Progress Report: Evaluation of Red Imported Fire Ant Treatment Programs to Preserve Federally Endangered Species of Concern and Endemic Cave Adapted Arthropod Species at Camp Bullis, Bexar County, Texas

Bastiaan 'Bart' M. Drees, Alejandro A. Calixto, Molly Keck, Natalie Cervantes,

Texas AgriLife Extension Service, Texas A&M System,

in collaboration with Chris Beck and Lucas Cooksey, Department of Defense

Camp Bullis, in Bexar Co., is a 28,000 acre U. S. Army training facility on which over 100 cave entrances have been located. Of these, 26 caves contain three federally listed endangered arthropods (*Rhadine exilis*, *Rhadine infernalis*, and *Cicurina madla*) and an additional 48 contain species of concern not listed but are managed similarly. The accidentally introduced pest ant species, *Solenopsis invita* Buren (Hymenoptera: Formicidae) commonly called the red imported fire ant, is believed to pose a threat to these endangered species. Efforts have been ongoing since September 1999 to suppress their population levels around cave entrances using ant mound treatments using periodic (2-3 times per year) injection of very hot water into ant mounds and restricted application of an insect growth regulator (IGR) bait product applied in bait cups around the karst formation followed by monthly assessment focused on counting the number of active fire ant mounds. Since the fall of 2003, half of the sites treated for imported fire ants in this manner have consistently been found to be below the critical density of 80 mounds per 7,850 m^2 or within a circular area around each cave entrance with a radius of 50 m. This expensive, labor-intensive treatment program may be limited in success due to ants in these hot, dry areas not forming the mounds characteristic of their surface nesting activity and difficulty injecting hot water in often rocky situations and dense tree/shrub vegetation.

Project Objectives:

1) To develop and assess alternate treatment and monitoring regime for management of the red imported fire ant and compare this approach to existing treatment protocol as well untreated reference sites;

2) To study ant behavior around karst formations (suspected cave openings) and inside caves and the ant-cricket interactions that serve as key component for protected species (optional);

3) Facilitate the potential release of and monitor for natural enemies of red imported fire ant.

Methods and Materials

Experimental approach. To compare the efficacy of the targeted treatment approach to current management practices (boiling water), we conducted manipulative field experiments on 18 karst formations at Camp Bullis not known to harbor endangered or threatened species , not in the current treatment program, and previously untreated. Three management practices are being compared, 1) fire ant management using very hot or boiling water to saturate active ant mounds detected (standard treatment regime and sole treatment approved for use by the U. S. Fish and Wildlife Department), 2) fire ant population suppression using a target-specific, action threshold-driven approach selectively employing insecticide bait stations (called the experimental "bait and switch" approach – a term coined and first used to describe this treatment by Zara Environmental in fire ant research at Ft. Hood), and 3) no treatment (untreated control reference karsts).

Treatment plot assessments. To assess red imported fire ant population levels, food lures (hot dog slices) were used on each of the designated 18 karst formation plots on April 13, 2009. Each lure was deployed on the ground (**Figure 1**), one in the plot center, and the along 4 transects (North, South, East and West) radiating from the central karst formation at intervals of 15, 30, 60 and 120 ft (5, 10, 20 and 40 paces, respectively), and left exposed for 45-60 minutes (17 food lures were used in total per plot) (**Figure 2**), before the number of ants on each food lure was estimated. This method was also used to assess treatment effects periodically following treatment applications in addition to direct ant mound number counts. Plots were numbered and arrayed from those with the highest number of ants estimated on food lures to the lowest, and grouped into three replicate blocks (Treatments A, B, and C) of six plots each (**Appendix 1**). Thereby, one block contained heavily-infested plots and one contained low population levels in order to eliminate pre-treatment differences.

Application methods. The "standard treatment" was use of very hot or boiling water heated using a truck bed mounted Hotsy unit. This approach consisted of detecting fire ant active mounds within a 50 m (2-acre) radious circle around each karst formation of cave entrance and injecting boiling water on top of the mound until saturation occurred. Although scheduled for application in spring 2009, problems with the contract for these treatments between the Department of Defense and Zara delayed treatments until fall, preventing direct comparisons between the standard and experimental treatments.

The experimental "bait and switch method" is being evaluated by the Texas AgriLife Research and AgriLife Extension Service of the Texas A&M System. This approach relies on data gathered to assess pre-treatment population levels by attracting foraging worker ants using a food lure (hot dog slices) as described above (**Figure 1**). Food lures were left exposed for 45-60 minutes before the number of ants on each hot dog slice was estimated (**Figure 2**).

As shown in previous studies, discovery and dominance by fire ants on food resources and lures occurs during this short period of time. This method is useful to detect other ant species attracted to the same food source, and to estimate species' relative abundance in the area. The approach can also incorporate an "action level" or "threshold" which is used to justify the need for treatment(s), such that applications are triggered for control only when lures are occupied with a significant number of fire ants, e.g., more than 300 ants per 10 lures or 30 ants per lure.

Treatments employed a different food lure and grid for applying insecticidal ant bait: 10 by 10 meter (33 ft or 11 yards) grid to encompass the circular 50 m diameter plot area, totaling 89 stations per plot (**Figure 2**). At food lure stations where imported fire ants were attracted, hot dog slices were replaced with B&G Perimeter bait stations containing 0.72 oz Esteem® Ant Bait (pyriproxyfen) (**Figure 3**). Thereby, no more than 2.0 pounds per acre of product was applied per plot as directed on the product label. This approach ensured that the treatments were only delivered to red imported fire ants, not to other ant species attracted so some food lures, and that the amount of active ingredient was sufficient to provide significant control. The protocol intended for bait stations to be removed from the ground 24 to 48 hours later. Weight of bait product remaining in the 89 stations was later weighted to enable calculation of estimate the amount of bait removed by the ants. Esteem® Fire Ant bait contains an active ingredient that mimics an insect growing hormone, known to leave very low residual in the environment (or none if taken all by the ants).

Red imported fire ant workers were collected from food lures from plots to determining if ants at Camp Bullis are infected with the microsporidian *Kneallhezia solenopsae* using molecular techniques, and to determine the fire ant social form which is important for its management and for establishing potential sites for colonization by natural enemies (i.e. phorid flies).

Figure 1. Food lure (hot dog) used to aid decision-making tool to evaluate fire ant relative density and determine treatment triggers.

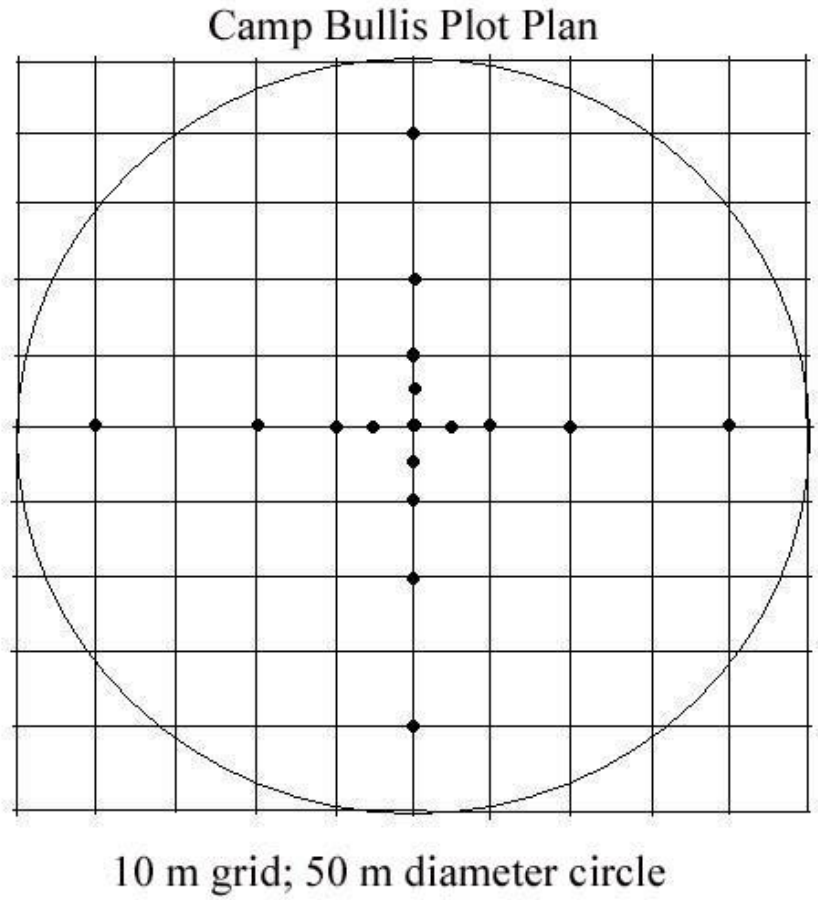

Figure 2. Food lure (hot dog) layout around karst formations, a total of 17 units were deployed on the ground spaced ~10 feet between each other.

Figure 3. B&G Perimeter ant bait station open containing Esteem® Ant Bait (left) and closed (right).

Results

Summary of work done May-August 2009.

- Fire ant relative abundance pre-treatment counts using food lures were conducted April 13th – 21st, no significant differences were found across karst formations in relation to fire ant abundance (**Table 1** and **Figure 4**, *P*=0.91).
- First "fire-ant specific" treatment was delivered May 20th – 28th. Stations were collected 24 hours later but weight measurement was not taken since the rain either soaked or washed out some of the material from the traps. It remained unclear how much bait the ants removed.
- Fire ant relative abundance post-treatment counts using food lures were conducted June 12th – 22st, significant differences were found across karst formations, baited treated formations had significantly lower number of ants compared to boiling (still untreated) and reference untreated sites (**Table 2** and **Figure 4**, *P*=0.27).
- Based on the first post-treatment counts, we were able to conclude the numbers met the requirement for triggering re-treatments (~30 ants average across all the food lure stations or +300 ants total across them). We decided then to schedule the second fire-ant specific treatment for the end of June (**Figure 4**).
- The second treatment was applied June 29th- July 3rd.
- Bait remaining for last treatment from the bait stations was collected and will be weighed to determine the amount picked up by ants.
- One round of mound counts on all 18 sites has been completed.
- One round of hot dog monitoring on all 18 sites has been completed.
- Zara began applying hot water treatments in Camp Bullis in late August 2009. Data will be compiled and analyzed, and we intend to observe methods used or equipment needed, labor required or time needed, etc.
- We were able to go with someone from Zara that does cave cricket counting at night and observe the crickets. Preliminary observations were taken and we plan on developing more intensive observation in the fall that would be used for comparing across the different managed/unmanaged caves.

Major accomplishments to the date.

1) We have significantly reduced fire ant densities around karst formations using the targeted treatment approach and we have minimized the impact on non-target (resident) native and exotic competitor ant species (**Figure 4**). As a consequence of the removal of red imported fire ants around the karst formations, we have observed a significant increase of resident ant abundance (~9 species from genera including *Crematogaster, Forelius, Monomorium, Diplorhoptrum, Brachymyrmex, Oheidole, Doryomyrmex,* and *Tapinoma*). This is a strong indication of the positive effect of the targeted treatment.

2) Although we have not been able to directly compare the boiling water approach, we believe our method provides better coverage, has proven to date to be environmental friendly and it is probably less expensive and less time consuming.

Tests of Between-Subjects Effects

Dependent Variable:Fire Ants

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	16.032	2	8.016	.009	.991
Intercept	38217.993	1	38217.993	42.116	.000
Treatment	16.032	2	8.016	.009	.991
Error	13611.649	15	907.443		
Total	51845.675	18			
Corrected Total	13627.682[a]	17			

a. R Squared = .001 (Adjusted R Squared = -.132)

Tests of Between-Subjects Effects

Dependent Variable:Residents

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	599.769	2	299.885	.921	.420
Intercept	5000.000	1	5000.000	15.354	.001
Treatment	599.769	2	299.885	.921	.420
Error	4884.660	15	325.644		
Total	10484.429	18			
Corrected Total	5484.429[a]	17			

a. R Squared = .109 (Adjusted R Squared = -.009)

Table 1. Relative fire ant and resident ant abundance before the treatments. Karst perimeters were surveyed by using food lures (ANOVA, significant differences when $P<0.05$).

Tests of Between-Subjects Effects

Dependent Variable:FireAnts

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2150.673	2	1075.336	4.613	.027
Intercept	22881.219	1	22881.219	98.161	.000
Treatment_	2150.673	2	1075.336	4.613	.027
Error	3496.482	15	233.099		
Total	28528.374	18			
Corrected Total	5647.155[a]	17			

a. R Squared = .381 (Adjusted R Squared = .298)

Tests of Between-Subjects Effects

Dependent Variable:Residents

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	73.054	2	36.527	.062	.940
Intercept	11997.248	1	11997.248	20.303	.000
Treat	73.054	2	36.527	.062	.940
Error	8863.829	15	590.922		
Total	20934.131	18			
Corrected Total	8936.884[a]	17			

a. R Squared = .008 (Adjusted R Squared = -.124)

Table 2. Relative fire ant and resident ant abundance before the treatments. Karst perimeters were surveyed by using food lures (ANOVA, significant differences when $P<0.05$).

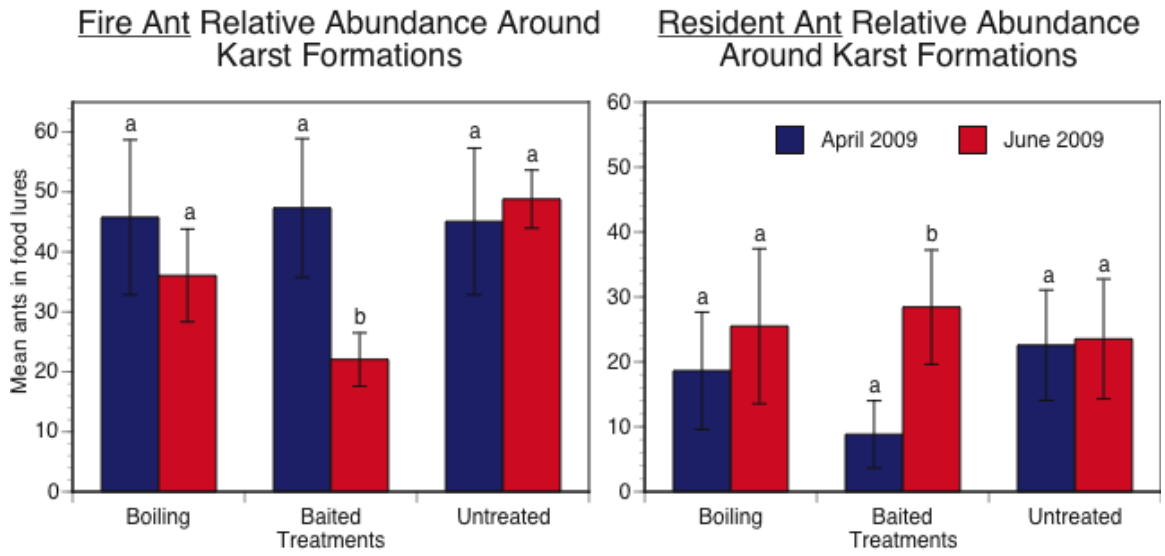

Figure 4. Relative fire ant and resident ant abundance before and after the treatments. Karst perimeters were surveyed by using food lures (ANOVA, significant differences when $P<0.05$ (a,b)).

Appendix 1. Pre-treatment assessment of red imported fire ants, plot array into replicate blocks and treatment assignments, Camp Bullis, Bexar Co., TX, 2009.

Rep		Karst	Mean	Treatment A	Treatment B	Treatment C	Std. Error	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
		08B-F1	3.529				2.09010	-.9014	7.9602
		10-084	5.882				4.77885	-4.2483	16.0131
		08B-F3	6.471				2.83637	.4578	12.4834
		7-019	8.235				4.94345	-2.2444	18.7149
	1								
A		06B-005	12.940	12.940			5.54200	1.1900	24.6900
C		11A-002	14.706			14.706	5.88603	2.2281	27.1837
B		11B-067	16.471		16.471		6.23918	3.2441	29.6971
	2								
C		10-002	22.500			22.500	8.29156	4.8270	40.1730
A		11B-121	24.118	24.118			9.51233	3.9524	44.2829
B		7-056	25.294		24.118		8.04937	8.2302	42.3580
	3								
B		C1-F7	30.588		30.588		7.14712	15.4370	45.7395
A		11B-001	34.118	34.118			10.18429	12.5279	55.7074
C		C1-F4	34.118			34.118	10.11183	12.6815	55.5538
	4								
A		10-044	35.294	35.294			10.88434	12.2203	58.3679
C		11A-015	35.882			35.882	11.85261	10.7559	61.0088
B		7-022	50.588		50.588		8.93072	31.6560	69.5205
	5								
B		6A-35	71.176		71.176		6.79978	56.7616	85.5914
A		6A-086A	72.353	72.353			8.38106	54.5859	90.1200
C		6A-027	80.588			80.588	7.44937	64.7963	96.3802
	6								
C		6A-036	84.118			84.118	6.36274	70.6292	97.6060
B		6A-17	90.000		90.000		6.18347	76.8916	103.1084
A		6A-029A	95.882	95.882			1.23038	93.2741	98.4906