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Field Cage Comparisons Of Wheat Bran, Apple Pumice And Food Waste Baits Containing Carbaryl Against Rangeland Grasshopper Species With Varying Susceptibilities To Standard Wheat Bran Baits.

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Introduction

Various baits (carriers and toxicants) have been used since the late 1800's to suppress grasshoppers and Mormon crickets (Foster 1996). However, since the early to mid 1970's, carbaryl on wheat bran has been the most commonly used bait on western US. rangelands. The level of acceptance by different rangeland grasshopper species to wheat bran bait containing carbaryl is well documented (Onsager et al. 1980 a, b; Quinn et al. 1989, 2000; Jech et al. 1993; Foster et al 1998, 1999). Some species are categorized as "sensitive" where control is expected to average about 70% with worst-case and best-case scenarios about 55% and 85% respectively. Some species are "vulnerable" where control is expected to average about 42% with worst-case and best-case scenarios about 12% and 72% respectively. Some species are categorized as "nonsusceptible" where control is expected to average about 15% with worst-case and best-case scenarios about 0% and 30% respectively (Onsager et al. 1996).

The Mormon cricket, a long-horned grasshopper, is extremely susceptible to wheat bran bait containing carbaryl (Foster et al. 1979). During the last three years, studies with alternative baits based on apple pumice and food waste (combined bakery, snack, cereal

and confectionary waste) have shown activity equal to the standard wheat bran bait against Mormon cricket. (Foster et al. 2003, 2004). These alternative baits may provide an opportunity to expand the usefulness of baits against grasshoppers.

Species that are moderately or nonsusceptible to wheat bran bait may be more susceptible to alternative baits. However, little information exists on the level of susceptibility of rangeland grasshopper species to these baits, particularly in individual studies containing all three baits. Foster et al. (2003) showed *Ageneotettix deorum* to be "sensitive" (68% to 76% mortality) to waste food bait, a range consistent with what is expected from this species on wheat bran bait, 55% - 85% (Onsager et a. 1996). In comparative tests with all three baits, (Foster et al.2004) showed both *A. deorum and Aulocara elliotti* to be equally "susceptible" in the highest category to all three baits. This level of susceptibility is consistent with expected mortality for these species and wheat bran bait (Onsager et al. 1996). With species that are categorized as "nonsusceptible" to wheat bran bait, Foster et al. (2006) showed *Cordillacris occipitalis* to be nonsusceptible to all three baits, and *Amphitornus coloradus* and *Trachyrachys kiowa* to exhibit nonsusceptible levels of mortality when exposed to food waste bait. While these initial data suggest that apple pumice and food waste baits will generally perform similarty to wheat bran bait, data on additional species should be gathered to lend further credence to this to proposition.

The following study was conducted to develop data on the level of susceptibility (expressed as mortality) that could be expected when alternative apple pumice or food waste based baits containing carbaryl are used against rangeland grasshoppers species categorized as "sensitive" (susceptible), "vulnerable" (slightly susceptible) or "nonsusceptible" to the current wheat bran bait standard. The study will test the hypothesis that grasshoppers species will respond similarly to wheat bran bait, apple pumice bait and food waste baits containing carbaryl and therefore the guidance charts for grasshopper species susceptibility to wheat bran bait can also be used for these and perhaps other baits.

Objectives

Generate additional data to complement existing data for testing the hypothesis that wheat bran, apple pumice and food waste based baits containing carbaryl produce similar mortality in specific rangeland grasshopper species.

Determine and compare the levels of mortality that result from wheat bran, apple pumice and food waste based baits containing carbaryl when applied to specific rangeland grasshopper species.

Specifically determine if two common species of rangeland grasshoppers (*Phlibostroma quadrimaculatum* and *Trachyrhachys kiowa*) categorized as "nonsensitive" to the standard wheat bran bait will respond similarly, in terms of mortality, to alternative apple pumice and food waste based baits.

Specifically determine if a common species of rangeland grasshoppers (*Melanoplus sanguinipes*) categorized as "sensitive" (highly susceptible) to the standard wheat bran bait will respond similarly, in terms of mortality, to alternative apple pumice and food waste based baits.

Methods and Materials

The study was conducted in Harding County of northwestern South Dakota ca. 7.5 miles east and 4 miles south of the town of Ludlow on the Eugene Stensland ranch during the period of July 8 - 17, 2006. The location was selected because of the diversity in grasshopper species and grasses, density of grasshoppers, recent 3-year history of grasshoppers in the area, better than average range condition, availability of rangeland without livestock for the time required in the study and proximity to another ongoing study.

Three species of rangeland grasshoppers, (*Phlibostroma quadrimaculatum* and *Trachyrhachys kiowa* categorized as "non-susceptible" and *Melanoplus sanguinipes* categorized as "sensitive" or highly susceptible to carbaryl wheat bran bait), were selected for separate field cage evaluations of three baits. The baits used in the studies were the Sevin Bait (apple pumice "crumbles", Wilbur-Ellis), Tast-E-Bait (food waste, Endres Processing Ohio, LLC) and the standard, Eco Bran (wheat bran, Peacock Industries Inc.), formulations, each containing 2% and 5% carbaryl. For each species, ten cages (two gallon bottomless buckets modified with screen sides and top) were established on rangeland for treatment with each concentration of each bait type (Figs. 1 and 2). Ten additional cages were established on untreated vegetation as controls for comparison. This design, including the untreated control, therefore utilized a total of 70 cages for each species. The appropriate amount of bait was pre-weighed in the laboratory and introduced into each cage at the rate of 10 lbs/ acre. Grasshoppers were captured in an adjacent untreated area and introduced into cages after bait was applied. In the first study (July 8-15) five adult *T. kiowa* species were introduced into each cage. In the second study (July 9-16) five 5th instar stage *P. quadrimaculatum* were introduced into each cage. In the third study (July 10-17) five adult *M. sanguinipes* were introduced into each cage.

These species were selected because of their known level of susceptibility to wheat bran bait (Onsager et al. 1996) and were some of the most prevalent rangeland grasshopper species in the area. After exposure to bait treatments, grasshoppers in all cages including untreated cages were monitored daily for 7 days for mortality (Fig. 3).

Results and Discussion

In the study with *T. kiowa* (Fig. 4), some treated populations started to show significantly more mortality at two days after treatment compared to the untreated populations (Table 1). Mortality resulting from all treatments increased slowly over the course of the study.

At 6 and 7 days after treatment, mortalities resulting from all treatments were significantly higher than in the untreated population. Mortalities at 7 days after treatment ranged from 40% - 44% in treated populations compared to 8% in the untreated population. There was no significant difference between any of the treatments at 6 and 7 days after treatment. The 2% and 5% concentrations were statistically equivalent for each bait type for the duration of the study. When adjusted for the mortality in untreated populations, the resulting values are consistent with earlier studies (Foster et al 2006). All three baits produced some mortality judged to be within the "nonsusceptible' category for wheat bran bait established by Onsager et al. (1996), albeit toward the upper level.

The known feeding behavior of *T. kiowa* helps explain the results. This specie feeds almost exclusively on grasses and sedges with a great preference for blue grama in mixedgrass and shortgrass prairies. Other plants known to be consumed in substantial quantities include western wheatgrass, needle-and-thread, Kentucky bluegrass, threadleaf sedge, needle-leaf sedge, and Penn sedge. Nymphs have never been observed eating plant litter although adults have been observed infrequently (Pfadt, 2002).

In the study with *P. quadrimaculatum* (Fig. 5), none of the bait treatments produced mortality significantly higher than occurred in the untreated populations at any time during the study (Table 2) indicating there was no evidence of consumption of any of the baits. Reductions at 7 days after treatment ranged from 34% to 40% in treated populations compared to 38% in untreated populations. These results place this species at the bottom of the "nonsusceptible" category and are consistent with those reported for wheat bran bait by Onsager et al.1996.

Phlibostroma quadrimaculatum feeds almost exclusively on grasses with blue grama and buffalograss it's preferred food plants. However, other species of grasses and sedges are ingested. It selects attached green leaves for its food and has never been observed to feed on dry plant matter in its natural habitat (Pfadt 2002). These behavioral traits help explain the lack of mortality produced by baits with this species.

In the study with *M. sanguinipes* (Fig. 6), all baits and concentrations produced equivalent mortality that was significantly greater than occurred in the untreated populations (Table 3). This result was consistent throughout the study. Mortalities at 7 days after treatment ranged from 98% to 100% in treated populations compared to 56% in untreated populations. When adjusted for the mortality in the untreated populations, the resulting value (76%) is consistent with that reported by Onsager et al. (1996) for wheat bran baits. The untreated populations in this study demonstrated much higher mortality than the untreated populations in any of the other studies, most likely indicating that the small cage area limited the availability of critical mixes of preferred host food sources in the field. Our data suggest that this species should be considered highly susceptible "sensitive" to all of the carbaryl baits studied and is again consistent with the categorization of Onsager et al. (1996) for wheat bran baits.

Melanoplus sanguinipes is a mixed feeder of grasses and forbs, usually feeding on several species of plants in its habitat. Although polyphagous, the migratory grasshopper

selects host plants from its habitat with preferred foods including dandelion, tumble mustard, wild mustard, pepperweed, western ragweed, downy brome, Kentucky bluegrass, barley, and wheat. Nymphs and adults ingest dry materials lying on the ground surface including plant litter, cattle manure, and bran flakes (Pfadt 2002). This preference as a mixed feeder helps explain the higher mortality in untreated populations in this study.

In an earlier study Foster et al. (2004) showed *A. deorum* and *A. elliotti* to exhibit similar levels of susceptibility to all three baits which was consistent with the sensitive level proposed by Onsager et al. (1996) for wheat bran baits. Additionally, Foster et al. (2006) showed responses of *C. occipitalis* to all three baits and *A. coloradus* and *T. Kiowa* to food waste baits to be consistent with the nonsusceptible category of Onsager et al.(1996). When our results are combined with those earlier studies it seems clear that the categorization of susceptibility level of individual rangeland grasshopper species to wheat bran bait holds true for apple pumice and food waste baits also. Knowledge regarding the feeding behavior of these species (Pfadt 2002) indicates that individual behavior and food preferences are more important to the successes of solid baits than attraction to the three baits we studied. Additionally, in behavioral studies that included these species, Jech (1996) found that species easily controlled with wheat bran bait feed on plant litter and detrital matter on the ground and species not controlled or slightly controlled with wheat bran bait prefer feeding on living host plants.

Conclusions

All three baits produced some mortality to *Trachyrhachys kiowa* that was statistically equivalent among bait types and judged to be within the "nonsusceptible" category established by Onsager et al (1996), albeit toward the upper level. None of the selected baits produced mortality to *Phlibostroma quadrimaculatum.* Therefore, placing it near the bottom of the "nonsusceptible"category. All three baits produced high levels of mortality to *Melanoplus sanguinipes* that is consistent with the highest category of susceptibility.

The level of susceptibility of individual rangeland grasshopper species to bait is largely affected by feeding behavior and food preferences. There was no advantage with the higher concentration of carbaryl in any of the baits tested.

The data strongly supports the hypothesis that the categorization for wheat bran bait may hold true for rangeland grasshopper species treated with apple pumice or food waste baits. In the original bait acceptance study, 31 separate species were evaluated and categorized as to being "sensitive", "vulnerable" or "nonsusceptible" to wheat bran baits (Onsager et al. 1996. Seven of those species (3 "sensitive' and 4 "nonsusceptible") have now been tested against one or both of the alternative baits. The combined results from this study and those of Foster et al. 2004 and Foster et al. 2006 are consistent for all three types of baits (wheat bran, apple pumice and food waste) and strongly suggest that results expected to be obtained from treatment with wheat bran bait on individual species can be extrapolated to the alternative apple pumice and food waste baits.

Study Improvement

This is a new section that the senior author has been considering for several years to include in all reports and is included here for the second time. It is an attempt to improve future similar studies and investigations by identifying design issues that result in less than optimal intended results. The operational question of this section is: What would the author/s do differently if able to repeat this study. The pitfall of such a section is that the weaknesses in the study are not only suggested, but highlighted for all to see. However, in the eyes of science, the potential for improving subsequent similar studies far outweighs such a concern.

Absence of critical mixes of preferred plant hosts for *M. sanguinipes* within the enclosed cage may have attributed to increased mortality in untreated checks. Placement of cages in the specific area where this species was captured may improve the untreated check mortality. The addition of an evaluation of a species categorized as "vulnerable" could provide additional information to strengthen the conclusion.

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Table 1. Mean mortality of adult *Trachyrhachys kiowa* treated with selected carbaryl baits in field cages – Ludlow, SD 2006.

	Days after exposure – mean % mortality *						
Treatment	1	2	3	4	5	6	7
Wheat bran 2%	0 a	2 b	4 ab	12 ab	34 a	42 a	44 a
Crumbles 2%	4 a	4 ab	12 ab	16 ab	26 ab	28 a	40 a
Tast-e-bait 2%	4 a	4 ab	6 ab	8 ab	28 ab	34 a	40 a
Wheat bran 5%	2 a	2 b	2 b	12 ab	30 ab	34 a	46 a
Crumbles 5%	2 a	4 ab	10 ab	12 ab	34 a	40 a	44 a
Tast-e-bait 5%	10 a	14 a	14 a	18 a	36 a	44 a	44 a
Untreated	2 a	2 b	2 b	2 b	4 b	4 b	8 b

* Means in a column followed by the same letter are not significantly different ($P \leq 0.05$)

Table 2. Mean mortality of 5th instar and adult *Phlibostroma quadrimaculatum* treated with selected carbaryl baits in field cages – Ludlow, SD 2006.

	Days after exposure – mean % mortality						
Treatment	1	2	3	4	5	6	7
Wheat bran 2%	2 a	2 a	6 a	20 a	20 a	26 a	40 a
Crumbles 2%	12 a	16 a	18 a	26 a	26 a	30 a	38 a
Tast-e-bait 2%	10 a	12 a	14 a	24 a	26 a	26 a	34 a
Wheat bran 5%	14 a	18 a	22 a	22 a	22 a	28 a	34 a
Crumbles 5%	6 a	14 a	28 a	36 a	36 a	36 a	38 a
Tast-e-bait 5%	16 a	16 a	20 a	26 a	26 a	26 a	38 a
Untreated	10 a	10 a	16 a	28 a	32 a	34 a	38 a

* Means in a column followed by the same letter are not significantly different ($P \leq 0.05$)

Table 3. Mean mortality of adult *Melanoplus sanguinipes* treated with selected carbaryl baits in field cages – Ludlow, SD 2006.

	Days after exposure – mean % mortality						
Treatment	1	2	3	4	5	6	7
Wheat bran 2%	58 a	78 a	90 a	94 a	98 a	100 a	100 a
Crumbles 2%	64 a	82 a	96 a	96 a	98 a	100 a	100 a
Tast-e-bait 2%	54 a	70 a	92 a	94 a	94 a	96 a	98 a
Wheat bran 5%	58 a	76 a	96 a	98 a	98 a	98 a	98 a
Crumbles 5%	66 a	86 a	96 a	96 a	100 a	100 a	100 a
Tast-e-bait 5%	76 a	88 a	96 a	98 a	98 a	98 a	98 a
Untreated	6 b	20 b	26 b	26 b	30 b	44 b	58 b

* Means in a column followed by the same letter are not significantly different ($P \leq 0.05$)

Figure 1. Bucket cage used in evaluating baits for rangeland grasshoppers.

Figure 2. Setup for evaluating selected grasshopper baits in cage studies near Ludlow, South Dakota, 2006.

Figure 3. Selecting the appropriate grasshopper species from sweep net collections for placement in field cages.

Figure 4. Monitoring bucket cages used in evaluating grasshopper baits.

Figure 5. *Trachyrhachys kiowa*, Adult male. (Pfadt 2002).

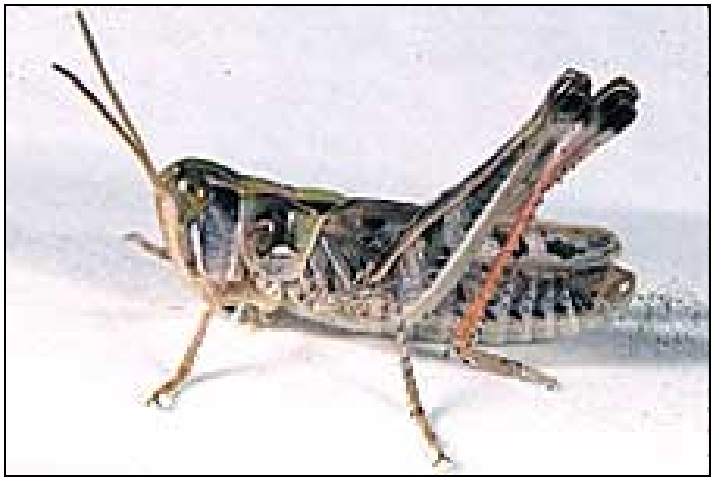

Figure 6. *Phlibostroma quadrimaculatum*, Adult male. (Pfadt 2002)

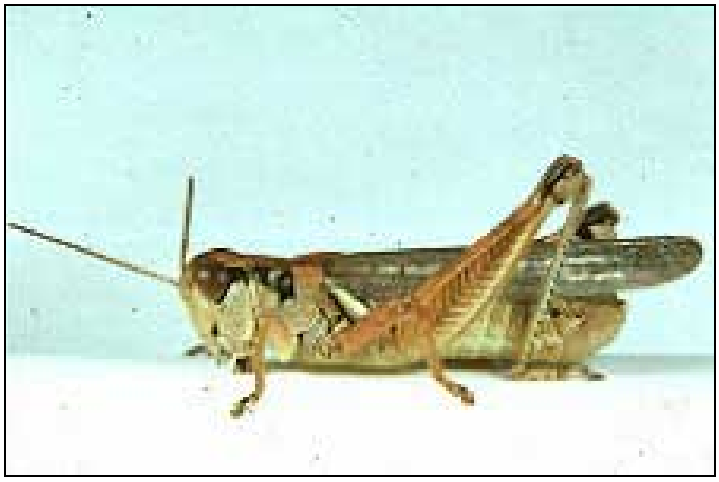

Figure 7. *Melanoplus sanguinipes*, Adult male. (Pfadt 2002)

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Extending the residual activity of Spinosad: Field cage evaluation of selected products in simulated aerial spray formulations

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Introduction

All rangeland grasshopper treatments in the U.S. rely on traditional insecticide sprays or baits, except for the insect growth regulator, diflubenzuron. Many geographical areas and situations increasingly preclude the traditional use of insecticides and could benefit greatly from the development of nontraditional treatments. This need has been seriously identified and pursued to some degree, although unsuccessfully in the United States, for the last 25 years.

Spinosad (the active ingredient (AI) in Tracer) is a bacteria derived toxin which has shown activity against grasshoppers (Foster et al. 1996, 2002, 2004 and 2006) and is registered for use against other pests on organically grown crops. If developed to be economical for controlling rangeland grasshoppers, its use in insecticide restricted areas could yield substantial benefits. Some areas in need of treatment are partially or completely composed of rangeland that is considered sensitive for one reason or another and may not allow treatment with traditional insecticides. These currently untreatable areas may directly jeopardize a proposed program effort and if sufficiently large could

indirectly impact a larger area-wide control effort. Treatments allowed in such areas could be extremely important to local and area wide control efforts. Spinosad doses of 42.5 and 85 g AI/acre (3 and 6 fluid oz/acre of Tracer) have shown excellent control when aerially applied to grasshoppers on rangeland. While the higher dose showed significant activity diminishing between 7 and 14 days after treatment, activity of the lower dose disappeared between 0 and 7 days after treatment (Foster et al. 2002). Although these rapid and high kill rates with short residual appear ideal for rangeland grasshoppers, the expected costs at such rates would probably preclude their use. Subsequent studies showed that 21.3 to 42.5 g AI/acre of Spinosad (1.5 to 3 fl oz/acre of Tracer) yielded acceptable control (84% to 98%) while 5.3 and 10.6 g AI/acre (0.375 and 0.75 fl oz/ac of Tracer) both yielded 78% mortality, probably slightly less than acceptable (Foster et al 2004). Most of the doses in the later study were considered too expensive. However, the lowest dose 5.3 g AI/acre (0.375 fl oz/ac of Tracer) was estimated to be economically competitive with existing spray treatment options. Additionally and as expected, this dose showed no residual activity beyond the day of treatment. Lengthening the residual activity of low dose treatments may result in higher mortality levels. Any improvements in the lower dose formulation that could even slightly increase resulting mortality levels would appear to make such a treatment acceptable in terms of both control and cost levels.

With some other pests (pink bollworm and gypsy moth), liquid formulations containing insect pheromones depend on extended persistence for economy. If the core components of those formulations could be combined with Spinosad to produce longer treatment activity, lower doses producing acceptable mortality that is cost competitive, may be realized. In an attempt to extend the residual activity of a low dose of Spinosad to increase mortality, the following study was conducted.

Objectives

Demonstrate that the length of residual activity of Spinosad could be extended by modifying the formulation.

Determine if extended residual activity can be translated into increased overall mortality.

Compare core formulation components of two separate pheromone formulations for potential use in Spinosad formulations.

Evaluate the utility of Field Aerial Application Spray Simulation Tower Technology (FAASSTT) for replacing an aerial application when the amount of material available for testing is limited.

Materials and Methods

The study was conducted in Harding County of northwestern South Dakota ca. 7.5 miles east and 4 miles south of the town of Ludlow on the Eugene Stensland ranch during the period of July 4 - 26, 2006. The location was selected because of the diversity in grasshopper species and grasses, density of grasshoppers, recent 3-year history of grasshoppers in the area, better than average range condition, availability of rangeland without livestock for the time required in the study and proximity to another ongoing study.

The design consisted of caging grasshoppers on (10) 14 inch diameter sprayed areas for each of 3 treatments and 10 untreated areas for controls (40 cages) at post-treatment intervals of 0, 3, 6, 9, 12, and 15 days after treatment (totaling 240 cages for the study). With this design each treatment was sprayed a total of 60 times. Treatment areas were pre- marked with 0.1 m^2 rings arranged in rows of 10 with rings within rows separated from adjacent rings by about 6 feet. Rows were also separated by about 6 feet. Groups of cages (40) for each interval were separated by about 12 feet (Fig. 1).

Each of three formulations (two containing potential extenders and one standard without extender) containing Spinosad from the commercial formulation, Tracer (Dow AgroSciences, Indianapolis, IN), were sprayed on the predetermined ring delimited areas of untreated rangeland (Fig. 2). The treatments were, Tracer and water applied at 32 fluid oz/acre, Tracer and the core component of a pheromone formulation (ISCA Technologies, Riverside, CA) applied at 64 fluid oz/ace and Tracer and the core component of another pheromone formulation (Pacific BioControl, Litchfield Park, AZ) applied at 32 oz/acre. The ISCA/Spinosad formulation was very viscous and was diluted with water at a higher total volume applied per acre to facilitate spraying. All sprays were applied at the rate of 1.5 fluid oz Tracer (21.3 g AI) / acre.

Each treatment was applied to areas 14 inches in diameter using the Field Aerial Application Spray Simulation Tower Technology (FAASSTT) (Fig.3), developed in the Phoenix Laboratory. The system consisted of a portable chamber fitted with an air brush (Paasche Type H with 75 regulator operating at 20 psi) and modified syringe (Fig. 4). Use of this system followed the established FAASSTT procedure involving three people, an applicator, a regulator/evaluator and a locator (Fig. 5). Each application was evaluated for deposition by using a 1 X 2 cm Tee Jet water sensitive spray card within the sprayed area.

After the areas were treated and on the appropriate post-treatment interval, cages (two gallon bottomless plastic buckets modified with screen sides and top) (Fig. 6), were placed on each of the treated areas. Each cage was then stocked with 5 adult *Ageneotettix deorum* grasshoppers. Cages were monitored for grasshopper mortality daily for 5 days following setup.

Results and Discussions

Compared to untreated populations, all three Spinosad treatments resulted in mortality significantly higher when grasshoppers were exposed to treated rangeland on the day of treatment (Table 1). At this interval the standard formulation of Spinosad produced mortality that was significantly higher than the treatments containing extenders. This level of mortality (90%) was considered consistent with that seen for the same dose in a 2003 study (85%) (Foster et.al 2004). Three day old residuals of the standard Spinosad formulation produced mortality significantly higher than in untreated populations. However, three day old residuals of both formulations of Spinosad containing extenders demonstrated no mortality significantly higher than untreated populations. There was no statistical difference in mortality resulting from any of the 6, 9 and 12 day old treatment residuals and untreated populations. Fifteen day old residuals, while producing higher mortalities than 12 day old residuals, produced no mortalities greater than occurred in untreated populations. Mortalities resulting from 6 and 15 day old residuals were slightly numerically higher than other residual ages and as of yet are unexplained.

Mortalities were expected to be similar for all treatments at the early residual intervals of 0 and 3 days after treatment, while treatments containing extenders were expected to show greater mortality than the standard Spinosad formulation at the post-treatment intervals of 6, 9, 12 and 15 days. Unfortunately, this did not occur. The authors believe the lack of performance was directly related to the extreme temperatures experienced during application (Fig. 7). Daily high temperatures were near or above 100° F for more than half of the study. High temperatures caused problems for two reasons. During any aerial application, total spray droplet deposition is greatly reduced when ground temperatures exceed air temperatures. These conditions existed during this study and most likely help to explain the unexpected results. While we initially thought the portable chamber would protect sprays from environmental conditions (wind), our smaller scale applications were not exempt from the laws of physics. Additionally, compared to a spray system on an aircraft, The FAASSTT relays on injecting liquid material into an air stream, and subsequently through a nozzle tip. This injection into the air stream coupled with the designed fast drying of the components within the formulations resulted in material drying before exiting the nozzle tip and significant nozzle plugging occurred. It is important to note that with an aircraft spraying system this drying and plugging of nozzles would not likely have occurred. Those systems force the liquid through the nozzle tip before becoming directly exposed to air. Spray cards showed evidence of both occurrences (reduced deposition and nozzle plugging). Sometimes no material was deposited on the cards, evidence of nozzle plugging and application was repeated. Sometimes when material was deposited on the spray cards, the pattern was weak, evidence that all of the fine droplets were not reaching the ground due to ground temperatures exceeding the surrounding air. In any case the authors had little option, the amount of experimental formulation was limited and was not of sufficient total volume to apply from a conventional aerial spraying system. Sufficient material to support an aerial application with a standard aerial application system should alleviate the drying problems associated with this study.

Conclusions

The water sensitive spray cards demonstrated two problems that caused the results of this study to be inconclusive. The injection of the experimental formulations into the air stream coupled with the designed fast drying of the components within the formulations resulted in material drying before exiting the nozzle, thus producing significant nozzle plugging. It is important to note that with an aircraft spraying system this drying and plugging of nozzles would not likely have occurred. Aircraft spraying systems force the liquid through the nozzle tip before becoming directly exposed to air. Additionally, when material was deposited on the spray cards, the pattern was weak, evidence that all of the fine droplets were not reaching the ground due to ground temperatures exceeding the surrounding air. Both of the problems experienced during this study can be alleviated with correct aerial application from an aircraft. The importance of extending the persistence of Spinosad warrants study being repeated with sufficient material to allow for traditional aerial application.

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Figure 1. Field cage layout in the study area near Ludlow, South Dakota.

Figure 2. Ring (0.1 m^2) set in place to delimit an individual spray area. (Note small spray card to confirm application)

Figure 3. Field Aerial Application Spray Simulation Tower Technology (FAASSTT) in operation. Applicator (center), regulator/evaluator (left) and locator (right).

Figure 4. Close-up of Field Aerial Application Spray Simulation Technology.

The FAASSTT procedure involves three people, an applicator, a regulator/evaluator and a locator.

The evaluator places spray card near the center of the area to be sprayed. Locator opens top inspection door and moves and precisely locates (centers) spray unit directly over the area to be sprayed. (Location identified by 0.1 m^2 ring to assist in centering). Locator closes top inspection door. Applicator agitates material and draws predetermined amount of material into syringe, removes bubbles, adjusts volume in syringe for accuracy, attaches hilt and inserts syringe into the side port of the airbrush. Regulator turns on the compressed air source and sets the pressure as predetermined. Applicator checks (through side service door) airbrush for air movement through system and wipes off any excess material from the airbrush tip. Locator closes side service door. Applicator dispenses material into the stream of air flowing through the airbrush. Applicator removes the syringe dispenser from the airbrush side port. Regulator dispenses two "puffs" of squeeze bottle supplied air through the side port of the airbrush to insure all material dispensed has been applied through the side port and then adjusts the pressure regulator to zero. Locator opens side inspection door and moves spray unit from treated area and places it on the next area to be treated. Evaluator retrieves and examines the spray card to determine positive deposition of material on the desired area. Process repeated.

Figure 5. Procedure for Field Application Spray Simulation Tower Technology (FAASSTT).

Figure 6. Field bucket cage used to evaluate Spinosad formulations near

Ludlow, South Dakota, 2006.

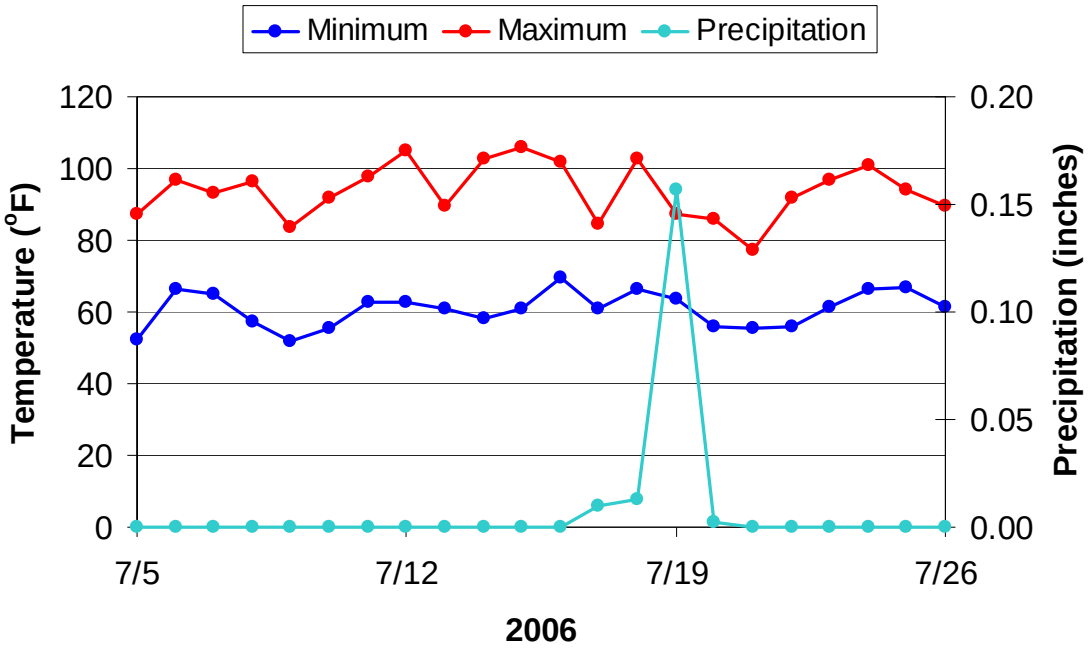

Figure 7. Daily minimum and maximum temperatures and precipitation recorded in the study area near Ludlow, South Dakota, 2006.

Table 1. Selected formulations of Spinosad applied by airbrush to range vegetation against *Ageneotettix deorum* grasshoppers in field cages near Ludlow, South Dakota, 2006.

	Residual day – mean % mortality [2]					
Treatment [1]	0	3	6	9	12	15
Spinosad - 32oz	90 a	62 a	24 a	6 a	2 a	10 b
Spinosad/ISCA - 64oz	48 b	8 b	34 a	4 a	4 a	16 ab
Spinosad/PBC - 32oz	34 b	2 b	20 a	4 a	0 a	34 a
Untreated	4 c	2 b	20 a	2 a	0 a	28 ab

[1] We applied all Spinosad treatments at 1.5oz AI/acre in a total volume of 32 or 64oz/acre.
[2] Mortality determined from data collected at 5 days after introduction of grasshoppers into field cages placed on range vegetation. Means in a column followed by the same letter are not significantly different ($P \leq 0.05$)

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Additional Evaluation of Selected Diluents and Doses of Malathion Against Older Grasshoppers Under Sub-average Rangeland Conditions in Northwestern Goshen County, Wyoming.

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Introduction

The currently used formulation of malathion (Fyfanon ULV) is typically applied undiluted at 8 (traditional) or 4 (reduced agent area treatments - RAATs) fluid oz per acre. Further reductions in AI use levels and percentage of acreage directly treated using the current Fyfanon ULV formulation or a longer lasting formulation of encapsulated malathion would greatly enhance the usefulness and economics of malathion in RAATs technologies and would help assure strong competition between the chemical tools available for private, state and federal use in grasshopper suppression on rangeland.

The recent developments in reduced agent-area treatments (RAATs) strategies (Foster et al. 2000; Lockwood et al. 2000) have greatly improved the economics of grasshopper treatments. However, room for additional improvement, particularly with malathion, still exists. With the RAATs strategy, maximum control is not the goal (Larsen and Foster, 1996) and greater attention is afforded to actual cost benefit parameters through economic analyses (Berry et al. 1996). Additionally, lower than traditional insecticide doses are used in combination with alternated directly treated and untreated swaths. However, material unintentionally deposited in the area not directly treated has an enhancing impact on the overall effectiveness of RAATs strategies. Carbaryl and

diflubenzuron treatments are used in RAATs treatments that cover a maximum of 50% of the acreage to be protected while malathion is used in a treatment that covers 80% of the acreage to be protected. Generally, this design is considered to be a result of the persistence of the insecticide used. Shorter persistence requires smaller areas left untreated between swaths. However, when malathion is used in RAATs treatments, a total volume of 4 fl oz/acre is applied compared to 16-31 fl oz/acre with carbaryl and diflubenzuron, respectively. The small total volume used with malathion appears insufficient to accommodate a larger spacing of the aircraft during application. Simply increasing the total volume of a malathion mix applied by adding a diluent may provide a sufficient volume of material to increase the swath spacing to approximate the spacing used with other chemicals; therefore decreasing the maximum amount of acreage that must be directly treated.

During a study to compare several doses of malathion and encapsulated malathion (Foster et al. 2002), data was developed which strongly indicated that the standard formulation of malathion Fyfanon ULV can be further enhanced for use in RAATs strategies by adding a cottonseed oil (CSO) diluent to increase the total volume applied per acre while further reducing the amount of AI applied per acre. Their data indicate that reductions from the current 4 fluid oz per acre of malathion to 3 or 2 and perhaps even 1 fluid oz per acre may be possible if CSO diluent is added. A reduction to the two oz AI level with 8 fluid oz of CSO and treated as RAATs in 50% coverage is estimated to cost a remarkable $0.64 for formulation and $0.78 for application per treated acre ($0.71 per protected acre in RAATs). This compares to the traditional uniform coverage of 8 fl oz/ac of malathion at a total of $2.86/ac. Such a treatment would become the least expensive aerial treatment for rangeland grasshoppers and would average one fluid oz AI per acre, an incredible one third as much AI per acre as mosquito treatments that rely on malathion.

In a detailed and replicated study conducted in 2004 to evaluate and compare selected diluents and doses of malathion on adult grasshoppers under extreme drought and poor rangeland conditions (Foster et. al, 2005), both the standard and lower doses resulted in mortality levels substantially lower than expected and inconsistent with results from similar treatments studied earlier (Foster et al. 2002). The authors ruled out degraded material or advanced population age as causing factors in the 2004 study. They strongly suggested that the substantial reduction in mortality was most likely attributable to minimal vegetation available for collection of spray droplets and subsequent ingestion by the grasshoppers. That suspicion gained substantial credence when, at the end of that study, the authors treated an additional un-replicated plot with much more vegetation for comparison with those in the full study. With the standard 8 oz/acre 100% coverage treatment, the plot with more vegetation than those in the replicated study showed 76, 82, and 92 % mortality at 1, 3 and 7 days after treatment compared to 62, 69 and 76 % mortality in worse rangeland conditions. As a result the authors suggested that malathion should not be used in situations where grasshoppers and drought damage have reduced range conditions to mostly bare ground with minimal vegetation, especially when a preponderance of the population are adults.

Because of the potential for improving the economics of malathion and impact on the environment, further evaluation of these selected treatments is still warranted and should be conducted on a younger assemblage of grasshoppers, but more importantly should be conducted on more typical rangeland conditions where less bare ground and more vegetation is present.

To generate data under conditions appropriate for malathion use on rangeland and for comparison with previous studies conducted under extremely poor rangeland and drought conditions, the following study was conducted.

Objectives

Determine in the field, the potential for increasing mortality with low doses of Fyfanon ULV by increasing the total volume applied / acre with a cottonseed or canola oil diluent.

Specifically, evaluate and compare the standard traditional malathion treatment of 8oz/acre applied to 100% of the acreage and 2, 3 and 4 fl oz/acre of malathion applied in a total volume of 10 fl oz/acre of CSO to 50% of the acreage for activity against grasshoppers.

Specifically, evaluate and compare 2 fl oz/acre plus 8 fl oz /acre of CSO and 8 fl oz/acre of malathion both applied to 100% of the acreage for activity against grasshoppers.

Compare the efficacy resulting from treatments conducted on rangeland under extreme drought and poor rangeland conditions in an earlier study to results from selected similar treatments conducted on younger grasshoppers on rangeland exhibiting somewhat better conditions and impacted with less severe drought.

Methods and Materials

Study Site

The center of the study was located in northwestern Goshen County, Wyoming approximately 12.5 miles north and 3.5 miles east of the town of Fort Laramie on the Jim O'Brien ranch. The general location was selected because of grasshopper density, adult age, history of grasshopper problems, and abundance of contiguous grasshopper infested land.

Treatments and Experimental Design

The undiluted Fyfanon ULV formulation was used for comparison and in all mixes with CSO. The treatments studied were 2, 3 and 4 fl oz/acre of Fyfanon ULV increased to a total volume of 10 fl oz /acre for application with CSO (0.155, 0.232 and 0.309 lb AI/ac, respectively). These treatments were applied at 50% coverage. Additional treatments studied were 2 fl oz/acre of Fyfanon ULV increased to a total application volume of 10 oz/acre with CSO and applied at 100% coverage and 8 fl oz/acre of undiluted Fyfanon

ULV applied at 100% coverage (0.155 and 0.618 lb AI/ac respectively). All treatments were aerially applied to square 40 acre grasshopper infested rangeland plots and were replicated four times. Four untreated plots were included in the experimental design. The replicated study consisted of 24 forty acre plots. To insure that any one treatment was not assigned exclusively to plots with high or low grasshopper densities and that all treatments were tested against similar population densities, pretreatment counts were arranged in descending order and divided into groups of six. Subsequently, each of the 6 treatments, including the untreated control, were randomly assigned to one of the six plots within each group.

The 8 oz at 100% coverage and 4 oz plus 6 oz CSO at 50% coverage were applied on June 27. The 3 oz plus 7 oz CSO at 50% coverage was applied on June 28. The 2 oz plus 8 oz CSO at 50% coverage and 2 oz plus 8 oz CSO at 100% coverage were applied on June 29. Treatments were 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 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. All applications occurred from an altitude of 30 to 50 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 aircraft was calibrated for a 100 feet wide swath for all treatments. Treating 100% and 50% of a plot was achieved by calibrating the aircraft for a 100 foot wide swath and spacing the aircraft during treatment at 100 and 200 feet respectively during application.

The specific mix, total volume applied per acre, number of nozzles, nozzle screen size, nozzle tip size, boom pressure, aircraft speed, and swath width used for each of the 5 different treatments is summarized in Table 1. Winds during application ranged from 0 to 5 mph and averaged 1.7 mph. Ground temperatures did not exceed air temperatures at any time during applications. Other meteorological conditions recorded during application are summarized in Table 2. The precipitation and daily minimum and maximum temperatures recorded by a temporary weather station established in the treated area for the duration of the study are shown in Figure 1.

Sampling Methods

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 1, 3 and 7 days after treatment. Untreated control plots were also 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 an approximate 100 yard diameter circle near the center of each 40 acre plot. Rings were separated from adjacent rings by ca, 5 yards.

The abundance of each species was determined from uniform sweep samples taken at each site (Foster and Reuter, 1996). Each sample consisted of 50 high and fast sweeps and 50 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.

Analysis

For the general population, data were expressed as percent survival based on pretreatment counts in the same plot and 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.

Percentage control data were converted to rank data (Conover and Iman, 1981). An analysis of variance was performed with the Tukey's HSD multiple comparison test used to separate means. All analyses were performed with Systat 6.1 For Windows.

Results and Discussion

Pretreatment densities averaged 10.7 grasshoppers/m^2 for all of the plots in the study. At the time of treatment, the population was composed of 0.94 % first instars, 0.91 % second instars, 2.91 % third instars, 12.61 % fourth instars, 52.02 % fifth instars and 30.62 % adults. The total mean age was 5.06. A value of 1-5 coincides with the first 5 instar stages and a value of 6 represents the adult stage. The seven most abundant species were *Ageneotettix deorum* (48%), *Cordillacris occipitalis* (18%), *Melanoplus infantilis* (9%), *Melanoplus sanguinipes* (7%), *Amphitornus coloradus* (5%), *Phlibostroma quadrimaculatum* (5%) and *Aulocara elliotti* (4%). The relative abundance of all species in pretreatment samples is shown in Table 3.

All treatments resulted in significantly greater mortality than occurred in the untreated controls at 1, 3 and 7 days after treatment except the 2 oz of malathion plus 8 oz of CSO applied at 50% coverage at 1 day after treatment. (Table 4). Within each coverage group, 50% and 100% coverage, treatments generally performed in dose rank order. The exception was 4 oz of malathion plus 6 oz of CSO compared to 3 oz malathion plus 7 oz CSO at 7 days after treatment. When data was corrected for changes in the untreated population (Table 5) both treatments that were applied at 100 % coverage (8 oz malathion/acre and 2 oz malathion plus 8 oz CSO/acre) resulted in statistically equivalent mortality and were numerically higher than the other treatments at all of the intervals evaluated. While the three doses applied at 50% coverage generally performed numerically in dose rank order, there was no significant difference in mortality produced by the three treatments. However, the lowest dose, 2 oz malathion plus 8 oz CSO/acre applied at 50% coverage, resulted in significantly lower mortality than the 8 oz treatment at all intervals and the 2 oz treatment applied to 100% of the acreage at 1 day after treatment.

While these data unquestionably demonstrate higher mortality than seen with similar treatments on poor rangeland (Foster et. al 2005) they, at first, also appear inconsistent with and lower than earlier findings under substantially better rangeland conditions (Foster et. al 2002). The varying results among the three different studies are believed to be generally a result of different rangeland conditions. Compared to average conditions, poor range conditions with sparse vegetation result in less grass for collection of aerially applied spray droplets and translate into lower mortality. Particularly, since the major component of mortality attributed to malathion in grasshopper control is a result of ingestion rather than contact. (Pfadt et. al 1970). The varying range conditions for 2002, 2004 and 2006 studies can be seen in Figures 2, 3 and 4 respectively. The extra plot in the 2004 study with more vegetation and that produced higher mortality compared to those in the main replicated study is seen in Figure 5. Conditions during this 2006 study were judged by the authors to be closer to those experienced in 2004 than 2002 and as such would seem to suggest that further improvement in results could be achieved under better range conditions.

A detailed comparison between the results of the 2004 and this 2006 study can be seen in Table 6. Mortalities in the 2006 study averaged 20% higher than those in the 2004 study which was conducted under extremely bad range and drought conditions. Cursory examination of 2006 and 2002 data could lead one to conclude that the 2002 results are substantially superior. However, when the average AI/acre doses, that are common to both studies, are considered (Table 7), 2006 results are fairly consistent with 2002. Therefore, a substantial significant improvement in results may not be expected even if the study was conducted under better range conditions.

A comparison between 4 oz of malathion plus 6 oz of CSO at 50% coverage and 2 oz malathion plus 8 oz CSO at 100% coverage (both treatments have the same average AI of 2oz per acre) indicates that a total coverage treatment may produce better control. However, the cost would be substantially higher (Table 8). In another comparison, one would suspect that numerically the 4 oz malathion + 6 oz CSO would be better than 3 oz

malathion +7 oz CSO. This was not the case in this study, 71 % vs 78% respectively (Table 5) and this result was consistent with results in the 2004 study, 47% vs 50% respectively.

The average mortality of the standard RAATSs 4 oz 80% coverage malathion treatment is ca. 80% (Foster 2000). In terms of mortality, two of our treatments compared very favorably, 3 oz of malathion plus 7 oz CSO at 50% and 2 oz malathion plus 8 oz CSO at 100% coverage producing 78% and 79% mortality, respectively. One of the major reasons for the development of RAATs was the economy of treatments. Costs must be compared to fully evaluate the potential RAATs treatments in this study (Table 8). The standard RAATS treatment costs an estimated $1.77 / acre . Our best RAATs treatments 3 oz malathion plus 7 oz CSO at 50% coverage and 2 oz malathion plus 8 oz CSO at 100% coverage are comparatively estimated to cost $1.31 and $2.52/ acre, respectively.

The efficacy of these low AI /acre treatments is quiet remarkable. The lowest use rate registered for malathion is for treatment of mosquitoes at 2-4 oz/acre. All of the treatments we studied containing 4 oz or less of malathion are equivalent to or lower than the lowest AI/acre rate registered for adult mosquito control. The potential environmental and economical advantages of these remarkably low AI treatments are so great that further evaluations on an operational scale (at least 640 acres) are warranted.

Conclusions

Rangeland conditions are extremely important in predicting the success of an aerial treatment of malathion. Malathion should not be used on rangeland that exhibits extremely sparse vegetation. Treatments containing reduced levels of malathion may still afford significant mortality under less than average rangeland conditions. However, significant vegetation must be present to capture the spray droplets. Even though only slight improvements in the range condition were seen in 2006 compared to 2004, substantial improvements in mortality levels resulted.

Treatments containing 3 oz malathion plus 7 oz CSO/acre applied at 50% coverage and 2 oz malathion plus 8 oz CSO/acre applied at 100% coverage resulted in mortality levels (78 % and 79 % respectively) that could be considered acceptable as an alternative RAATs treatment when compared to mortality (80%) expected from the standard 4 oz malathion applied to 80% of the acreage . While providing essentially the same level of control, the 3 oz plus 7 oz CSO at 50% coverage is estimated to cost 26% less than the standard RAATs treatment while the 2 oz plus 8 ozs CSO at 100% coverage is estimated to cost 42% more. In this study, treatments containing lower AI/acre levels of malathion resulted in either unacceptable levels of control or expense. The addition of a diluent apparently increases the total volume applied to a level sufficient to allow for reduction in AI below the standard 4 oz undiluted rate applied at 80% coverage.

The efficacy of these low average AI /acre treatments is quiet remarkable. A standard use rate of malathion against adult mosquito is 2-4 oz/acre. The best alternative treatment in

our study averaged 1.5 oz/acre. The potential environmental and economical advantages of these remarkably low AI treatments are so great that further evaluations on an operational scale are warranted. Additionally, higher levels of diluent should also be studied. The diluent could be increased another 6 oz/acre without exceeding the cost of the standard RAATS treatment and may provide even higher levels of mortality.

Study Improvement

This is a new section that the senior author has been considering for several years to include in all reports. It is an attempt to improve future similar studies and investigations by identifying design issues that result in less than optimal intended results. The operational question of this section is: What would the author/s do differently if able to repeat this study. The pitfall of such a section is that the weaknesses in the study are not only suggested, but highlighted for all to see. However, in the eyes of science, the potential for improving subsequent similar studies far outweighs such a concern.

The study should be conducted under more average rangeland conditions. This will minimize grasshopper movement within the evaluation areas as vegetation dries and is consumed and additionally will allow for better collection of aerially applied droplets by the vegetation which must be consumed to realize the major component of mortality (ingestion rather than contact) that the treatments will produce.

A location should be selected with higher densities of grasshoppers. This will improve the precision of analyses that are performed on the data.

A larger study site (320 additional acres) that would allow for two additional treatments, should be used. Including the standard RAATs treatment of 4 oz per acre of malathion applied to 80% of the acreage would provide for a comparison with the experimental RAATs treatments applied to 50% of the acreages. Including an additional lower dose treatment with a canola oil diluent would allow for comparison of CSO and canola oil diluents, a question as yet unanswered.

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Table 1. Summary of treatments and calibration parameters.

Treatment AI/acre	Coverage	Fyfanon ULV fl oz/ac	Diluent[1] fl oz/ac	Total fl oz/ac	Nozzle no.	Tip size	Nozzle screen size	Pressure psi	Aircraft speed	Swath width
0.618	100	8	0	8	8	8002	50	32	120	100
0.309	50	4	6 cso	10	10	8002	50	27	120	100
0.232	50	3	7 cso	10	10	8002	50	27	120	100
0.155	100	2	8 cso	10	10	8002	50	27	120	100
0.155	50	2	8 cso	10	10	8002	50	27	120	100

[1] cso = cottonseed oil

Table 2. Meteorological conditions recorded during aerial application of treatments in the grasshopper study plots near Fort Laramie, Wyoming, 2006.

Treatment [1]	Plot no.	Date	Time (AM) Start	Time (AM) End	Temperatures ºF. Ground Start	Ground End	Air Start	Air End	Pilot	Wind (mph) Start	Wind (mph) End
8oz @100%	17	6/27	5:15	5:27	45	50	54	60	55	1-2 N	1-2 N
	19	6/27	5:29	5:42	48	50	51	53	56	4 NW	5 NW
	13	6/27	5:42	5:57	49	51	54	54	56	< 1 N	3-4 N
	24	6/27	5:58	6:09	51	52	53	56	56	2-3 NW	3 N
4oz+6oz @50%	9	6/27	7:04	7:10	61	63	67	68	68	< 1 NE	< 1 NE
	23	6/27	7:10	7:16	66	68	69	70	68	1-2 N	1 NW
	3	6/27	7:24	7:29	61	70	68	71	69	< 1 W	1-2 W
	21	6/27	7:32	7:38	68	69	72	70	69	2-3 SW	1-2 SW
3oz+7oz @50% [2]	2	6/28	5:12	5:19	50	53	60	57	59	3-4 N	4-5 N
	11,12	6/28	6:05	6:23	50	56	53	61	59	3-4 N	2-3 NE
	18	6/28	6:23	6:29	56	58	59	63	59	1-2 NE	< 1 N
2oz+8oz @50%	8	6/29	5:12	5:19	57	53	61	56	68	1-2 NW	3-4 SW
	14	6/29	5:20	5:27	51	52	59	57	66	1-2 W	< 1 W
	6	6/29	5:34	5:42	51	54	56	59	66	0-1 W	2-3 W
	10	6/29	5:43	5:55	52	53	57	64	66	2-3 NW	< 1 W
2oz+8oz @100%	1,5	6/29	5:56	6:11	53	56	58	58	64	0	1-2 SW
	7	6/29	6:15	6:24	54	56	64	64	64	1-2 W	< 1 W
	28	6/29	7:30	7:40	62	62	66	74	72	2-3 NE	< 1 N

[1] Fluid ounces of malathion (Fyfanon ULV) + cottonseed oil diluent and percent coverage.
[2] Returned to airport after one pass in plots 11,12 to repair failed nozzle.

Table 3. Grasshopper species composition and age structure prior to treatment near Fort Laramie, Wyoming 2006.

	Instar							
Species (26-27June 06)	1	2	3	4	5	Adult	**Total**	%
Gomphocerinae								
Acrolophitus hirtipes						1	1	0.04
Ageneotettix deorum		1	8	114	1026	111	1260	47.57
Amphitornus coloradus			1	4	41	95	141	5.32
Aulocara elliotti			1	3	32	71	107	4.04
Cordillacris occipitalis				2	36	442	480	18.12
Mermiria bivittata						1	1	0.04
Opeia obscura		2	3	5	1		11	0.42
Phlibostroma quadrimaculatum		1	8	22	42	54	127	4.79
Psoloessa delicatula	6					3	9	0.34
Melanoplinae								
Melanoplus confusus						7	7	0.26
Melanoplus gladstoni	12	12					24	0.91
Melanoplus infantilis		4	26	105	88	7	230	8.68
Melanoplus packardii			5	13	4		22	0.83
Melanoplus sanguinipes		3	22	62	84	9	180	6.80
Melanoplus "species"	2						2	0.08
Phoetaliotes nebrascensis		1	2	1			4	0.15
Oedipodinae								
Arphia conspersa	2						2	0.08
Arphia pseudonietana	1			1			2	0.08
Hadrotettix trifasciatus					2		2	0.08
Metator pardalinus					2	3	5	0.19
Spharagemon collare					2		2	0.08
Spharagemon equale				1	3		4	0.15
Trachyrhachys kiowa			1	1	15	7	24	0.91
Xanthippus corallipes	2						2	0.08
Totals	25	24	77	334	1378	811	**2649**	
%	0.94	0.91	2.91	12.61	52.02	30.62		

Table 4. Mean percentage mortality of grasshoppers treated with selected doses and diluent mixes of malathion – Fort Laramie, Wyoming, 2006.

Treatment[1]	1 day	3 day	7 day
8oz malathion 100%	87 a	99 a	98 a
4oz malathion + 6oz cso 50%	58 ab	69 ab	74 ab
3oz malathion + 7oz cso 50%	59 ab	62 b	87 ab
2oz malathion + 8oz cso 50%	32 bc	68 ab	55 b
2oz malathion + 8oz cso 100%	71 a	84 ab	74 ab
Untreated	4 c	23 c	18 c

[1] Means in a column followed by the same letter are not significantly different ($P \leq 0.05$).

Table 5. Mean percentage mortality of grasshoppers (adjusted for natural mortality) treated with selected doses and diluent mixes of malathion – Fort Laramie, Wyoming, 2006.

Treatment [1,2]	1 day	3 day	7 day
8oz malathion 100%	86 a	99 a	97 a
4oz malathion + 6oz cso 50%	57 ab	64 bc	71 ab
3oz malathion + 7oz cso 50%	57 ab	52 c	78 ab
2oz malathion + 8oz cso 50%	39 b	66 bc	63 b
2oz malathion + 8oz cso 100%	74 a	83 ab	79 ab

[1] Percentage mortalities were corrected for population changes in the untreated plots. Connin and Kuitert 1952.
[2] Means in a column followed by the same letter are not significantly different ($P \leq 0.05$).

Table 6. Mean percentage mortality of grasshoppers (adjusted for natural mortality) treated with selected doses and diluent mixes of malathion in 2 Wyoming studies.

Treatment [1,2]	Coverage	DAT [3]	2004	2006
8oz	100%	1	67	86
		3	75	99
		7	80	97
4oz	80%	1	45	
		3	66	
		7	70	
4 oz + 6oz cso	50%	1	46	57
		3	54	63
		7	47	71
3oz + 7oz cso	50%	1	32	56
		3	41	52
		7	50	78
2oz + 8oz cso	50%	1	32	39
		3	41	66
		7	50	62
2oz + 8oz cso	100%	1	38	74
		3	53	82
		7	57	79

[1] First number in treatment = malathion/acre, second number = cottonseed oil diluent/acre
[2] CSO = cottonseed oil
[3] DAT = days after treatment

Table 7. Mean percentage mortality of grasshoppers (adjusted for natural mortality) treated with acre average doses common to 3 separate Wyoming studies.

		Year of study		
Acre average AI dose [1]	DAT [2]	2002	2004	2006
8 oz	3	90	75	99
	7/9	94	80	97
2 oz	3	87	53	82
	7/9	88	57	79
1 oz	3	45	41	66
	7/9	51	50	62

[1] Acre average AI dose : i.e. 4 oz malathion at 50% coverage = an average of 2oz AI/acre dose.
[2] DAT = days after treatment : 2002 9DAT; 2004 and 2006 7DAT

Table 8. Comparison of estimated costs of selected treatments. [1]

Treatment			Cost / protected acre			
insecticide	diluent	coverage	insecticide	diluent	application	TOTAL
8oz malathion[2]		100%	1.312	0.00	1.55	2.86
4oz malathion		80%	0.525	0.00	1.24	1.77
4oz malathion[3]	6oz cso	50%	0.328	0.24	0.78	1.35
3oz malathion	7oz cso	50%	0.246	0.28	0.78	1.31
2oz malathion	8oz cso	50%	0.164	0.32	0.78	1.26
2oz malathion	8oz cso	100%	0.328	0.64	1.55	2.52

[1] Based on malathion @ $21/gallon; cottonseed oil @ $10.31/gallon; application costs/directly treated acre @ $1.55
[2] Standard 100% coverage treatment.
[3] Standard RAATs treatment.

Figure 1. Minimum and maximum temperatures and precipitation recorded at the study area near Fort Laramie, Wyoming 2006.

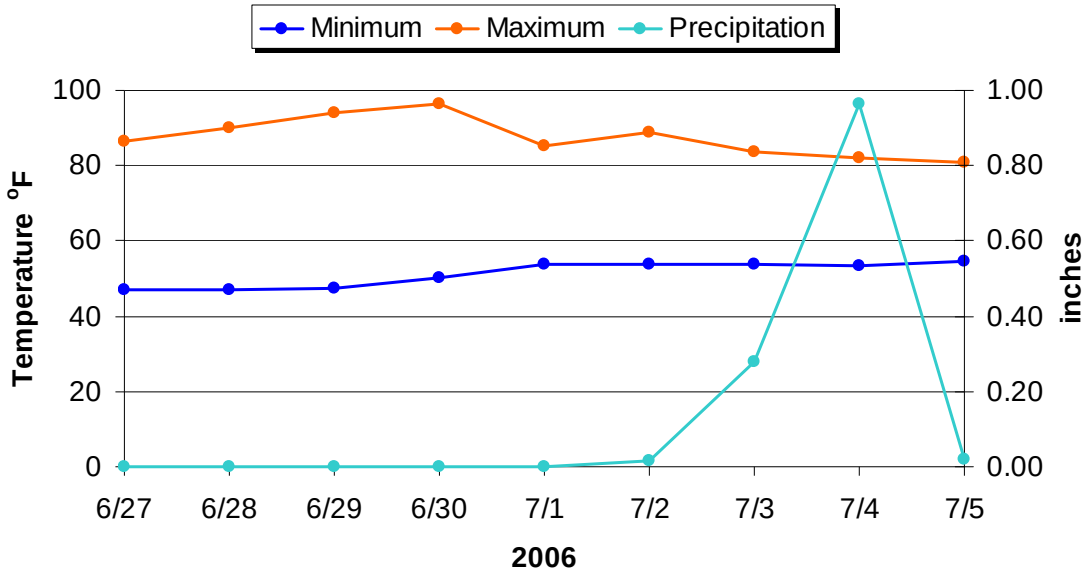

Figure 2. An example of typical range vegetation conditions near Edgemont, South Dakota in 2002.

Figure 3. An example of range vegetation during drought conditions encountered in this study near Jay Em, Wyoming 2004.

4. An example of sub-average rangeland conditions near Ft. Laramie in 2006.

Figure 5. Additional non-replicated 40 acre plot with more typical range vegetation treated for comparison with sparse vegetation plots near Jay Em, Wyoming 2004.

Simulated aerial sprays for field cage evaluation of *Beauveria bassiana* and *Metarhizium anisopliae* against *Anabrus simplex* in Montana

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Abstract

Both *Beauveria bassiana* strain GHA and *Metarhizium anisopliae* strain F52 demonstrated success, although minor, in reducing immature Mormon cricket populations in field cages. High field temperatures and or thermoregulation by the Mormon crickets prevented higher mortality from being expressed in the field. Field treated individuals moved to the laboratory and held under optimal thermal conditions demonstrated 90% and 100 % mortality in 12 days for *B. bassiana* and *M. anisopliae* respectively, indicating a lethal dose was applied and additionally confirming that sufficient feeding by the crickets in the field to pick up a lethal dose had occurred. Thermal surrogates, used to develop models for predicting the time required for fungal development in vivo to produce mortality, indicated mortality would start to occur between 8 and 27 days after treatment for *M. anisopliae* and 12-34 days after treatment with *B. bassiana*. The timing of mortality in field cages was consistent with the upper boundaries of the temporal models developed from the thermal surrogates. Our field mortality occurred between 28 and 39 days after treatment. Cage effects, in terms of temperature, which have historically been a problem in evaluating fungal populations in the field, were essentially eliminated with the newly designed hardware cloth cages used in this study. The new cages resulted in internal temperatures very near un-caged situations at both canopy and ground levels. The newest version of Field Aerial Application Spray Simulation Tower Technology used in the study resulted in the application of a lethal dose in an aerially simulated spray and in combination with the new field cage appears to be an efficient and economical method of applying sprays, particularly biologically based, for evaluation in very small field tests.

Introduction

In the early 1990's, extensive work on the development of the fungus *Beauveria bassiana* GHA by Mycotech Corporation and USDA-APHIS "Methods" (Foster et al. 1991, 1992, 1993, 1996-1999) led to its registration in the US, even though final development for wide acceptance and use was never achieved. While the "stressor concept " (Foster et al. 1996; Reuter et al. 1996), where sub-lethal doses of insecticides were added to the fungus in an attempt to alter normal behavior of a grasshopper to increase fungal susceptibility, indicated potential promise, the USDA-CPHST efforts in its development were redirected from 1995 until 2002. Interest in *B. bassiana* has recently been renewed with work conducted by the University of Wyoming which indicated that canola oil as an attractant and diluent may improve results. Collaborative work conducted by the University of Wyoming (Lockwood, 2003) and the USDA- ARS (Jaronski et al. 2002) 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 indicated substantial differences between *B. bassiana* treatments applied in canola oil compared to paraffinic oil and untreated control populations. In 2003, a joint APHIS and ARS replicated study (Foster et al. 2003) conducted to verify the improvement with canola oil compared to a paraffinic oil diluent, showed that no significant increase in grasshopper mortality compared to untreated populations could be attributed to either of the oils studied. Subsequent unpublished laboratory studies conducted by Jaronski, have revealed grasshoppers exhibited greater infection levels and consumption amounts when exposed to paper disks treated with raw canola oil and *B. bassiana* compared to the refined canola oil and *B. bassiana* mixture that had been used in both the 2003 and 2004 studies. However, a subsequent joint APHIS and ARS study to evaluate unrefined and refined canola oil mixtures with *B. bassiana* in field studies, revealed no statistical difference in population reductions among the *B. bassiana* treatments or between those treatments and untreated control populations or populations treated with unrefined canola oil without *B. bassiana*. (Foster et al. 2004).

The most popular current belief concerning ineffective results of *B. bassiana* against grasshoppers, in unconfined field populations, supposes that by positioning themselves to increase sun exposure, grasshoppers thermo-regulate and increase body temperatures (Inglis et al. 1996; Inglis et al. 1999). This higher temperature places the insect in a temperature environment much of the day that is non-conducive to fungal growth within the insect. Additionally, in past field studies we have observed fungal growth slowed greatly after sundown because body temperatures tracked the dropping ambient temperatures. The combination of both occurrences yield few hours each day during which grasshopper body temperatures are permissive, much less optimal, for fungal growth. As a result, the mycosis takes so long to grow to a level to cause mortality that detection of population reductions attributable to the fungus, compared to a natural population decline, is difficult at best. Therefore, lower daylight environmental temperatures may promote more easily detectable mortality that could be attributed to treatment induced fungal infection. Additionally, because damaging populations of grasshoppers are generally present in hotter conditions for longer periods during the day

than Mormon crickets, and because Mormon crickets did not demonstrate significant "behavioral fever" (thermoregulation) after infection with *B. bassiana* (Turnbow 1998), optimal temperature parameters for fungal development within an insect may be more easily encountered with Mormon crickets, particularly with immatures early in the year.

Another entomopathogen, *Metarhizium anisopliae* F52, has recently been registered for control of Coleoptera in horticulture and managed turf, and soft bodied ticks by Earth Biosciences Corp. (Danbury, CT), now Novozymes Biologicals (Salem,VA). In the laboratory, bioassays with immature and adult Mormon crickets, Jaronski (unpublished data) indicated that the F52 strain was highly infectious and virulent, more so than *B. bassiana* GHA, therefore presenting a potential alternative.

Previous work (Foster et al. 2006) has shown that longer than expected post-treatment intervals may be required to produce measurable mortality in the field attributable to artificially induced fungal infections. In those studies post-treatment field observations were conducted for only 14 days. However, the surrogate temporal data indicated that (depending on cricket location) 12 – 45 days and 19 – 80 days were estimated to be required before mortality could be seen with *M. anisopliae* and *B. bassiana*, respectively.

Significant work remains to develop fungal pathogens in the U.S. against Orthopteran insects. In an attempt to maintain detailed observation in the field through the required post-treatment interval necessary for measurable mortality to occur with both fungal isolates and to further evaluate their potential in the field, the following study was conducted.

Objectives

Validate the current concepts of temperature limitations on speed of fungal produced mortality.

Determine the accumulated hours of optimal and sub-optimal temperature parameters necessary for fungal growth to produce mortality under field conditions near Sidney, Montana using thermal surrogates of Mormon crickets.

Translate the required thermal parameters of the specific location into a prediction of the necessary post-treatment days required for measurable mortality (model) and compare with actual mortality.

Evaluate and compare *Beauveria* bassiana GHA and *Metarhizium anisoplinae* F 52 for potential against immature Mormon cricket.

Evaluate the operational effectiveness of Field Aerial Application Spray Simulation Tower Technology (FAASSTT).

Evaluate a newly designed field cage for maintaining temperatures similar to the surrounding environment (minimize cage effect).

Materials and Methods

Field Cage Study

The study area was located on the outskirts of Sidney, Montana. This location was selected because of the ease for almost continuous observation and proximity of the USDA-ARS-Northern Plains Agricultural Research Laboratory in Sidney.

Conidia of *B. bassiana* Strain GHA, as technical grade active ingredient Lot 0304-1, were supplied by Leverlam International, Butte, MT, (previously Emerald BioAgricultural Corporation, Lansing, Michigan). Conidia of *M. anisopliae* Strain F52, supplied by Novozymes Biological Inc. Salem, Virginia (previously Earth BioSciences, New Haven, Connecticut) were produced by USDA ARS NPARL using a biphasic, liquid-solid substrate fermentation process (Bradley et al. 2002). Prior to formulation, conidial viabilities were determined by plating dilute aqueous suspensions of each technical powder onto potato dextrose agar, incubating at 27-28° C. for 16-19 hr, and then examining the conidia with 400X phase contrast microscopy. The *Metarhizium* required a preliminary step to determine viability, in which a small quantity of conidia was exposed to 100% relative humidity for 1-2 hr before suspension and plating. A minimum of 400 conidia were examined for germination; a conidium was considered viable (germinated) if it had produced a visible germination peg during the specified incubation time. Viability of the *B. bassiana* and *M. anisopliae* technical powder was 95% and 85%, respectively.

The conidia were formulated in Orchex 796 paraffinic oil (Exxon Corp.) at the USDA ARS, Northern Plains Agricultural Research Laboratory (NPARL) in Sidney, Montana. Sufficient spores of *B. bassiana* GHA or *M. anisopliae* F52 were added to oil to achieve 8×10^{12} viable conidia/liter. The fungal formulations were applied at an intended rate equivalent to 4×10^{13} conidia / 5 liters (1.32 gal) / acre). A carrier control (oil carrier only applied at the same volume per acre) and an untreated control were included in the experimental design. Each of the treatments, including oil carrier and untreated controls, was replicated 6 times with 10 cages (each containing one Mormon cricket) per replicate. Therefore, the study utilized a total of 240 cages.

Treatments were applied to small areas of improved rangeland delimited by 0.1 m^2 rings (Figure 1) arranged in 24 rows of 10. Rings were separated from adjacent rings by 2 m. Treatments were applied by using Field Aerial Application Spray Simulation Tower Technology (FAASSTT). Sprays of treatments were specifically applied by using an airbrush (Paasche Type H with 75 regulator) modified with a customized syringe needle for liquid injection to produce droplets which simulate aerially applied sprays (Foster et al. 1996) to the area to be enclosed by each cage (Fig.2). The spraying system was attached to the top of a portable containment chamber which prevented drift (Fig. 3). The detailed protocol can be seen in Figure 4. Because of the time required to treat each of

the cages with sprays, three replications of the spray portion of study were treated on June 1 and 2**,** 2006 respectively.

Following treatment, cages 8.5 in high x 7 in diameter, fashioned from 1/8 inch hardware cloth (22 cm high by 17.8 cm diameter fashioned from 0.32 cm hardware cloth) (Fig. 5) were established on each sprayed area (Fig.6). Immediately following treatment and cage placement, each of the cages was stocked with a single fourth – fifth instar Mormon cricket. Stocking cages with single crickets prevented cannibalism, a common occurrence when more than one cricket is confined in a cage. Crickets were previously captured on May 31 near Lodge Grass, Montana and transferred to the Sidney location in cooled Styrofoam containers. Within the containers, crickets were individually held in 120 ml specimen cups with screw cap lids. Each lid had a screened 3-4 cm diameter opening for ventilation. Cricket mortality within cages was monitored daily.

The study was conducted from June 1 through July 16, 2006. After three days in the field cages, two of the six replicates were transferred to the laboratory for incubation at (28 C) 82.4 ^{0}F. to determine efficacy under known, constant, optimal developmental temperature for the fungi. The other 4 replicates remained in the field for the duration of the study.

Crickets that died during the study were removed from the field cage on the day of death and placed individually in a labeled petri dish for observation. The dishes were maintained at near saturated relative humidity. Each dish contained a cotton ball moistened with distilled water to provide very high humidity, to promote fungal outgrowth from the cadaver and sporulation. The presence of fungal outgrowth characteristic of *Beauveria* or *Metarhizium* was an indication of mycosis (Fig.7).

Mormon cricket thermal surrogates (Fig. 8) were used to measure the temperature that a cricket would generate through body positioning on the ground and at canopy level within and outside of a cage. They were also used to determine the duration of time above and below selected temperature thresholds for fungal growth as determined from the literature and data generated by USDA ARS Sidney, MT (Jaronski, unpublished). Surrogates consisted of 1.5 ml Eppendorff tubes filled with soy sauce and fitted with a copper-constatin thermocouple wire connected to a data logger recording device (DualogR®, Cole Palmer) These thermal surrogates have been shown to reasonably represent the ability of Orthoptera to absorb solar radiant heat as well as indicate typical body temperatures when Orthoptera are unable to thermo-regulate (Lactin and Johnson 1998). The recorder was set to record temperatures in the surrogates **every 15 min.** for the duration of the field aspect of the study. In addition, air temperatures and precipitation were recorded daily by a weather station established in the study area for the duration of the study (Figure 9).

Statistical Analysis.

Field data were analyzed using an analysis of variance with the Tukey multiple comparison test to separate means when the ANOVA F ratio was significant. Analyses were performed with SYSTAT for Windows® (SPSS Inc. 1977). Effects of fungal treatments on in-field Mormon Crickets were also assessed using Fisher's Exact test and

Kaplan Meier Survivorship analysis (Campos-Filho and Franco, 1988; Kleinbaum, 1996) to provide Average Survival Time and associated statistics under each treatment. These latter analyses were performed using Statistix® 8 (Analytical Software Inc. 1998) and KMSURV.exe (Campos-Filho and Franco, 1988). Mortality in laboratory incubations was manually analyzed with Fisher's Exact Test.

Results and Discussions

Field Mortality

No significant mortality was detected for 0-27 days after treatment (DAT) in the populations sprayed with *B. bassiana*, *M. anisopliae*, or oil only when compared to untreated populations (Table 1 and Fig. 10). Numerically, *M. anisoplinae and B. bassiana* treated populations declined 18 and 15% respectively while untreated populations and oil only treated populations declined 5% during this time (Fig.10). In an initial analysis at weekly intervals, significant differences between treatments were seen only at 28 days after treatment (Table 1). At that interval, *M. anisopliae* produced mortality significantly greater than in both untreated and oil only treated populations. *B. bassiana* produced mortality statistically similar to *M. anisopliae* but not statistically different than in untreated and oil only treated populations.

During the 42 day study, the Mormon cricket survival was affected by two severe spikes in the ambient temperature which occurred on June 29 (27 DAT) and July 10-11 (40-41 DAT) (Fig.9). On the succeeding days, untreated and oil only treated populations experienced increases in mortality but less than in fungus treated populations (Fig. 10).

Analysis of ranked grasshopper feeding days (GFD) from June 29 through July 10 (Table 2) showed *M. anisopliae* producing significantly higher mortality than occurred in the untreated population (P = 0.032) and the oil only treatment (P = 0.062). There was no significant difference between the *B. bassiana* treatment and untreated populations (P = 0.165) or the oil only treatment (P = 0.294). No significant difference in resulting mortality was seen when the two fungi were compared (P = 0.764) or when the oil only treatment and the untreated population were compared (P = 0.979).

In additional analysis using, Kaplan-Meier Survivorship Curves (Fig. 11) and Gehan-Wilcoxon means separation, when all data was considered, no significant difference in Median Survival Time was seen between untreated and oil only treated populations (P = 0.90, 1 DF). Both *M. anisopliae* and *B. bassiana* produced mortality significantly greater than in pooled untreated and oil only treated populations (P = 0.025, 3 DF). Median Survival Time was 41.0 (95% C.V. 37.0-42.0) days for untreated and oil only treated populations, 29.5 days (95% C.L. 29.0-41.0 days) for *B. bassiana* and 29.0 days (95% C.L. 29.0-39.0 days) for *M. anisopliae*. During the interval June 30 – July 9 when mortalities immediately following the high temperature peaks on June 29 and July 10-11 were censored (removed from data set) both fungal treatments produced mortality significantly greater than the pooled untreated and oil only treated populations (P = 0.02,

3 DF). The survivorship trends after day 29 in both fungal treatments becomes much shorter than those in the treatments without fungus (Figure 11).

The sudden mortality in the untreated and oil only treated populations can be explained by the extreme high temperature. Sparse vegetation and lack of hiding places within the cages prevented the Mormon crickets from avoiding the high temperatures. In nature they climb very high on vegetation or seek the shady side of vegetation and other objects and or cracks in the ground to minimize excessive exposure to heat. However, both *B. baassiana* and *M. anisopliae* treated populations demonstrated mortality significantly higher than either the untreated or oil only treated populations thus supporting the assumption of some field activity. The survivorship analysis implied that fungal infection greatly increased cricket mortality associated with the first heat spike which is consistent with daily mortality and GFD analysis.

Thermal surrogates and Model Estimates

Data from the thermal surrogates provided time estimates for when mortality attributable to the fungal treatments should have begun to occur. To better understand this, we must first review the optimal temperature parameters under which both fungi can reproduce to a level sufficient to result in mortality. Good growth of both fungi in vitro occurs at 19-30° C (66.2-86.0 ^{o}) with some development but less than 50% of optimum at intervals of 31-32°C (87.8-89.6 F) and 9-18°C (48.2-64.4^{o} F). Fastest fungal growth occurs at 27-28° C (80.6 – 82.4). No fungal growth occurs above 32° C (91.4^{o} F) or below 9 ^{o}C (48.2 ^{o} F) (Table 3) (Fargus et al. 1997; Ouedraogo et al. 1997; Jaronski unpublished). Additionally, laboratory bioassays conducted with nymphal and adult Mormon crickets and/or grasshoppers (Jaronski unpublished) demonstrate that under optimal conditions, *M. anisopliae* F 52 and *B. bassiana* will typically grow to produce mortality starting in 5-7 days (120 – 168 hrs) or in 7-9 days (168-216 hrs), respectively. With LC_{90} doses, at 28° C, *M. anisopliae* F52 and *B. bassiana* GHA demonstrate initial mortality at 160 and 200 hours of exposure respectively.

It is apparent that the recently designed cages (Fig 5) produced little if any cage effect in terms of temperature, as very little difference in temperatures were recorded inside and outside of cages (Table 4). The temperatures measured in the surrogates inside and outside cages on the ground and at the plant canopy demonstrate the number of possible hours of the day where fungal growth could occur, Figure 12 a, b, c. Further breakdown of each situation illustrates the actual number of hours each day that could produce optimal fungal growth at 18 – 30° C (64.4-86° F), Figures 13-16). The accumulated number of hours for temperatures from 18 thru 30° C, are shown daily for surrogates inside cages at ground and canopy levels, (Figure 17) and surrogates outside cages on ground and canopy levels, (Figure 18).

The cumulative surrogate data indicates that *M. anisopliae* treatments in cages should produce mortality beginning between 18 to 27 days and 9 to 12 days after treatment at ground and canopy levels respectively. Thus Mormon crickets inside cages should demonstrate mortality beginning between 8 to 27 days after treatment with *M. anisopliae*

(Table 4). Similarly, un-caged crickets should demonstrate mortality beginning at 8 to 28 days after treatment.

The cumulative surrogate data indicates that *B. bassiana* treatments in cages should produce mortality beginning between 27 to 34 days and 12 to 15 days at ground and at canopy levels respectively. Thus Mormon crickets inside cages should demonstrate mortality beginning between 12 to 34 days after treatment (Table 5). Similarly, un-caged crickets should demonstrate mortality beginning at 12 to 35 days after treatment.

Comparison with Model

Our field cage data shows mortality starting between 28 to 40 days after treatment, post treatment intervals that are somewhat consistent with those predicted by the surrogates. Ground predictions for both *B. bassiana* (27-34 DAT) and *M. anisopliae* (18-27 DAT) were closer than canopy predictions. As indicated earlier, outside and inside cage temperatures were almost identical indicating un-caged populations may experience similar temperatures to those experienced inside cages. However, care must be taken in extrapolating such considerations to normal field situations. The cage does restrict the insect from other normal behavior which can affect its body temperature and therefore the potential growth of an inoculated fungus. However, altered behavior due to cage restriction would favor underestimating permissive days when crickets move to cooler environs which could permit fungal development. Thermo-regulating behavior to raise body temperature should not be effected by the cages. Because of the extreme high temperatures experienced in this study, the cage data is more likely to be an under-estimation of the number of permissive days for fungal development in the natural environment near Sidney, Montana.

Additional Analysis

The above discussion yields a broad range of expected estimates for mortality to start, based on a continuous specific location of the Mormon Cricket. We can estimate the time for mortality to start from another view. The mean number of daily hours permissive for fungal growth during our study, are further summarized for each of 5 temperature ranges in Table 6. During the course of the study "permissive" temperatures (18-30° C.) averaged 6.5-6.9 hr /day for surrogates on the ground, while those for the surrogates in the canopy were 15-15.4 hr/day. This temperature range (18-30° C.) encompasses the range in which fungal growth is at least 50% of the fastest rate. If one examines the temperature profiles for a wider range, 9-32° C., which encompasses the entire range in which any fungal growth occurs, the number of permissive hours increases considerably, to 15.8-16.4 hr/day for surrogates on the ground and 21.1-21.6 hr/day for surrogates in the canopy. However, we believe the 18-30° C. range is more relevant for predicting fungal efficacy. Since Mormon crickets do not spent 100% of their time in any one plane, canopy or ground, until we know more, a more realistic estimate of average daily permissive hours would simply be an average of the daily time spent on the ground and in the canopy, 11 hrs (6.5 + 15.4 / 2 = 10.95). The predicted days required for *M. anisopliae* and *B. bassiana* to produce initial mortality in the Sidney, Montana cages during the study is summarized in Table 7. Using these estimates we can expect mortality attributable to *M. anisopliae* to start in 10.9 to 15.3 days after treatment and mortality

attributable to *B. Bassiana* to start in 15.3 to 19.6 days after treatment. While this estimate, based on an average of canopy and ground position, would intuitively seem more accurate, it was less consistent with field cage results than the earlier range estimate.

Laboratory Incubation

Incubation in the lab at 28 C of two of the six replicates after 3 days in field cages (Fig. 19) resulted in *M. anisopliae* producing initial and 100% mortality at 5 and 12 days respectively. *B. bassiana* produced initial and 90% mortality at 6 and 12 days respectively. Therefore a lethal dose of both fungi were applied. Only non-optimal parameters (high field temperatures) and or behavioral thermoregulation by the crickets prevented higher mortality from being expressed in the field. Field cage cadavers resulting from *M. anisopliae* showed 17.5% (7 of 40) sporulation in the laboratory. Field cage cadavers resulting from *B. bassiana* showed 37.5% (15 of 40) sporulation in the laboratory. This data indicates, in spite of the disadvantages experienced in the field, some individuals (a portion of the population) were susceptible to the treatments which is consistent with the field data showing low mortality in the cages.

Field Aerial Application Simulation Tower Technique

It is important to note that an analysis of microscope slides sprayed in the field immediately prior and after applications initially indicated spore concentrations to be 2.6 x 10^{13} and 2 x10^{13} spores/acre for *M. anisopliae* and *B. bassiana* respectively rather than the intended 4 x 10^{13} spores/acre. However, subsequent evaluation of this spore concentration estimating technique revealed it to be grossly underestimating concentration levels. It is also important to note that some spray was observed to be deposited on the inner wall of the tower. Therefore, an amount slightly less than intended ,reached the target. However, FAASTT still delivered a lethal dose as demonstrated by the test insects held initially outside 3 days in cages and then moved to optimal laboratory conditions. Subsequent laboratory study has indicated that static charges generated by the plastic walls of the tower were the cause of less than desired rates of application. These problems have been solved for the future by using non-static forming materials in the construction of the tower walls.

Conclusions

Beauveria bassiana strain GHA and *M. anisopliae* strain F52 demonstrate some, although minor, success in reducing immature Mormon cricket populations in field cages after 28 days post-treatment. High field temperatures and or thermo-regulation by the Mormon crickets prevented higher mortality from being expressed in the field. More importantly, the study demonstrates the doses delivered were sufficient to cause infection and produce mortality. Field treated individuals moved to the laboratory under optimal thermal conditions demonstrated 90% and 100 % mortality in 12 days for *B. bassiana* and *M. anisopliae* respectively. The field treated individuals moved to the laboratory additionally confirmed that sufficient feeding in the field to pick up a lethal dose by the

crickets occurred. Cadavers from field cages that sporulated in the laboratory also indicated some mortality in the field could be attributed to the fungal treatments.

The timing of mortality in the field cages was somewhat consistent with the cumulative surrogate data (model) that indicated mortality would occur between 8 and 27 DAT for *M. anisopliae* and 12-34 DAT with *B. bassiana*. Results of this study were complicated by ambient temperatures (ranging from 93 to 97° F on June 28 and 29 and 93 – 102° F from July 10 -16) that not only retarded and prevented fungal growth but also directly caused some death within the insect test population.

Cage effects, which have historically been a problem in evaluating fungal populations in the field, have essentially been eliminated with the use of the newly designed hardware cloth cages used in this study. The new cages resulted in internal temperatures very near un-caged situations.

The version of FAASSTT used in the study resulted in the application of a lethal dose even though some material was observed deposited on the inner walls of the tower, which resulted in slightly less than desired application rates being deposited on the target. However, post-study modifications have alleviated the static charges produced by the plastic walls of the tower which attracted some of the sprayed material. In combination with an appropriate field cage the FAASSTT appears to be an efficient and economical method of applying sprays for evaluation in very small field tests.

The field temperatures experienced during the study coupled with the thermoregulatory behavior of the Mormon crickets, caused less than optimal results. The thermal surrogates indicated few optimal hours for fungal development were available to yield quick mortality. Therefore, these isolates in their present form, may not be suited for use against Mormon crickets under these thermal, temporal and spatial conditions. However, these issues may be alleviated in the future with (1) strains that are more heat tolerant or possess broader thermal parameters for optimal growth, (2) synergism of existing strains with other treatments or additives and (3) development of the stressor concept where the insect is weakened with a sub-lethal dose of an insecticide sufficient to allow low doses of fungus to cause mortality. The need for alternatives to traditional chemical insecticides in the environment warrants the further development of these fungi as useful and economical tools.

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Figure 1. Ring (0.1 m^2) used to mark area to be treated.

Figure 2. Field Aerial Application Spray Simulation Tower Technology (FAASSTT) used to apply treatments.

Figure 3. Close-up of FAASSTT.

The FAASSTT procedure involves three people, an applicator, a regulator/evaluator and a locator.

The evaluator places spray card near the center of the area to be sprayed. Locator opens top inspection door and moves and precisely locates (centers) spray unit directly over the area to be sprayed. (Location identified by 0.1 m^2 ring to assist in centering). Locator closes top inspection door. Applicator agitates material and draws predetermined amount of material into syringe, removes bubbles, adjusts volume in syringe for accuracy, attaches hilt and inserts syringe into the side port of the airbrush. Regulator turns on the compressed air source and sets the pressure as predetermined. Applicator checks (through side service door) airbrush for air movement through system and wipes off any excess material from the airbrush tip. Locator closes side service door. Applicator dispenses material into the stream of air flowing through the airbrush. Applicator removes the syringe dispenser from the airbrush side port. Regulator dispenses two "puffs" of squeeze bottle supplied air through the side port of the airbrush to ensure all material dispensed has been applied through the side port and then adjusts the pressure regulator to zero. Locator opens side inspection door and moves spray unit from treated area and places it on the next area to be treated. Evaluator retrieves and examines the spray card to determine positive deposition of material on the desired area. Process repeated.

Figure 4. Detailed protocol for FAASSTT

Figure 5. Hardware cloth cage and delimiting ring in the field.

Figure 6. Field cages on improved rangeland near Sidney. MT.

Figure 7. Cadavers showing typical compact green *Metarhizium anisopliae* (left) and white *Beauveria bassiana* (right) fungal outgrowth under high humidity in the laboratory

Figure 8. Mormon cricket thermal surrogates positioned at ground and canopy levels.

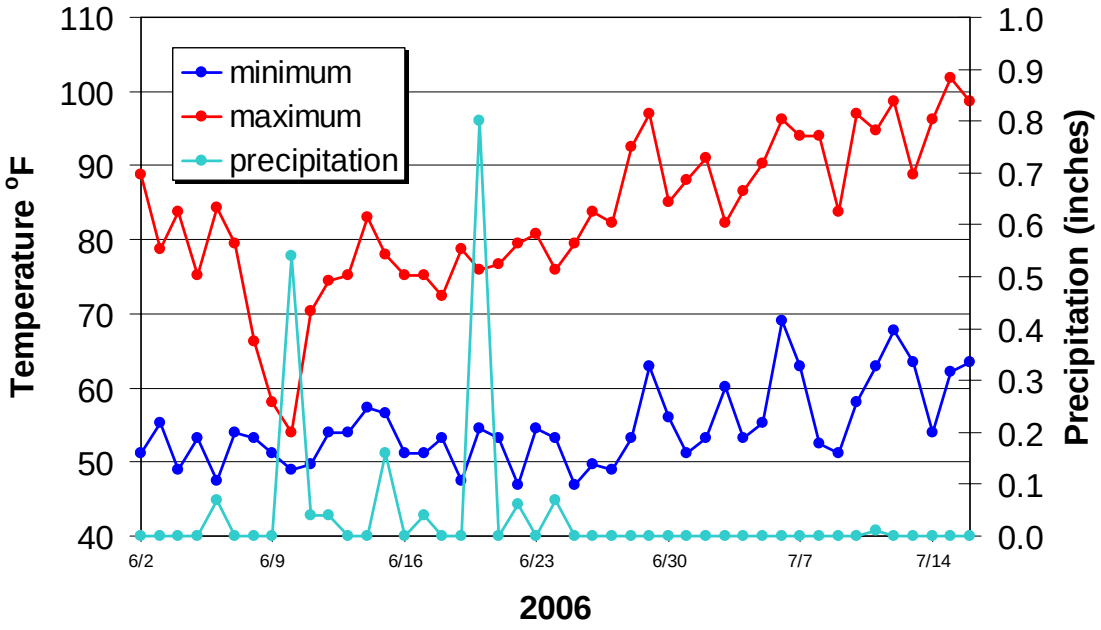

Figure 9. Daily minimum and maximum temperatures and precipitation recorded during the study near Sidney, MT, 2006.

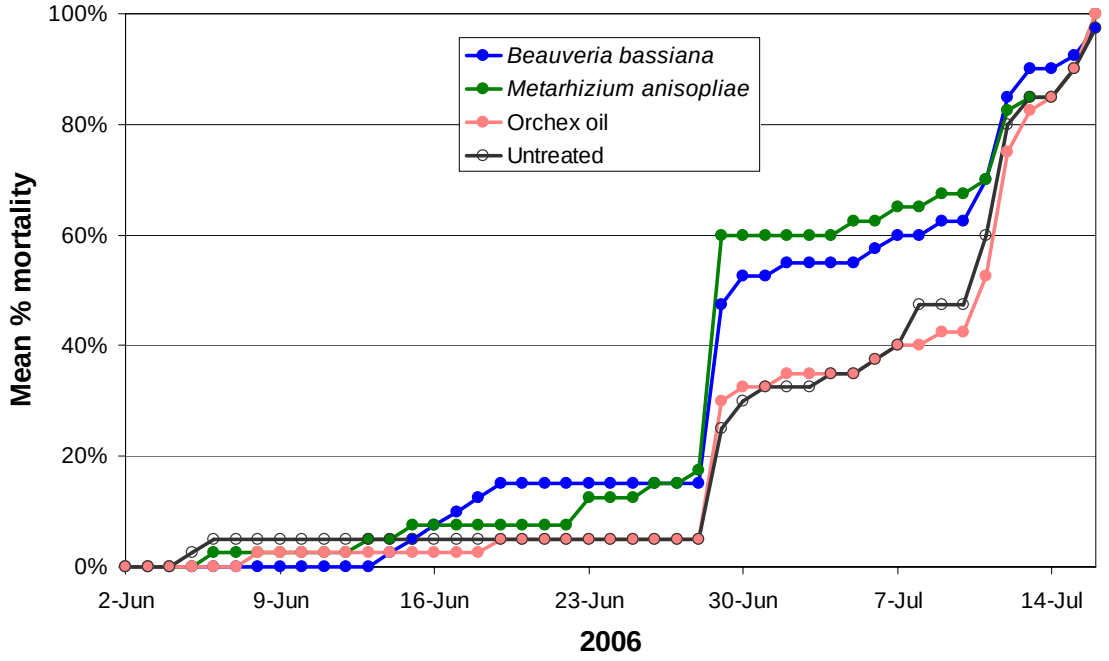

Figure 10. Mortality to immature Mormon crickets resulting from *M. anisopliae* and *B. bassiana* sprays.

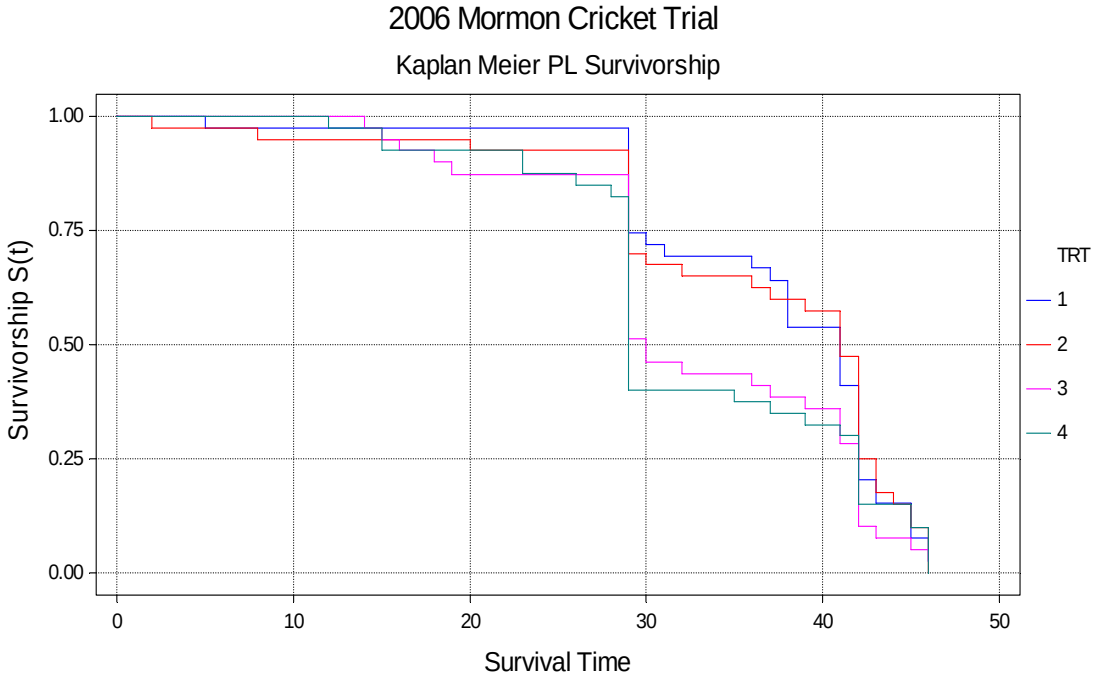

Figure 11. Kaplan Meier survivorship curves for field-incubated Mormon Crickets: 1 (blue) untreated control; 2 (red) oil carrier control; 3 (magenta) *B. bassiana* GHA; 4 (green) *M. anisopliae* F52. Survival time is in days.

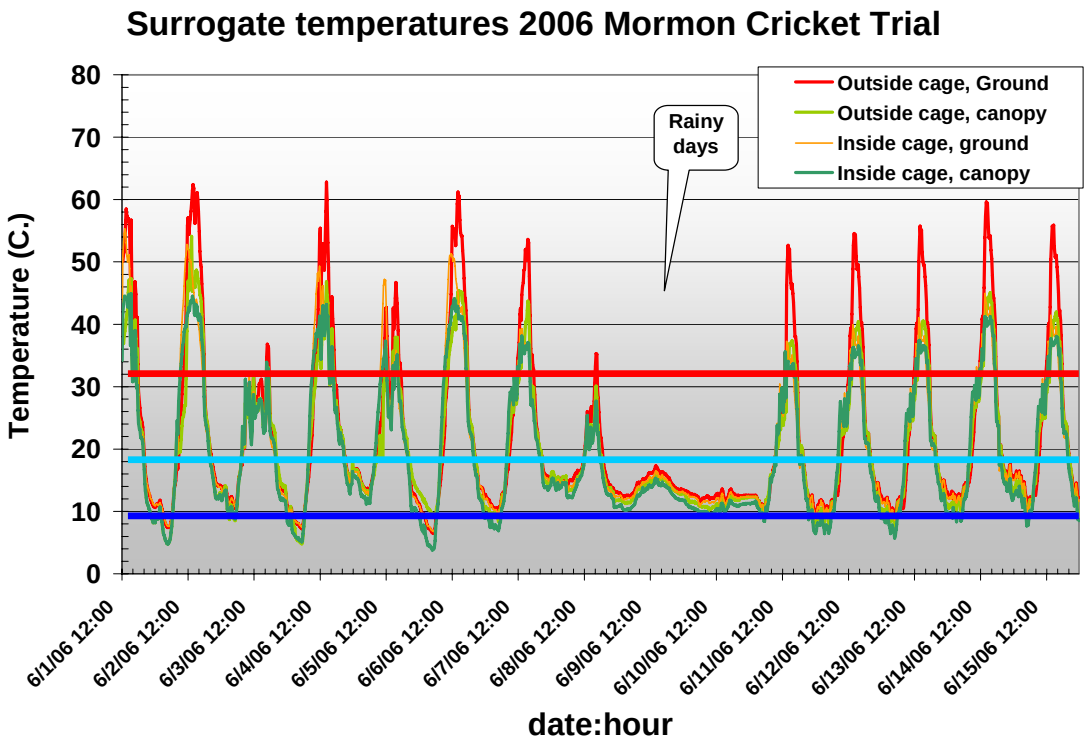

Figure 12 a. Surrogate temperatures inside and outside field cages at ground and canopy levels for Week 1 and 2 of the study. Upper (red) horizontal line represents upper thermal limit for fungal growth, 32° C.; light blue line is the temperature at which fungal growth falls below 50% of fastest rate (18° C.); dark blue line is the lower temperature limit for fungal growth (9° C.).

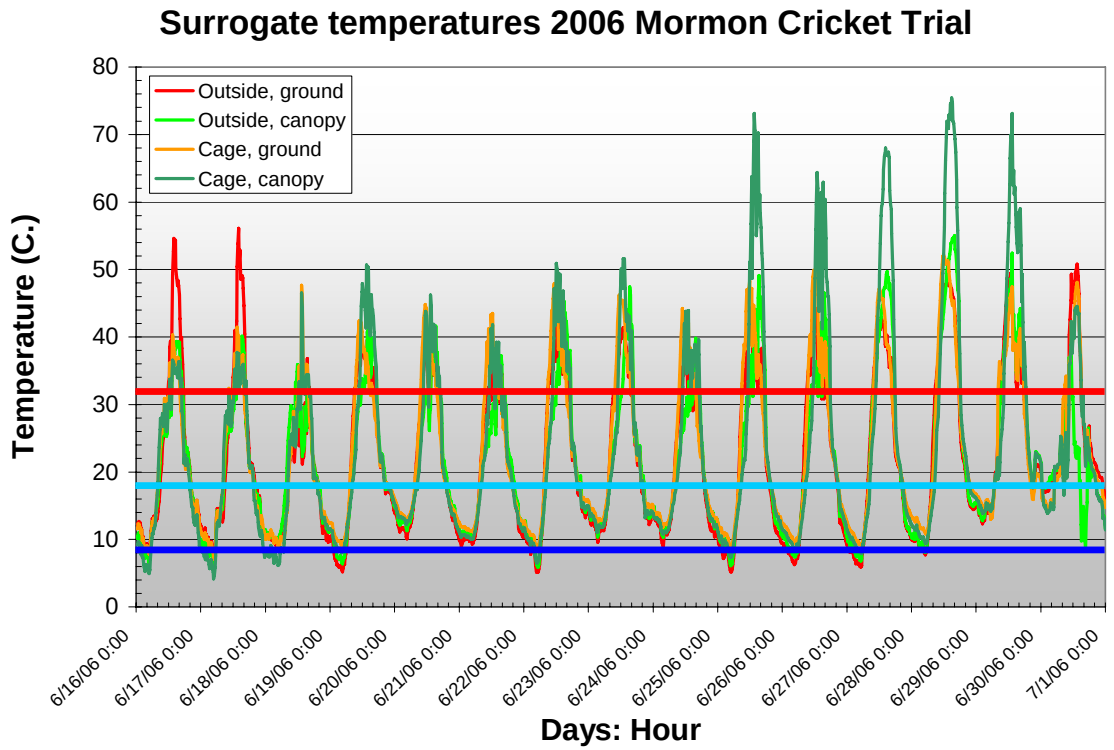

Figure 12 b. Surrogate temperatures inside and outside field cages at ground and canopy levels for Week 3 and 4 of the study. Upper (red) horizontal line represents upper thermal limit for fungal growth, 32° C.; light blue line is the temperature at which fungal growth falls below 50% of fastest rate (18° C.); dark blue line is the lower temperature limit for fungal growth (9° C.).

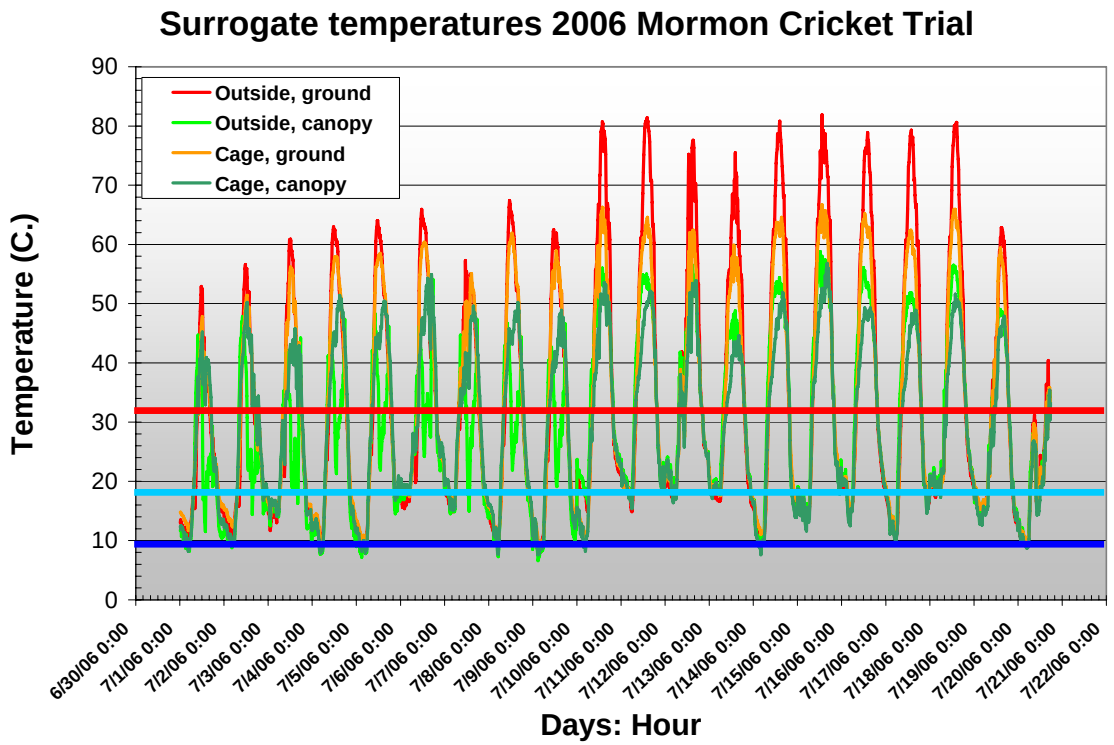

Figure 12 c. Surrogate temperatures inside and outside field cages at ground and canopy levels for Week 5 and 6 of the study. Upper (red) horizontal line represents upper thermal limit for fungal growth, 32° C.; light blue line is the temperature at which fungal growth falls below 50% of fastest rate (18° C.); dark blue line is the lower temperature limit for fungal growth (9° C.).

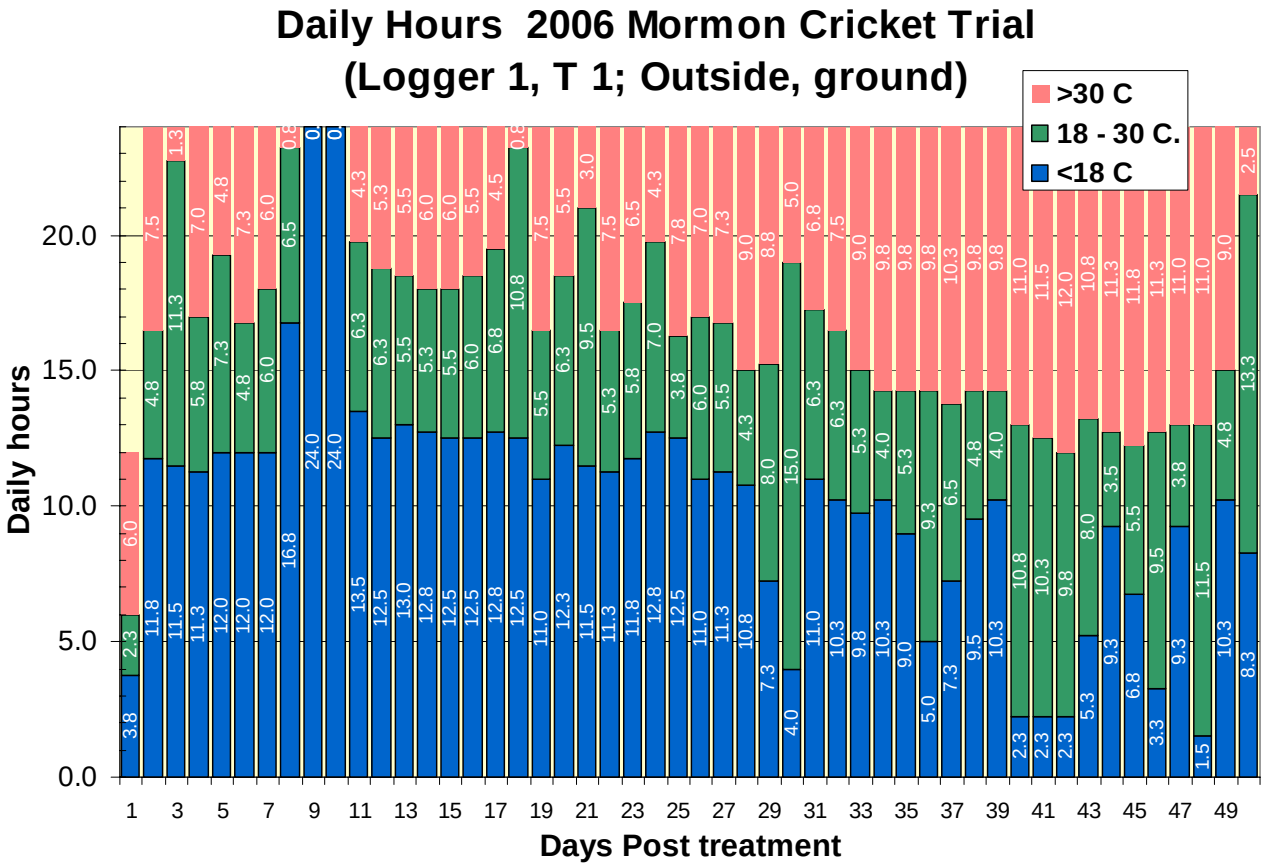

Figure 13. Daily hours of optimal, supra- and suboptimal temperatures in surrogates outside cage on ground.

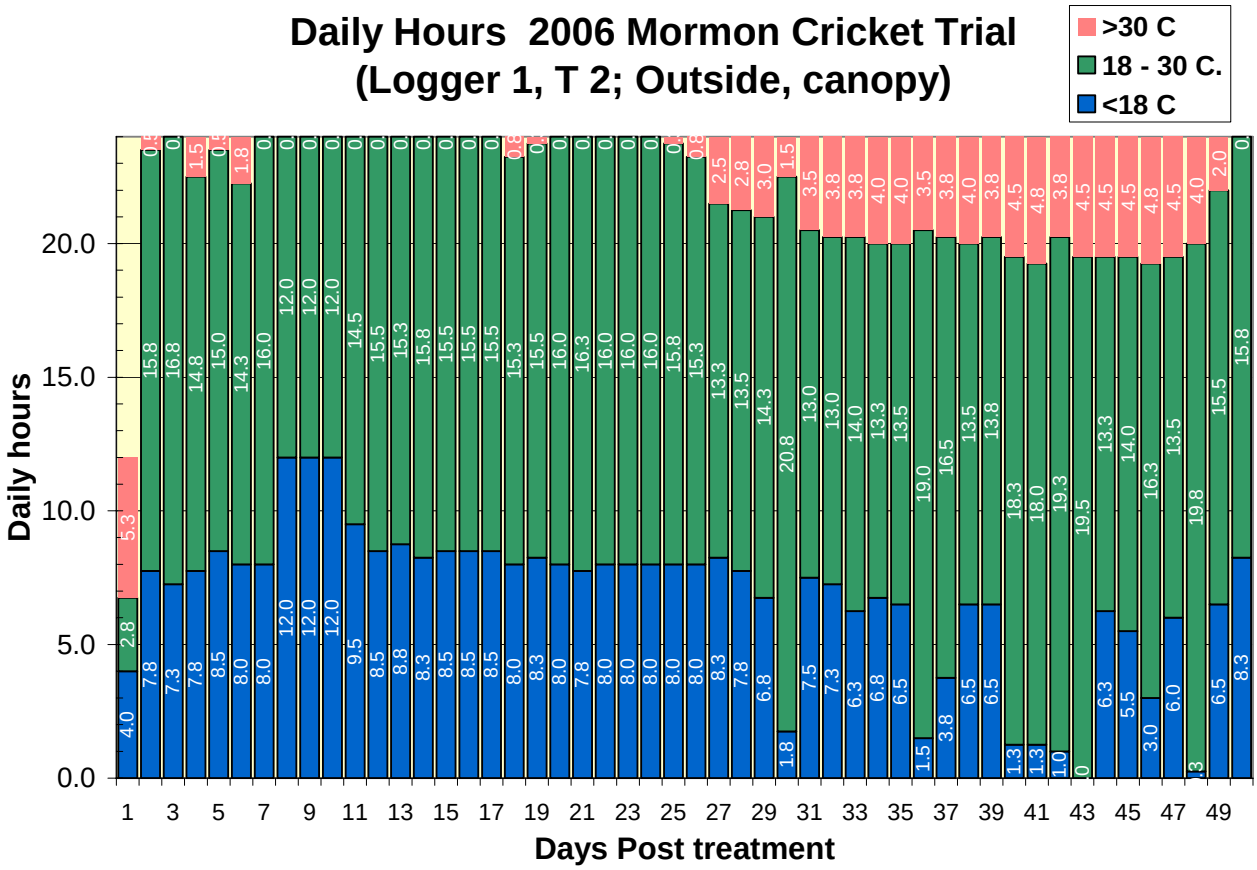

Figure 14. Daily Hours of optimal, supra- and suboptimal temperatures in surrogates outside cage in plant canopy.

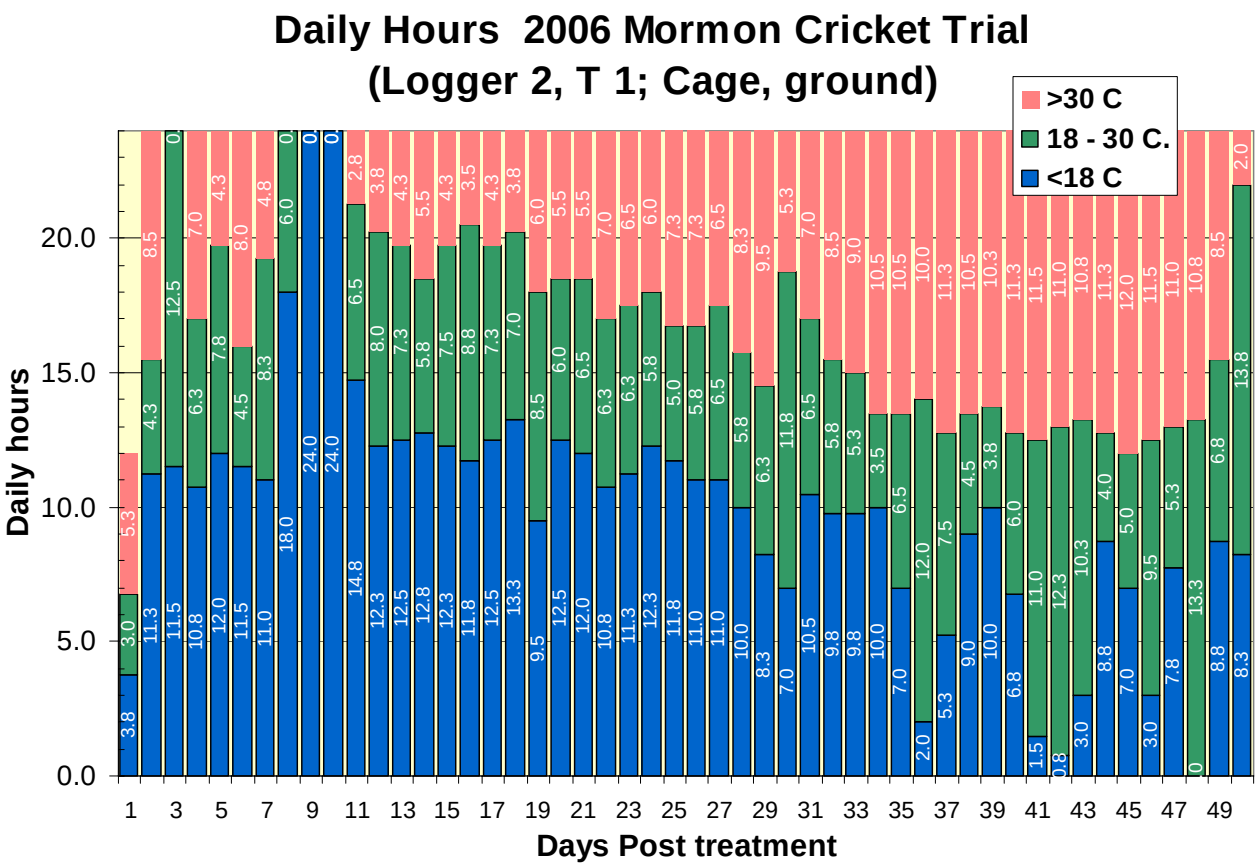

Figure 15. Daily hours of optimal, supra- and suboptimal temperatures in surrogates inside cage on ground.

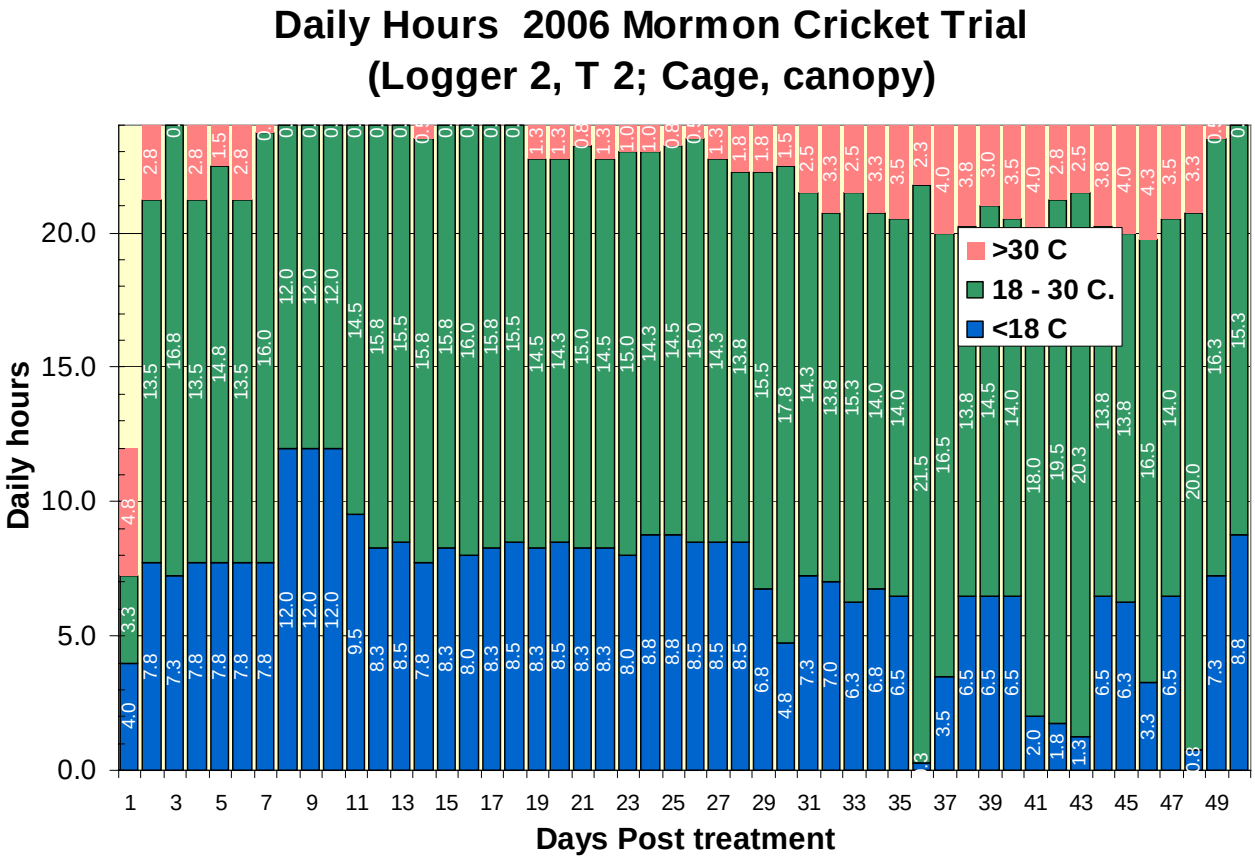

Figure 16. Daily hours of optimal, supra- and suboptimal temperatures in surrogates inside cage at canopy.

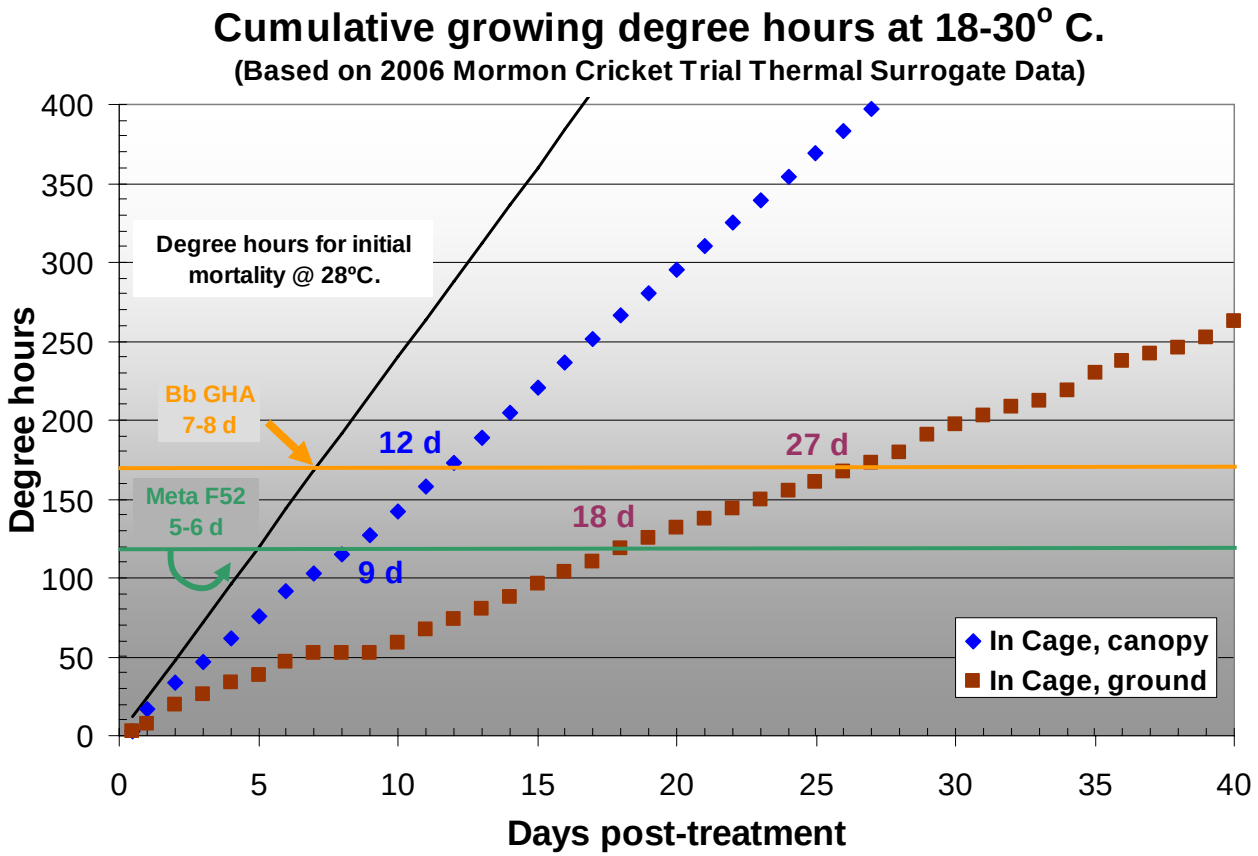

Figure 17. Accumulated growing degree hours for *Beauveria bassiana* GHA and *Metarhizium anisopliae* F52 using temperatures between 18-30° C. as measured by thermal surrogates at canopy and ground levels within cages. Green and orange lines indicate threshold for typical mortality.

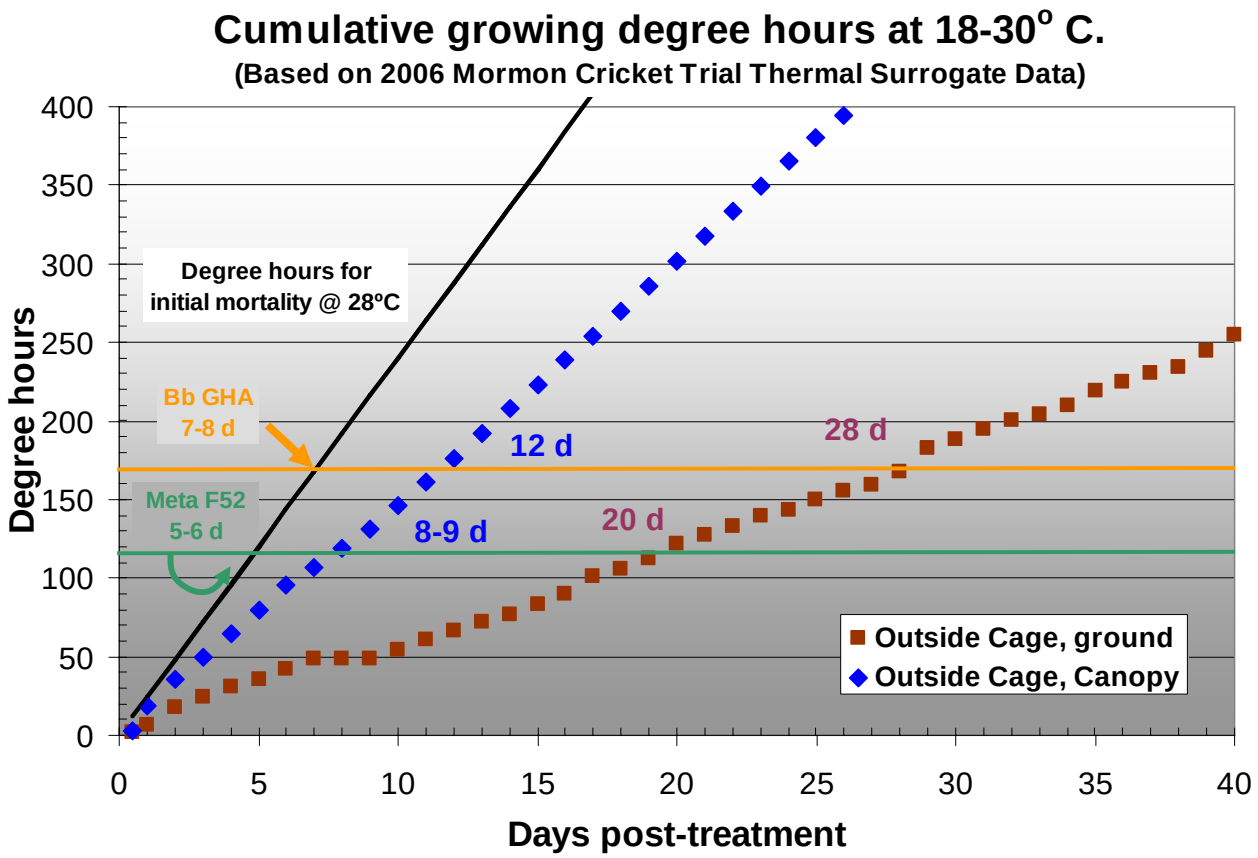

Figure 18. Accumulated growing degree hours for *Beauveria bassiana* GHA and *Metarhizium anisopliae* F52 using temperatures between 18-30° C as measured by thermal surrogates outside cages at canopy and ground levels. Green and orange lines indicate threshold for typical mortality.

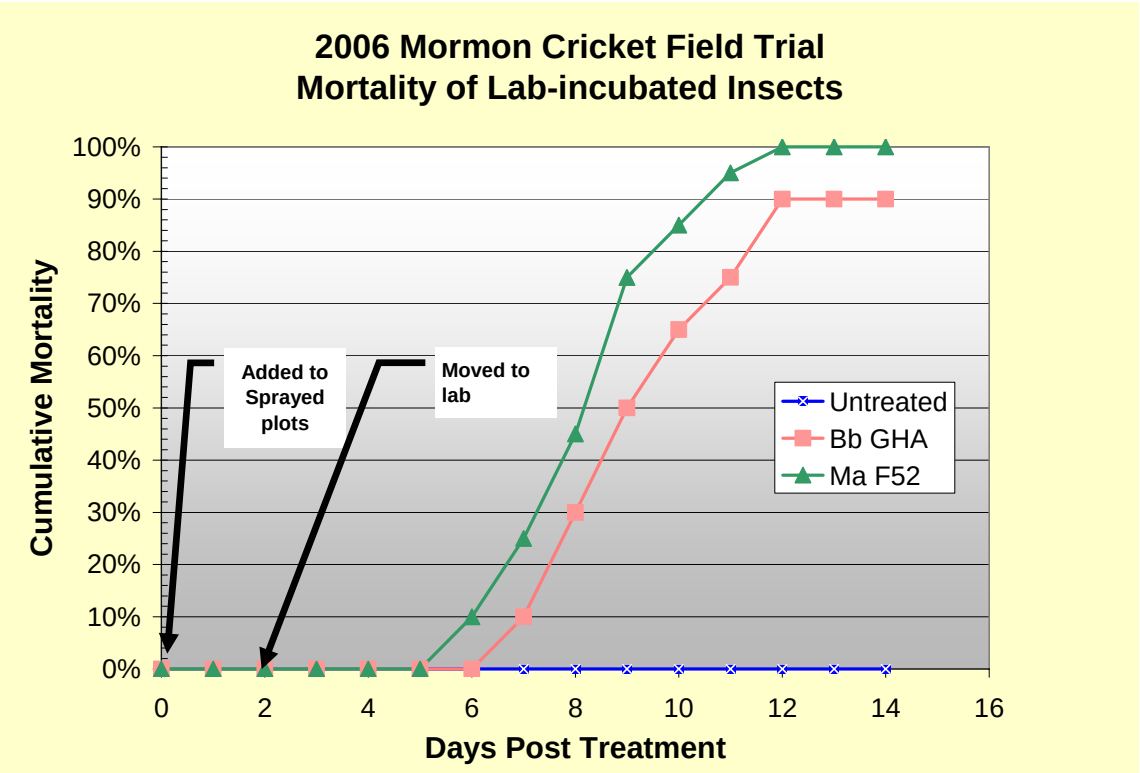

Figure 19. Cumulative mortality of Mormon Crickets exposed for two days to treated plots then brought indoors for incubation at constant 28° C.

Table 1. Analysis of weekly mortality of immature Mormon crickets in field cages exposed to vegetation sprayed with *M. anisopliae*, *B. bassiana* and oil only treatments.

	Days after treatment – mean % mortality [1]					
Treatment	7	14	21	28	35	42
Bb $4x10^{13}$	0 a	5 a	15 a	48 ab	58 a	90 a
Ma $4x10^{13}$	3 a	8 a	8 a	60 a	63 a	85 a
Orchex oil	3 a	3 a	5 a	30 b	38 a	83 a
Untreated	5 a	5 a	5 a	25 b	38 a	85 a

[1] Means in a column followed by the same letter are not significantly different ($P \leq 0.05$).

Table 2. Grasshopper Feeding Days accumulated between June 29 and July 9, 2006 near Sidney, MT.

Treatment	Mean grasshopper feeding days (28-38 DAT) [1]
Beauveria bassiana $4x10^{13}$	48.75 ab
Metarhizium anisopliae $4x10^{13}$	41.75 a
Orchex oil	70.50 b
Untreated	70.50 b

[1] Means in a column followed by the same letter are not significantly different ($P \leq 0.05$).

Table 3. Summary of optimal temperature parameters for fungal growth.

> 32° C	No Growth	(> 89.6° F)
31 - 32° C	< 50% 0f optimal growth	(86.0 – 89.6° F)
19 - 30° C	Optimal growth	(64.4 – 86.0° F)
9 - 18° C	< 50% 0f optimal growth	(48.2 – 64.4° F)
< 9° C	No Growth	(< 48.2° F)

Table 4. Comparison of accumulated heat (degree hours) for the range of 18-30° C, within and outside cages for thermal surrogates on the ground or placed in plant canopy.

Daily degree hours, 18-30° C.				
Days post-treatment	Inside cage, ground	Outside Cage, ground	Inside cage, canopy	Outside cage, Canopy
0.5	2.3	3.0	2.8	3.3
1	4.8	4.3	15.8	13.5
2	11.3	12.5	16.8	16.8
3	5.8	6.3	14.8	13.5
4	7.3	7.8	15.0	14.8
5	4.8	4.5	14.3	13.5
6	6.0	8.3	16.0	16.0
7	6.5	6.0	12.0	12.0
8	0.0	0.0	12.0	12.0
9	0.0	0.0	12.0	12.0
10	6.3	6.5	14.5	14.5
11	6.3	8.0	15.5	15.8
12	5.5	7.3	15.3	15.5
13	5.3	5.8	15.8	15.8
14	5.5	7.5	15.5	15.8
15	6.0	8.8	15.5	16.0
16	6.8	7.3	15.5	15.8
17	10.8	7.0	15.3	15.5
18	5.5	8.5	15.5	14.5
19	6.3	6.0	16.0	14.3
20	9.5	6.5	16.3	15.0
21	5.3	6.3	16.0	14.5
22	5.8	6.3	16.0	15.0
23	7.0	5.8	16.0	14.3
24	3.8	5.0	15.8	14.5
25	6.0	5.8	15.3	15.0
26	5.5	6.5	13.3	14.3
27	4.3	5.8	13.5	13.8
28	8.0	6.3	14.3	15.5
29	15.0	11.8	20.8	17.8
30	6.3	6.5	13.0	14.3
31	6.3	5.8	13.0	13.8
32	5.3	5.3	14.0	15.3
33	4.0	3.5	13.3	14.0
34	5.3	6.5	13.5	14.0
35	9.3	12.0	19.0	21.5
36	6.5	7.5	16.5	16.5
37	4.8	4.5	13.5	13.8
38	4.0	3.8	13.8	14.5
39	10.8	6.0	18.3	14.0
40	10.3	11.0	18.0	18.0
41	9.8	12.3	19.3	19.5
42	8.0	10.3	19.5	20.3
43	3.5	4.0	13.3	13.8
44	5.5	5.0	14.0	13.8
45	9.5	9.5	16.3	16.5
46	3.8	5.3	13.5	14.0
47	11.5	13.3	19.8	20.0
48	4.8	6.8	15.5	16.3
49	13.3	13.8	15.8	15.3
Total	324.5	343.0	755.0	748.8

Table 5. Predicted days for *M. anisopliae* and *B. bassiana* to begin causing mortality in the field in Sidney, MT during the trial period, June 1 – July 21, 2006. (Based on thermal surrogates position as indicated).

Range of beginning mortality	*Metarhizium anisopliae* F52			
	outside cages		inside cages	
	ground	canopy	ground	canopy
120 hrs (5 days)	20 d	8 d	18 d	9 d
168 hrs (7 days)	28 d	12 d	27 d	12 d

	Beauveria bassiana GHA			
	outside cages		inside cages	
	ground	canopy	ground	canopy
168 hrs (7 days)	28 d	12 d	27 d	12 d
216 hrs (9 days)	35 d	15 d	34 d	15 d

Table 6. Mean and (standard deviation) observed daily hours of temperatures that were permissive for growth of *Beauveria bassiana* GHA and *Metarhizium anisopliae* F52. Thermal surrogates were positioned on the ground and in canopy both inside and outside cages.

Surrogate Position	Hr > 30° C.	Hr < 9° C.	Hr 9-32° C.	Hr < 18° C.	Hr 18-30° C.
In cage, ground	7.1 (3.26)	0.9 (1.70)	15.8 (4.03)	10.2 (4.51)	6.5 (2.95)
In cage, canopy	1.9 (1.59)	1.0 (0.17)	21.1 (1.63)	6.8 (3.07)	15.4 (2.60)
Outside cage, ground	7.0 (3.44)	0.4 (0.91)	16.4 (3.76)	9.9 (4.72)	6.9 (2.95)
Outside Cage, canopy	1.8 (1.50)	0.4 (0.91)	21.6 (2.68)	7.0 (2.60)	15.0 (2.61)

Table 7. Predicted days (from Table 6) required for *M. anisopliae* and *Beauveria bassiana* to produce initial mortality in Sidney, MT field cages June 1 – July 21, 2006.

Metarhizium anisopliae F52

	Fungal growth hours / day	Fungal growth hours needed to start mortality 120 (5 days)	168 (7 days)
cage ground	6.5	18.5 days	25.8 days
cage canopy	15.4	7.8 days	10.9 days
average	11.0	10.9 days	15.3 days

Beauveria bassiana GHA

	Fungal growth hours / day	Fungal growth hours needed to start mortality 168 (7 days)	216 (9 days)
cage ground	6.5	25.8 days	33.2 days
cage canopy	15.4	10.9 days	14.0 days
average	11.0	15.3 days	19.6 days