Preliminary assessment of the impacts of red imported fire ants on communities of insects that serves as major food source for broods of the endangered Attwater's prairie chicken

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The endangered Attwater's Prairie-Chicken (APC) (*Tympanuchus cupido attwateri*) is on the brink of extinction (Morrow et al 1996, 2004, Silvy et al 2004). Currently, fewer than 50 individuals remain in 2 geographically separated populations in Texas (Attwater Praire Chicken National Wildlife Refuge (APCNWR) near Eagle Lake and Texas City) (Morrow et al 2004). Both of these populations have been supplemented with captive-reared birds since 1996 (Morrow et al 2004). Through the use of predator management and predator-deterrent fences around nest sites, 2001 – 2005 nesting success averaged 61% (APCNWR, unpublished data), compared to the historic average of 32% (Peterson and Silvy 1996). However, poor brood survival has been identified as a one of the major limiting factors affecting Attwater's prairie-chicken recovery (APCNWR, internal data). Like most gallinaceous birds, prairie-chicken chicks are primarily insectivorous during the first weeks of life (Lehmann 1941, Jones 1963, Thomas 1987, Johnson and Boyce 1990). Preliminary data collected collaboratively by APCNWR and the Society of *Tympanuchus cupido pinnatus* suggest that availability of insects as a food source for newly hatched chicks are contributing to the observed poor brood survival. Comparisons of insect abundance in APC brood habitat (APCNWR) with that from an increasing Minnesota greater prairie-chicken (GPC) (*T. c. pinnatus*) population found that while total insect biomass did not differ between the 2 areas, APC brood habitat supported < 30% of insect numbers compared to the GPC brood habitat (Pratt et al. 2003, unpublished report). Based on these preliminary data we argue that the Red Imported Fire Ant (RIFA), *Solenopsis invicta* Buren, might contribute to the reduction of insect diversity and abundance at APC brood habitat.

Disruptive impacts of RIFA on native insect communities are relatively well documented. In a study in Texas, Porter and Savignano (1990) reported that species richness and abundance of non ant arthropods were respectively 30% and 75% lower in sites infested by RIFA. In another study, Calixto et al (2007) investigates how the reduction of RIFA benefited other ant species resulting on increases of up to 25% indicating the negative impact of RIFA. Allen et al (2001) described how the presence of this invasive species altered the abundance of the loggerhead shrike by decimating insect abundance, the main source of food for this species.

Although experimental work links RIFA to negative impacts on arthropods, claims about the impact of this species on vertebrates are built mostly on anecdotic reports rather than on experimental evidence (Allen et al 2004, Tschinkel 2006). To cite a few examples, RIFA is linked to the declining of horned lizards in Texas where it is believed to competitively exclude the red harvester ant, *Pogonomyrmex sp.*, considered for many the primary food source of the horned lizard (Taber 1998). In another study Barlett (1997) speculates that the declining of snakes in certain areas is due to egg predation by RIFA. In yet another descriptive study, the authors conclude that the survival of the federally endangered black capped vireo is severely compromised by the presence of RIFA (Stake and Cimprich 2003). On this study video monitoring of 142 black-capped vireo nests identified RIFA as the primary nest predator on Fort Hood, TX. The impact at population level remains unclear. Despite the poor understanding on RIFA impacts on vertebrates there are a few studies that merit to be mentioned. For example Drees (1994) conducted a removal experiment using broadcast baits in a spoil island off Texas coast where he investigated the breeding success of several species of water birds. In the study he found that survival of water birds was 90% lower in untreated areas compared to those where RIFA was reduced. Furthermore Allen et al (1995) studied the impact of RIFA on bobwhite quail, white-deer tailed deer and loggerhead shrike populations. In this study bobwhite survival was significantly higher in areas were RIFA was removed.

The lack of manipulative experiments and appropriate replication, limits the inference that can be drawn from these experiments. These are strong justification for studying the impact of RIFA on food resources of the endangered Attwater's prairie-chicken in Texas. Understanding the effects of RIFA on food sources for APC brood is detrimental for the successful reintroduction and establishment of this endangered species in their native areas. We need long term comprehensive ecological studies with a larger scope, conducted with controls and adequate temporal and spatial replication to provide an understanding of the effects of RIFA on vertebrate and invertebrate populations.

The goal of this preliminary study is to collect baseline data that will help in the future to determine the impacts of RIFA on insect populations at the APCNWR. To address this goal first determined the impacts of RIFA on 1) insect abundance and 2) insect.

Materials and Methods

Study site

This study was conducted at the Attwater Prairie Chicken National Wildlife Refuge (NWR) located approximately 60 miles west of Houston, Texas. It is one of the largest remnants of coastal prairie habitat remaining in southeast Texas and home to one of the last populations of the critically endangered Attwater's prairie- chicken. All activities were conducted on coastal prairie grassland during the APC brood rearing period 15 April – 30 June, 2007 and 2008 respectively).

Experimental Design

In this study we used an Impact- Reference design with match pairs with 5 sets of replicates in space and 6 replicates in time (15 April – 30 June) with measurements of biological and environmental data (site by time factorial design).

Experimental plots. They consisted of two hectare plots (5 acres) as experimental units. The software ArcGIS (ESRI) was used for selecting the units. Plots were located in the field using Trimble GPS (submeter accuracy). To reduce the variation pair of plots were blocked according to the habitat observed both in the field and on aerial photographs when working on ArcGIS (**Figure 1**).

Treatments. Consisted of 1) RIFA reduced using broadcast bait (Amdro Fire Strike - Hopper blend - Extinguish, 0.5% s-methoprene and 0.036 % hydramethylnon labeled for pastures) and 2) untreated Controls. The treatments were assigned to the experimental units at random. Bait treatments was applied early on March 2006.

Sampling Methods. We used three complementary sampling techniques 1) sweep netting, 2) pitfall traps and 3) fatty food lures on each experimental plot. Samples were collected on a weekly basis from 15 April-30 June 2006 (APC brood rearing period) and will process them during the fall and winter.

1) *Sweep net sampling*: This method was used to estimate the abundance and species composition of active insects in lower vegetation (Southwood 1978). The sweep-net measures 38 cm (15 inches) in diameter and is attached to a 1 m wooden pole via a steel ring. The net is constructed of sailcloth to withstand the rigors of thorny bushes common at the refuge. We will take samples in four points inside each experimental plot. To minimize sample variation, the same individual who swept at the treated plot also swept at the untreated, on the same day. A sweep consists of two180° arcs (across and back) through the vegetation, quickly turning and reversing direction at the end of the first arc. A sample consisted on 25 sweeps taken on each transect walking at a constant speed. Each sweep-net sample was then transferred to a one gallon plastic bag and labeled. Bags were stored in a freezer for post processing.

2) *Pitfall traps*: This method was used to estimate the abundance and species composition of ground active insects (Southwood 1978). A trap consisted of a 120 ml plastic cup filled with propylene glycol (commercial non-toxic antifreeze). We deployed 5 informingly distributed traps with lids on the ground of each plot (systematic sampling; it gives uniform coverage of the whole of the population of interest (Morrison et al 2001)) (**Figure 2**), and open them 48 hours later to minimize "digging in" effects (Greenslade 1973). We left the units open for seven consecutive days, and then samples were collected and returned to the laboratory for post processing.

3) *Fatty food lures ("hot dogs")*: This method was used to estimate and examine RIFA activity and behavior in the ground (Bestelmeyer et al 2000). We assessed pre- and post-treatment RIFA surface activity on each one of the 2 hectare plot, by determining the number of RIFA/30-45 minutes attracted to 10 uniformingly distributed food lures (hot dog slice) (Porter and Tschinkel 1987, Calixto et al 200X). We estimated the number of ants per slice using a scale system ranging from 0 to 100 on increments of 10 (Pereira and Porter 2005, Calixto et al 200X).

Samples processing. Insects were freeze immediately after collection. Later, insect were sort insects from each sample (winged, non-winged); and measured (mm), weight (wet and dry to nearest 0.01 gr) and identify them as winged/unwinged. Dry weight was determined after drying the samples in a forced-air oven for least 24 hours.

Data Analysis

Analyses focused on 2 aspects; 1) the effect of RIFA reduction (treated vs untreated) and time on insect abundance and 2) the effect of RIFA reduction on insect biomass.

Data obtained from the different sampling methods were analyzed using a Linear Mixed Model (Repeated Measures - Type III sum of squares and diagonal repeated covariance) comparing the effect of different treatments on the response variable (i.e insect abundance) per sampling interval. In this model treatment and sampling interval are considered fixed factors and plot as random factor. The statistic software SPSS 14.0 (SPSS Inc. 2005) was used to perform these analyses, values are significantly different when $P < 0.05$.

Results

Fire ant density: Data collected using pitfall traps and food lures shown the effectiveness of the treatment. RIFA density was significantly lower on bait treated plots compared to those untreated (**Table 1**). Density remained low throughout the sampling periods.

Insect biomass: measurements (gr - wet and dry) for both winged/unwinged in pitfall traps and sweep netting showed no differences between sites where RIFA was reduced vs those untreated (**Table 2**).

Insect abundance: numbers of winged/unwinged insects in pitfall traps showed no differences between sites where RIFA was reduced vs those untreated (**Table 2**).

Discussion

This preliminary study has shown that for the 2006 season removal of RIFA did not exert significant impacts on insect guilds (winged/unwinged), further analyses at order/family level and size classes might be necessary to understand the impacts of RIFA on insect assemblages. This study also suggests that further and more detailed analysis of samples (by order/family) is needed to effectively determine the impact of fire ants on insect abundance. At this time samples are being processed to order level. Analysis will be once again performed to establish the effects of RIFA on insect communities at a smaller scale. If differences are found, research hypotheses will be developed where effect sizes (magnitude of the effect) are used to determine biological significance of the impact of RIFA on insect assemblages. This information will tailor strategies for the management of RIFA on this area and for the restoration and conservation of the Attwater's Prairie-Chicken.

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Figure 1. Plots location at APCNWR

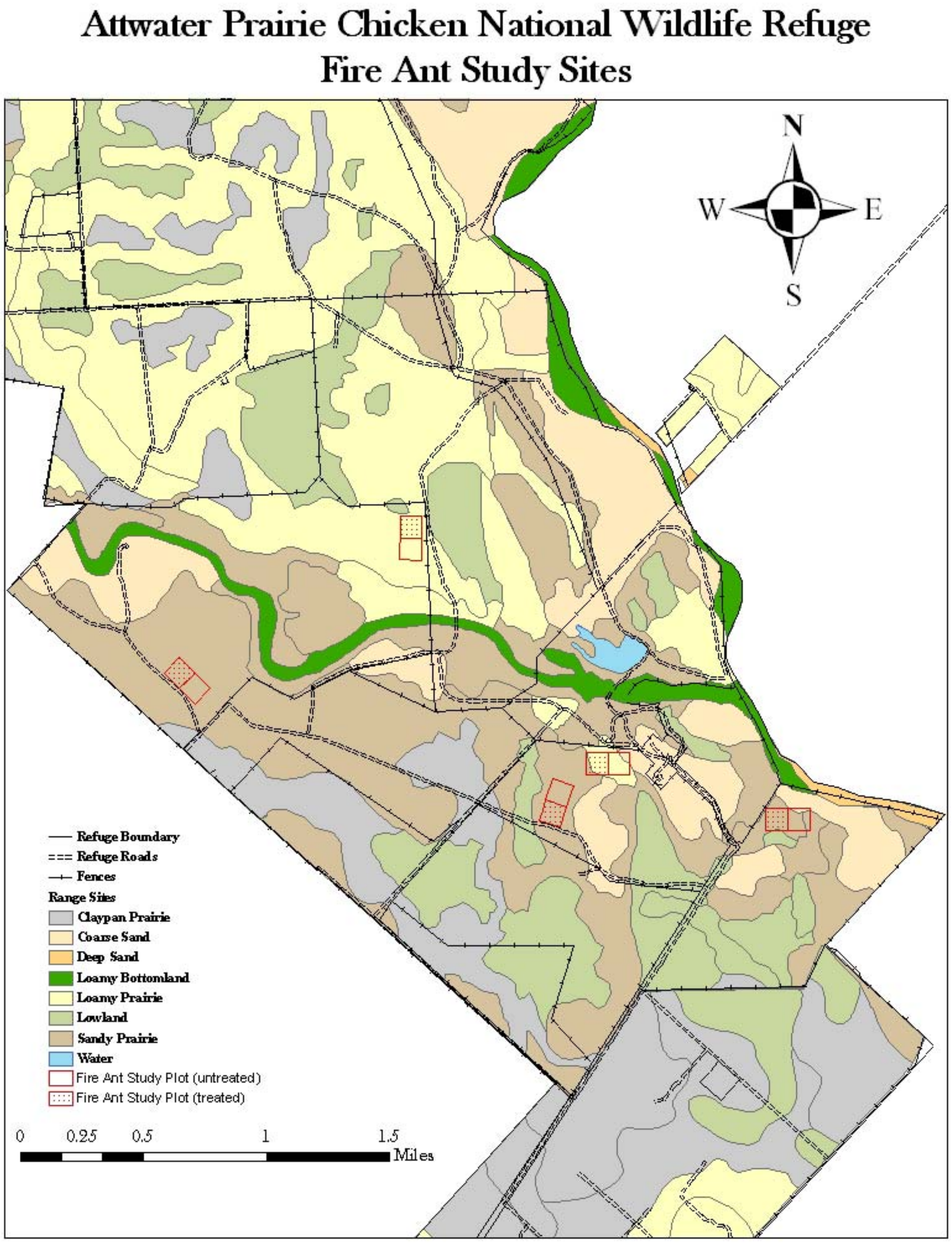

Figure 2. **Pitfall traps and food lures location (systematic sampling)**

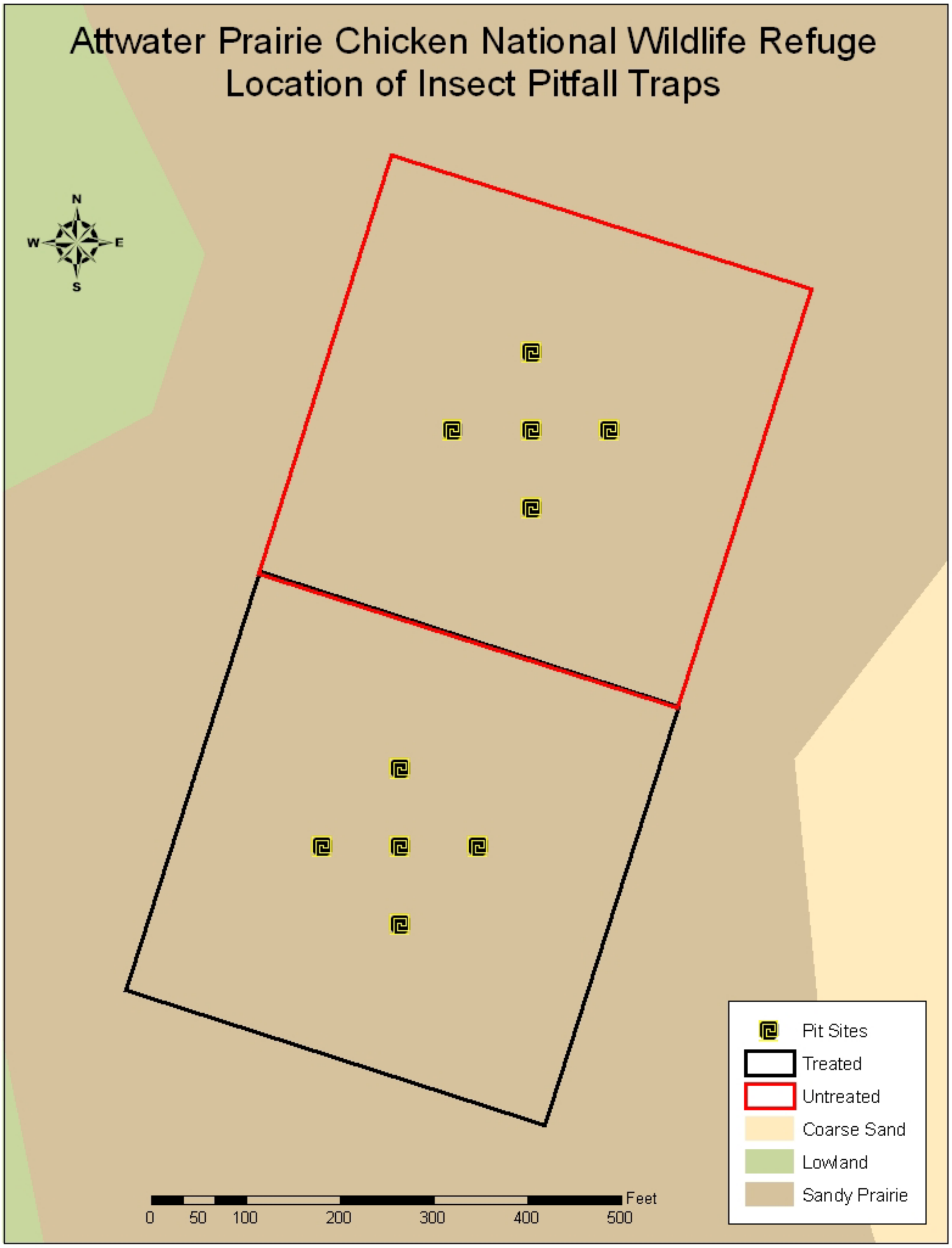

Table 1. Fire ant density (mean±se) in bait treated and untreated plots based on pitfall traps and food lures, 2006. (Mixed Linear Model (MLM), differences at $P < 0.05$).

	Fire Ant Abundance		
	Treated	Untreated	*P*-Value
Pitfall traps	1.50±0.49	27.28±5.66	**0.007**
Food lures	14.04±6.47	215.96±28.33	**0.002**

Table 2. Insect biomass and density (mean±se) in bait treated and untreated plots based on pitfall traps and sweep netting, 2006. (Mixed Linear Model (MLM), differences at $P < 0.05$).

	Wet Weight					
	Winged			Unwinged		
	Treated	Untreated	*P*-Value	Treated	Untreated	*P*-Value
Pitfall traps	1.25±0.23	0.73±0.09	0.54	0.32±0.06	0.01±0.003	0.44
Sweep netting	0.34±0.04	0.66±0.05	0.09	0.12±0.01	0.22±0.02	0.57

	Dry Weight					
	Winged			Unwinged		
	Treated	Untreated	*P*-Value	Treated	Untreated	*P*-Value
Pitfall traps	0.32±0.06	0.19±0.02	0.66	0.19±0.02	0.01±0.002	0.33
Sweep netting	0.34±0.08	0.66±0.09	0.06	0.18±0.02	0.25±0.02	0.49

	Insect Abundance					
	Winged			Unwinged		
	Treated	Untreated	*P*-Value	Treated	Untreated	*P*-Value
Pitfall traps	7.22±0.93	6.40±0.57	0.06	2.62±0.18	3.04±0.19	0.75
Sweep netting	41.26±3.94	48.04±4.00	0.22	26.25±1.79	31.35±3.16	0.57