – a total of 1028 lbs of bait applied would cost $2,570. Best case scenario – a total of 555 lbs of bait applied only once (April 30) during the entire 8 week period would cost $1387.50. This best case scenario also assumes that no further treatment is needed and no migration into the plot occurs, both unlikely. In both scenarios, the estimated costs are substantially higher (38% to

158%) than the Dimilin treatment. Additionally, the Dimilin treatment required ground personnel for only a portion of the day of treatment and the ground applied bran bait treatment was estimated to have required ground personnel present for at least a portion of 4 separate days.

Droplet deposition.

Examination of the spray cards revealed that a mean number of 7.6 ± 3.13 droplets /cm^2 and 4.4 ± 1.97 droplets /cm^2 were deposited on the oil sensitive and water sensitive cards respectively. The former value was consistent with what we expected. Previous studies with carbaryl (32 fluid oz/acre) applied through (20) 8003 tips resulted in 7.4 droplets/cm^2 in 1997 and 7.0 droplets/cm^2 in 1998 (Foster et al. 2000). Even though application occurred in winds ranging from 2 mph to 5 mph and the aircraft was spaced at 200 ft while calibrated for 100ft swath, droplets were recorded on 100% of both the oil and water sensitive spray cards, including those cards that occurred in the 50% of the acreage not "directly" treated. It is noted that the dye was critical for determining the presence of fine droplets. These data represent the first successful operational size evaluation of spray cards for Dimilin spray on rangeland. Previous attempts had resulted in difficulty with water sensitive cards in early morning high humidity conditions and with oil sensitive cards that failed to maintain droplet mark integrity for more than a short period of time after application. Mean droplet deposition/ cm^2 across the center one half mile of the plot can be seen in Figure 11. Noteworthy is a recent study conducted by Uniroyal with 31 oz/acre of Dimilin mix applied aerially through 8 nozzles at 4:00 pm from an altitude of 15 to 20 feet in wind (undetermined speed), that indicated detectable droplets with and without dye on both water and oil sensitive cards (Stephen Colbert, 2002).

Dimilin residual.

These cage studies were conducted at residual ages of 0, 7, 14, 21, 28, 35 and 42 days. Generally, Dimilin demonstrated activity for at least 21 to 28 days after treatment, (Fig. 12). Dimilin treated grass showed 70% more mortality with residuals 0 and 7 days old and 80% more mortality with residuals 14 days old compared to untreated grass. Dimilin treated grass with 21 day old residuals showed 62% more mortality compared to the untreated grass. No differences in mortality were seen between Mormon crickets exposed to untreated grass and grass with 28 day old Dimilin residues. In the studies with residual ages of 35 and 42 days, untreated populations inexplicably died soon after set up.

Dimilin bait.

Numerically, all doses performed in dose rank order (Fig. 13, Table 3). Only the 1% dose at 5 lbs/acre produced mortality significantly greater than the untreated control at all of the intervals of the study. Except for 15 and 17 days after exposure the 1% dose resulted in significantly higher mortality than the 0.5% dose. The 0.5% dose did not produce mortality significantly higher than the 0.0025% dose until 17, 20 and 22 days after treatment. The 0.0025% dose did not result in mortality significantly greater than the untreated control until 9 days after treatment.

Dimilin bait with and without attractant.

The coating additive, viewed as a potential feeding attractant produced no advantage in terms of significantly increasing mortality. The comparison of the 0.0025% dose with and without attractant resulted in no significant differences in mortality at any of the 8 intervals evaluated in the study (Fig. 14, Table 4).

Fipronil bait, initial kill - lab. study.

The three doses 0.02 g, 0.32g and 8.0 g preformed in dose rank order and resulted in 71.25%, 83.75% and 91.6% mortality respectively in this study at 21.5 hours after exposure to Mormon crickets (Fig. 15). Mortality was not recorded beyond 21.5 hours. None of the untreated control died during this time. It is important to note that in a later study where initial kill crickets were being used as bait in a field study of secondary kill, that all three of the same doses produced mortalities greater than 90% when mortality was not recorded until 28 hours after exposure.

Fipronil bait, secondary kill – lab. study.

Dead Mormon crickets from the previous initial kill - lab study were subsequently set up in new cup cages with new unexposed Mormon crickets to determine secondary kill. All treatments performed in dose rank order (Fig. 16, Table 5). There was no significant difference in mortality produced by the two highest doses at any of the intervals examined. The lowest dose failed to produce mortality significantly greater than occurred in the untreated control at any time during the study. After 3 days of exposure, mortality in the untreated control increased rapidly. This was not unexpected since no additional food or water was provided.

Fipronil bait initial kill - field study.

All three doses performed in dose rank order (Fig. 17, Table 6). All doses except the low dose (0.02 g) at one day after treatment resulted in significantly greater mortality compared to the untreated control for the duration of the study. These doses produced statistically equivalent levels of mortality except at one day after exposure where the low dose was equivalent to the untreated control and at 6 and 7 days after exposure where the low dose resulted in mortality statistically equivalent to the 0.32 g dose but significantly lower than the highest dose. Mortalities at 3 days after treatment were similar to those at 21.5 hours after treatment in the initial kill lab study. The faster mortality in the laboratory study most likely occurred because no alternative food or water was available in comparison to the field cages which contained actively growing grass.

Fipronil bait secondary kill - field study.

Mormon crickets killed with the appropriate doses of bait in additional squat cup cages were placed in field cages with new unexposed Mormon crickets. As in all the previous studies with Fipronil, treatments performed in dose rank order (Fig. 18, Table 7). The 8g and 0.32g treatments resulted in statistically equivalent mortality and produced mortality significantly greater than the 0.02g dose and the untreated control at all post exposure intervals. Mortality resulting from the low dose was not significantly greater than the untreated control. Mortality in this field study occurred substantially slower compared to

the laboratory study, again most likely a result of alternative food and water sources present in the field cages.

CONCLUSIONS:

Aerial application of Dimilin resulted in significant mortality from two to five weeks after treatment. Residual studies indicated measurable activity for 21 to 28 days. Spray droplets were recorded on 100% of both oil and water sensitive cards including those cards placed in the 50% of the acreage not directly treated in the RAATs strategy. The addition of dye to the mix was critical for determining presence of fine droplets on the spray cards. Almost twice as many droplets were recorded on oil sensitive cards compared to water sensitive cards. In this study, estimated costs for controlling Mormon crickets with ground applied bran bait was 38% to 158% (mean 98%) more than costs of aerially applied Dimilin spray. Regardless of the study, overall costs of both types of treatments indicate that 640 acres could be protected by treating 320 acres with Dimilin spray for the same costs required to ground treat 40 acres (with a potential for protecting up to 200 acres) with 2% carbaryl bran bait.

The 1% dose of Dimilin bait applied at 5 lbs /acre was significantly superior to the other doses tested. The addition of a potential feeding attractant had no effect on the mortality achieved with Dimilin bran bait. All three doses of Fipronil bait applied at 5 lbs/acre produced statistically equivalent high mortality in both laboratory and field initial kill studies. In both laboratory and field studies, the highest two doses of Fipronil bait produced equivalent and high secondary kill which was statistically greater than the low dose or the untreated control. The low dose of Fipronil bait demonstrated no statistical increase in secondary mortality compared to the untreated control.

Aerially applied Dimilin spray in a RAATs strategy offers an attractive economical alternative to ground applied carbaryl bran bait for combating Mormon crickets. Both Dimilin and Fipronil baits show promise and with additional development of data may play an important role in large or small scale integrated approaches for controlling Mormon crickets on rangelands in the western U.S.

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The authors thank T. Wieland, Curtis Sandberg and Dan Largent, Crompton-Uniroyal Chemical for providing the Dimilin 2L, Dimilin bait , Herbimax Oil-Surfactant Adjuvant, Hi-Light dye and assisting with some of the spray card and field cage set-up; Henry Yang and Terry Gouge, Aventis Crop Science for providing and formulating respectively the Fipronil Bait. Thanks are extended to Greg Kesler and Wesley Stephenson for providing land used in the study. Thanks is also extended to Bruce Radsick, the USDA, APHIS project pilot for his cooperation in the project and special service as a scientists in the sky.

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Figure 1. Schematic of the sampling protocol for estimating densities of field populations of Mormon cricket.

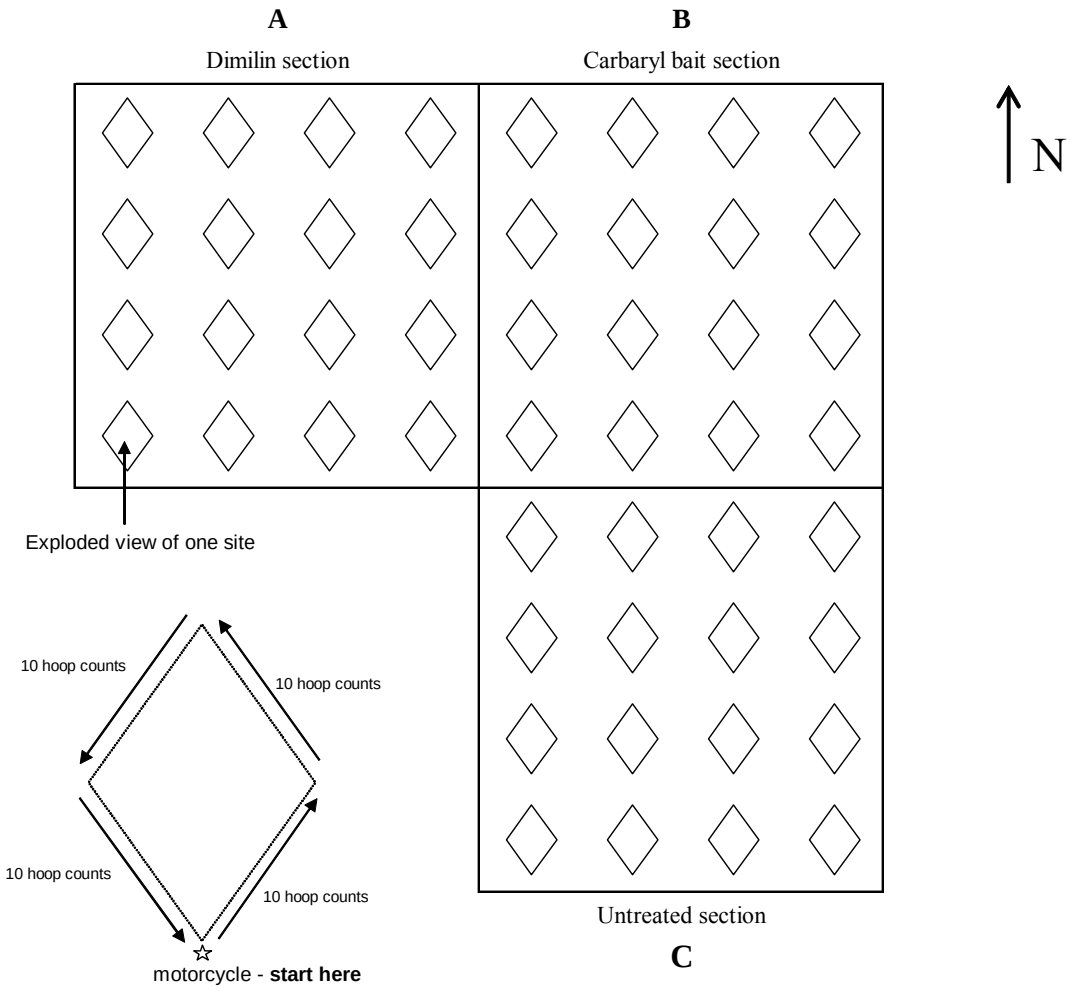


Figure 2. Mormon cricket densities in the three section blocks including counts conducted prior to the major storm.

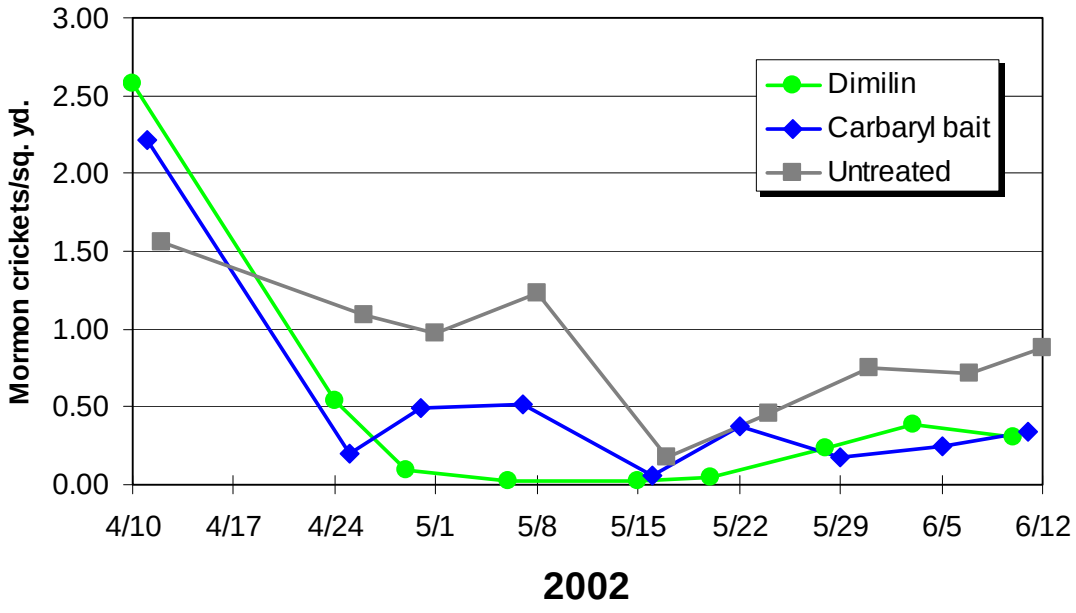


Figure 3. Mormon cricket densities in the three section blocks excluding counts conducted prior to the major storm.

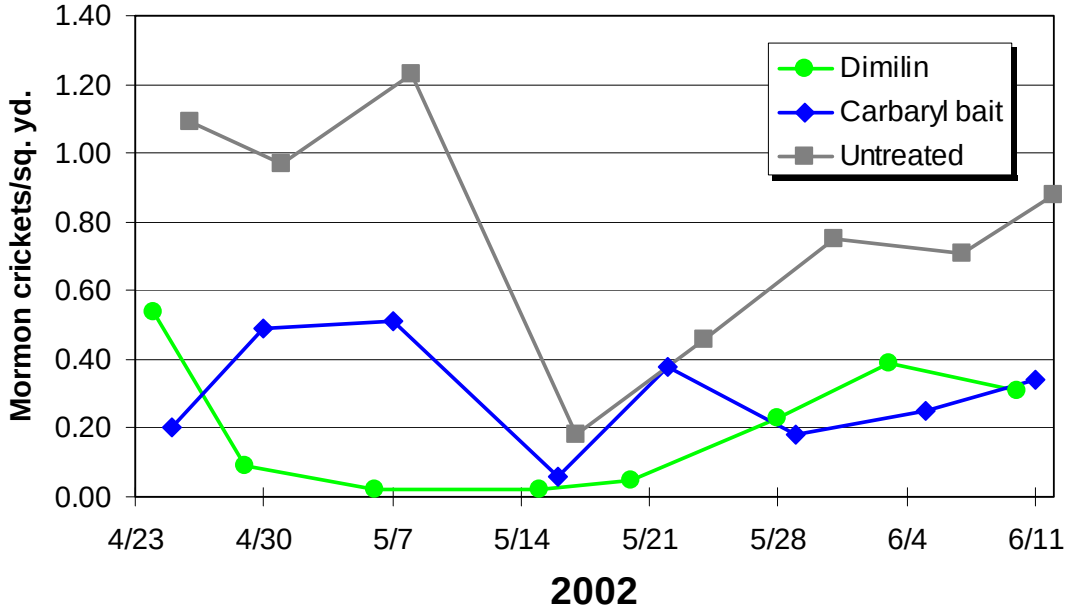


Figure 4. Mormon cricket counts in the three section blocks with corresponding color shading for density. (4/10 – 4/12)

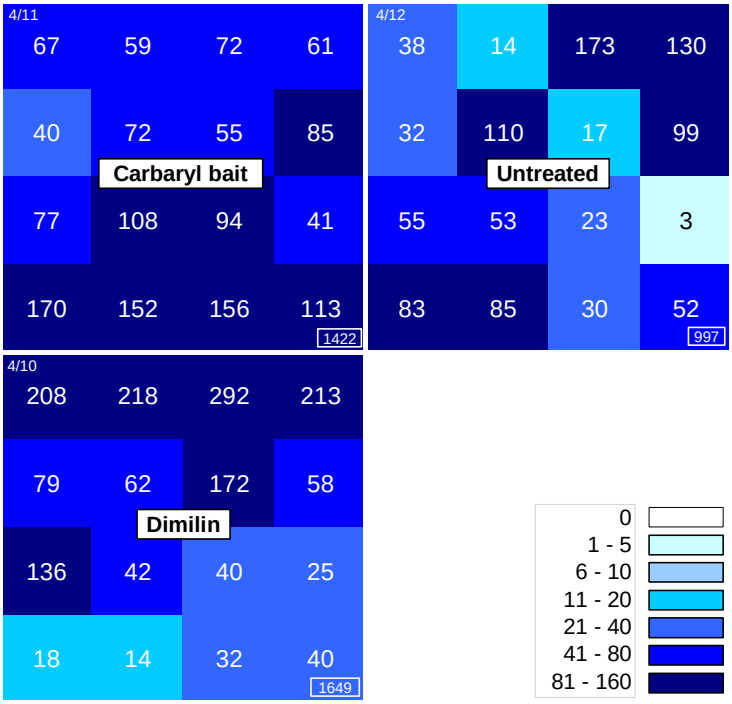


Figure 5. Mormon cricket counts in the three section blocks with corresponding color shading for density. (4/24 – 4/26)

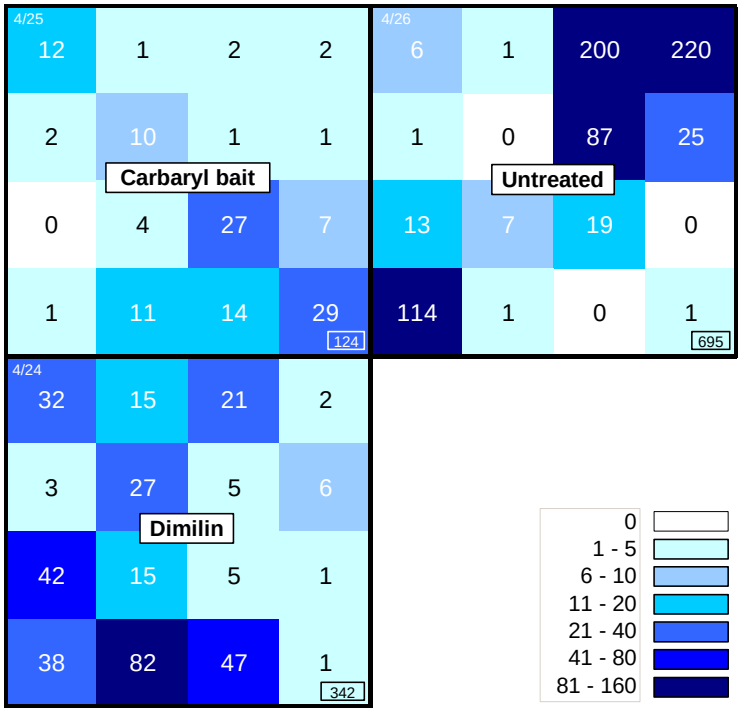


Figure 6. Mormon cricket counts in the three section blocks with corresponding color shading for density. (4/29 – 5/1)

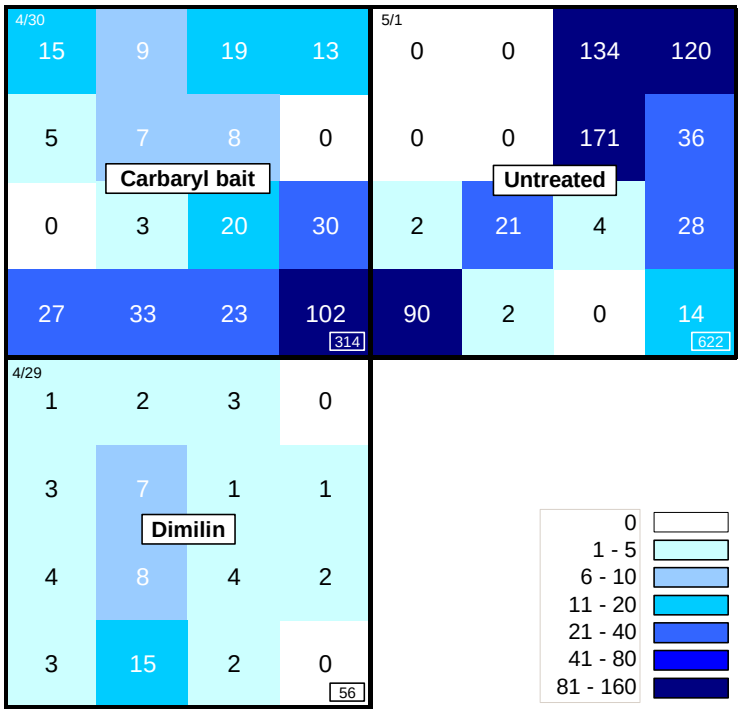


Figure 7. Mormon cricket counts in the three section blocks with corresponding color shading for density. (5/6 – 5/8)

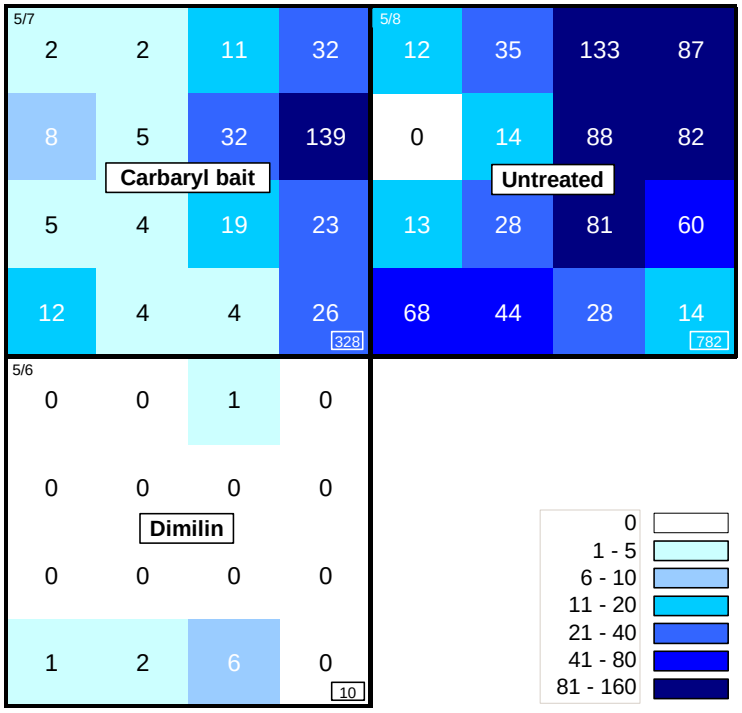


Figure 8. Mormon cricket counts in the three section blocks with corresponding color shading for density. (5/29 – 5/31)

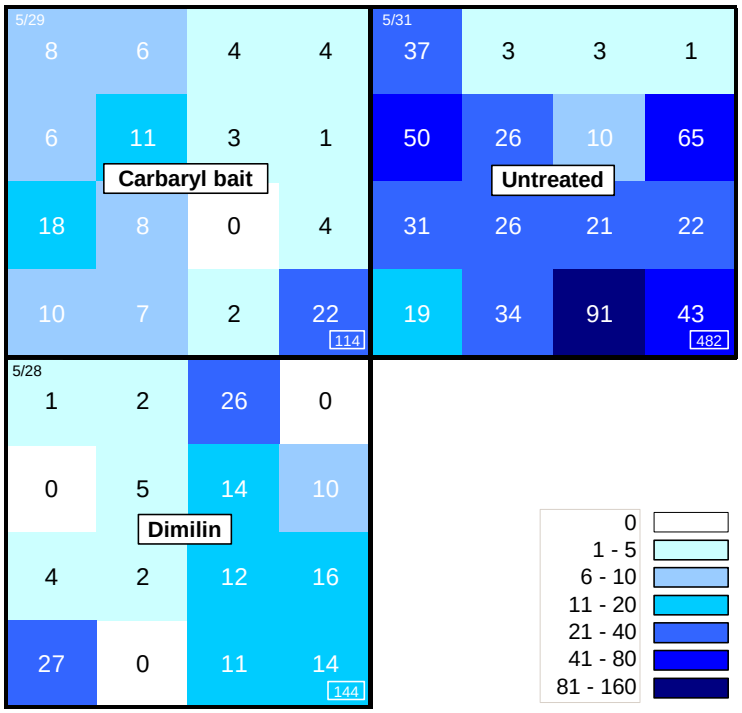


Figure 9. Mormon cricket counts in the three section blocks with corresponding color shading for density. (6/3 – 6/7)

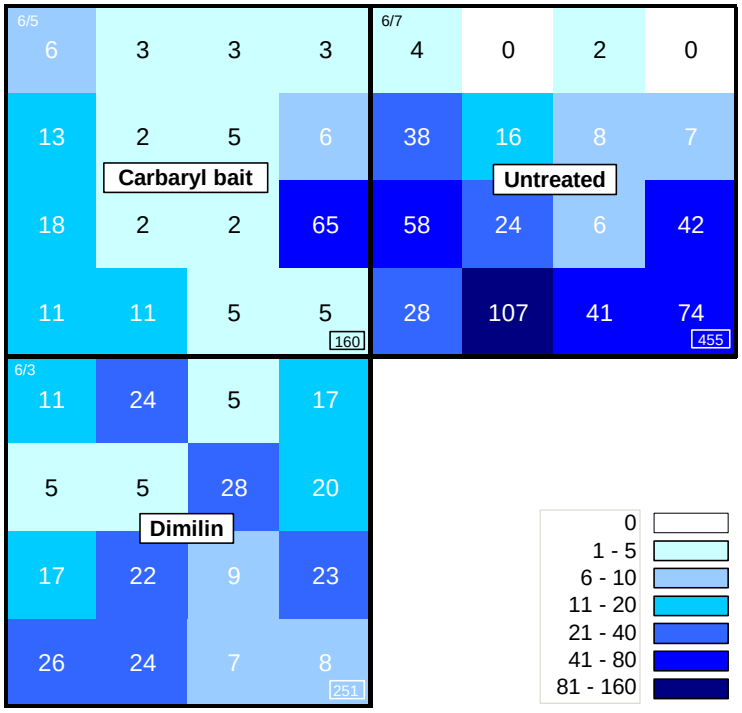


Figure 10. Mormon cricket counts in the three section blocks with corresponding color shading for density. (6/10 – 6/12)

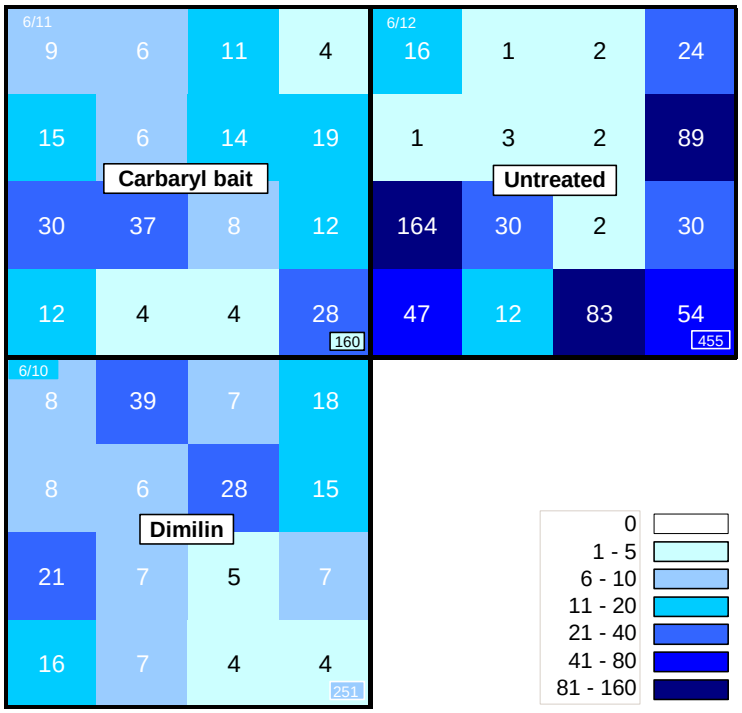


Figure 11. Mean droplet deposition/cm^2 across the Dimilin block using both oil and water sensitive spray cards.

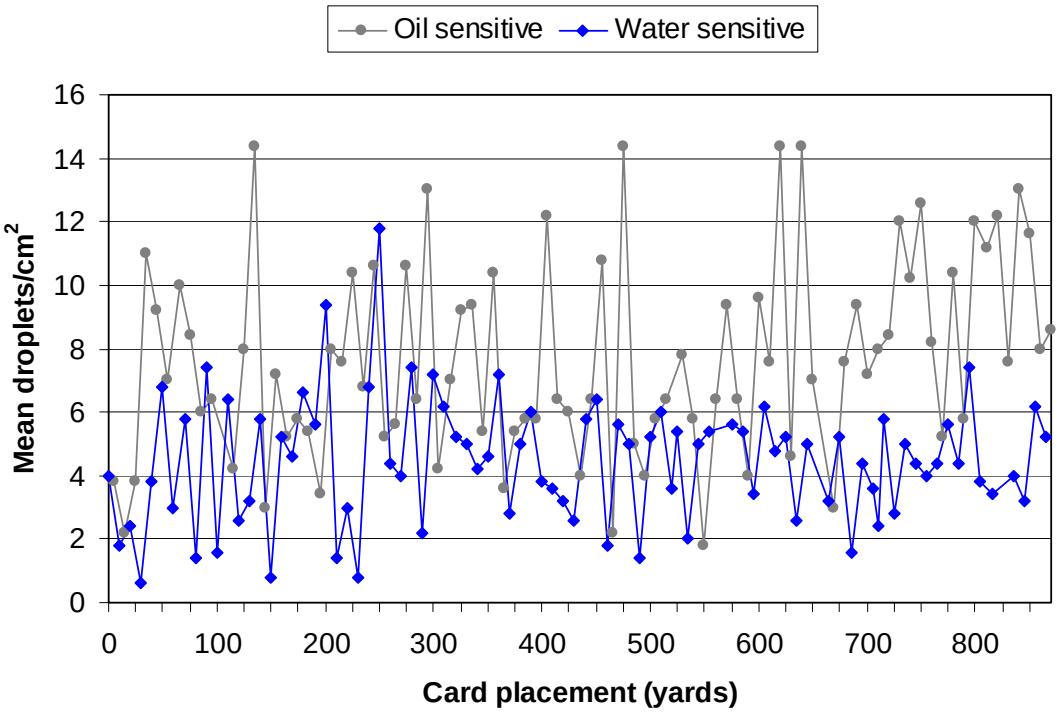
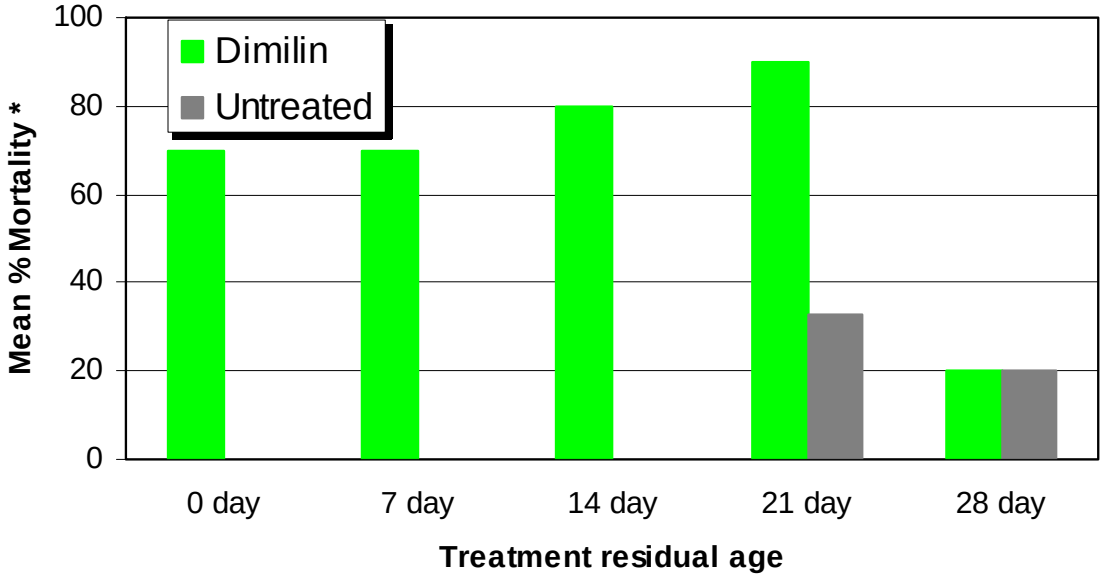


Figure 12. Mormon crickets exposed to Dimilin spray residual in field cages through 28 days after treatment application.



* (0, 7 and 14 day residual based on 16 days post-treatment; 21 day residual based on 7 days post-treatment; 28 day residual based on 9 days post-treatment)

Figure 13. Mean percentage mortality of Mormon crickets treated with selected doses of Dimilin bait in field cages.

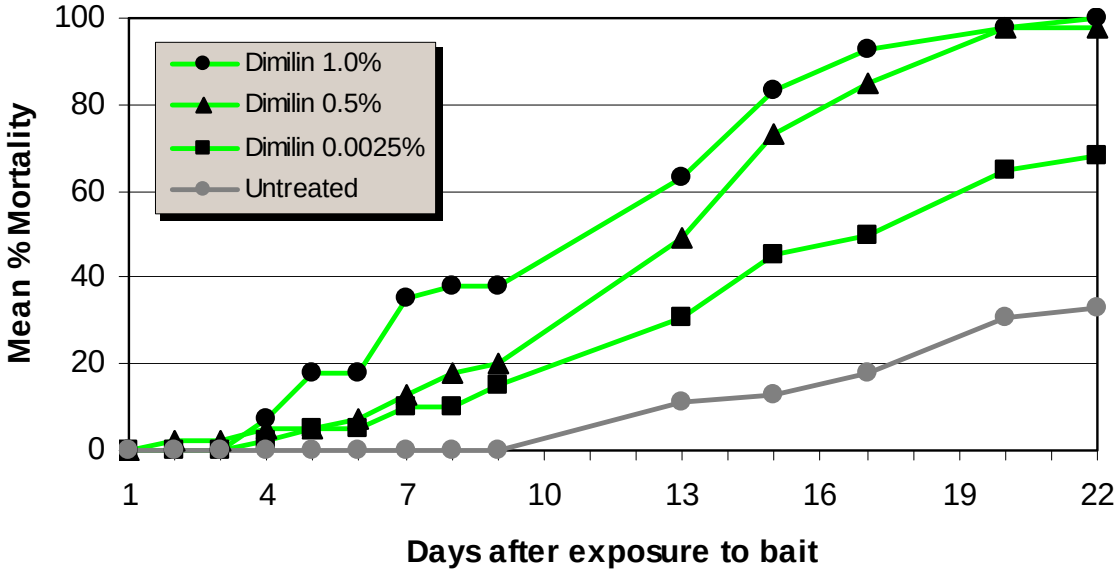


Figure 14. Mean percentage mortality of Mormon crickets treated with 0.0025% Dimilin bait with and without attractant in field cages.

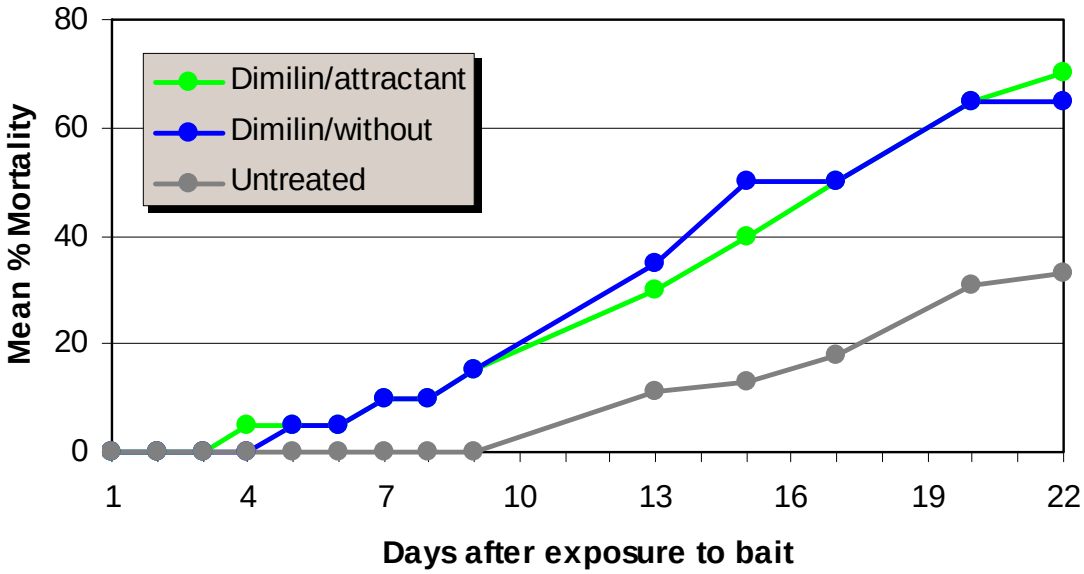


Figure 15. Initial kill with Fipronil bait against Mormon crickets in a laboratory dose study in cages.

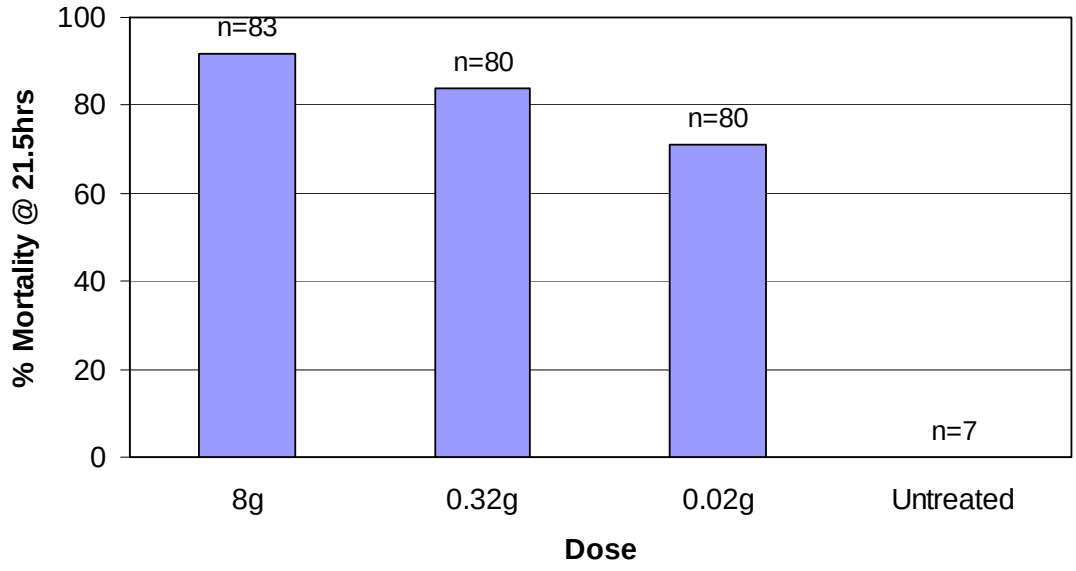


Figure 16. Mean percentage of secondary mortality of Mormon crickets treated with selected doses of Fipronil bait in laboratory cages.

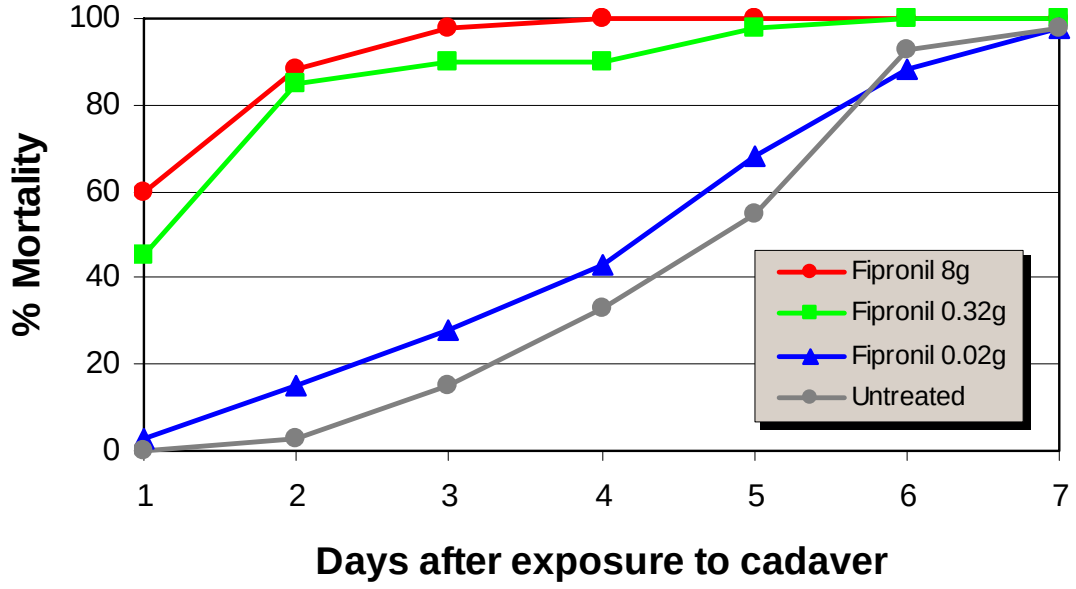


Figure 17. Mean percentage of initial mortality of Mormon crickets treated with selected doses of Fipronil bait in field cages.

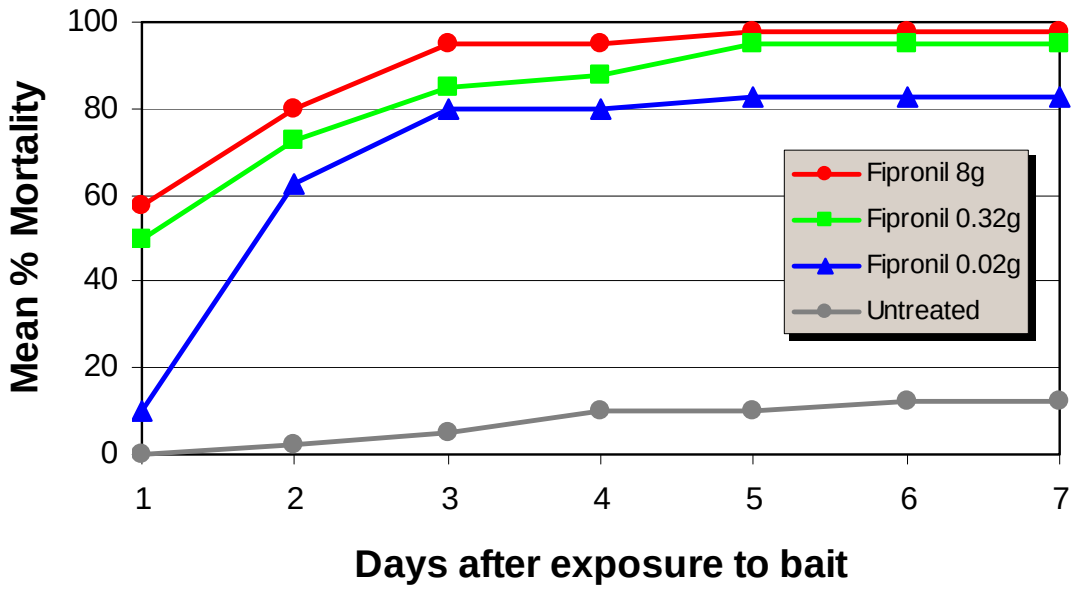


Figure 18. Mean percentage of secondary mortality of Mormon crickets treated with selected doses of Fipronil bait in field cages.

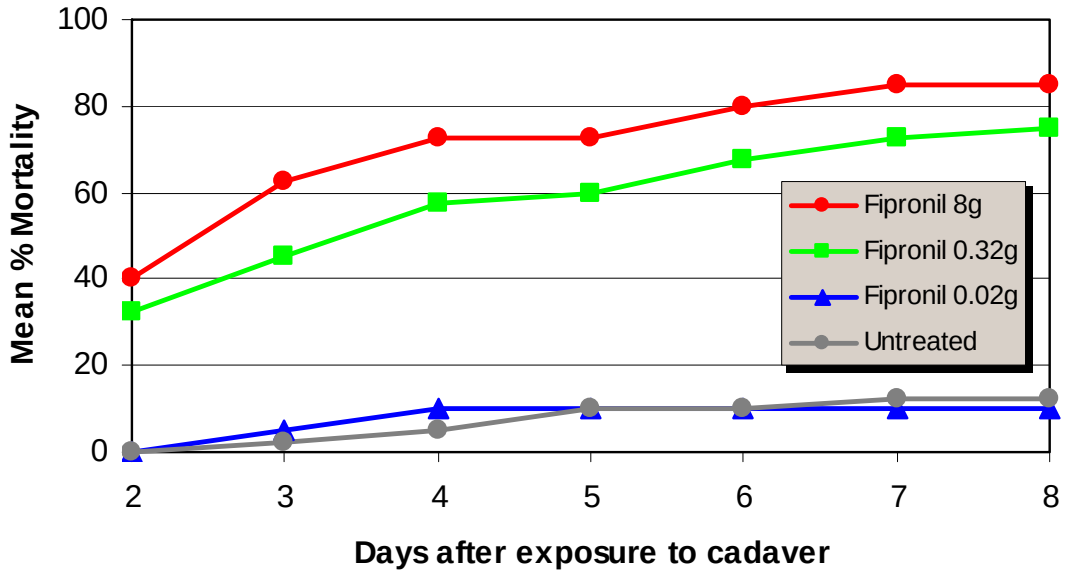


Table 1. A comparison of Mormon cricket population changes in the Dimilin and untreated section plots.

	% reduction – days post-treatment *				
Treatment	6-8	13-15	35-38	41-45	48-50
Dimilin	74	95	52	-4	31
Untreated	7	-38	7	12	30
P =	0.203	<0.001	0.051	0.614	0.776

* Data analyzed by the Mann-Whitney Rank Sum Test.

Table 2. Estimated pounds of 2% carbaryl bran bait applied per week.*

Date	
4/25	160 lbs
4/30	555 lbs
5/7	520 lbs
5/16	0 lbs
5/22	400 lbs
5/29	80 lbs
6/5	100 lbs
6/11	240 lbs
Total	2055 lbs
mean	257 lbs

* Estimated values were based on detailed population density and location data gathered weekly in the bran bait plot.

Table 3. Mormon cricket response to Dimilin bait in a field cage dose study.

	Mean % Mortality - Days after exposure to bait *							
Dose	7	8	9	13	15	17	20	22
1.0%	35 a	38 a	38 a	63 a	83 a	93 a	98 a	100 a
0.5%	13 b	18 b	20 b	49 ab	73 ab	85 a	98 a	98 a
0.0025%	10 b	10 bc	15 b	31 b	45 b	50 b	65 b	68 b
Untreated	0 b	0 c	0 c	11 c	13 c	18 c	31 c	33 c

* Data analyzed by a one-way analysis of variance and an all pairwise multiple comparison procedure (Tukey Test). Means in a column followed by the same letter are not significantly different, $P \leq 0.05$

Table 4. A comparison of the low dose of Dimilin bait with and without attractant against Mormon crickets in field cages.

	Mean % Mortality - Days after exposure to bait *							
Dose	7	8	9	13	15	17	20	22
.0025% w	10	10	15	30	40	50	65	70
.0025% w/o	10	10	15	35	50	50	65	65
Untreated	0	0	0	11	13	18	31	33

* Data analyzed by a one-way analysis of variance and an all pairwise multiple comparison procedure (Tukey Test). No significant differences between means were detected, $P \leq 0.05$

Table 5. Secondary kill of Mormon crickets with Fipronil bait in a laboratory cage study.

	Mean % Mortality – Days after exposure to cadaver *						
Dose	1	2	3	4	5	6	7
8.0g	60 a	88 a	98 a	100 a	100 a	100 a	100 a
0.32g	45 a	85 a	90 a	90 a	98 a	100 a	100 a
0.02g	3 b	15 b	28 b	43 b	68 b	88 b	98 a
Untreated	0 b	3 b	15 b	33 b	55 b	93 ab	98 a

* Data analyzed by a one-way analysis of variance and an all pairwise multiple comparison procedure (Tukey Test). Means in a column followed by the same letter are not significantly different, $P \leq 0.05$

Table 6. Initial kill of Mormon crickets with Fipronil bait in a field cage study.

	Mean % Mortality – Days after exposure to bait *						
Dose	1	2	3	4	5	6	7
8.0g	58 a	80 a	95 a	95 a	98 a	98 a	98 a
0.32g	50 a	73 a	85 a	88 a	95 a	95 ab	95 ab
0.02g	10 b	63 a	80 a	80 a	83 a	83 b	83 b
Untreated	0 b	3 b	5 b	10 b	10 b	13 c	13 c

* Data analyzed by a one-way analysis of variance and an all pairwise multiple comparison procedure (Tukey Test). Means in a column followed by the same letter are not significantly different, $P \leq 0.05$

Table 7. Secondary kill of Mormon crickets with Fipronil bait in a field cage study.

	Mean % Mortality – Days after exposure to cadaver *						
Dose	2	3	4	5	6	7	8
8.0g	40 a	63 a	73 a	73 a	80 a	85 a	85 a
0.32g	33 a	45 ab	58 a	60 a	68 a	73 a	75 a
0.02g	0 b	5 bc	10 b	10 b	10 b	10 b	10 b
Untreated	0 b	3 c	5 b	10 b	10 b	13 b	13 b

* Data analyzed by a one-way analysis of variance and an all pairwise multiple comparison procedure (Tukey Test). Means in a column followed by the same letter are not significantly different, $P \leq 0.05$