

USING TARGETED POISONING OF RED IMPORTED FIRE ANTS TO IMPROVE TEXAS  
HORNED LIZARD HABITAT

by

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## INTRODUCTION

Agricultural development, urbanization, climate change, and the invasion of non-native species have accelerated the risk of species extinction (Cox et al., 2022; Jantz et al., 2015). Herpetofauna are especially at risk, with 40.7% of amphibians and 21.1% of reptiles being threatened with extinction (Cox et al., 2022). The most direct way to mitigate these declines is to find land management strategies that specifically reduce the effects of these environmental challenges.

In the United State alone, invasive species cause between 121 and 220 billion dollars in damages annually to agriculture, human health, and ecological systems (Marbuah et al., 2014). The red imported fire ant (*Solenopsis invicta*; RIFA) is an organism of primary concern in the Southern United States. Since being introduced into Mobile, Alabama, in the 1930s (Allen et al., 1995), *S. invicta* has quickly expanded its range and occurs in the southern states ranging from Texas to North Carolina, with a disjunct range in California (Ascunce et al., 2011; Porter & Savignano, 1990). Red imported fire ants exhibit high aggression towards native ant species and non-nestmate conspecifics (Fadamiro et al., 2009; Jones & Phillips, 1987). They are regarded as a superior competitor due to their ability to tolerate a wide range of climatic conditions, their diverse diet, their potent venom used for prey capture, their large colony size, and their preference for disturbed areas (Porter & Savignano, 1990; Xu & Chen, 2023). Red imported fire ants have deleterious effects on wildlife communities and cause ecosystem-level shifts that result in a cascade of ecological problems (Allen et al., 1994; Allen et al., 2004; Kenis et al., 2009).

The Texas horned lizard (*Phrynosoma cornutum*; THL) is a Texas state listed threatened species that has experienced population declines and regional extirpations due to habitat loss, urbanization, the spread of RIFA, and reductions in their specialized food resource of native ants

(Donaldson et al., 1994; Texas Parks and Wildlife Department, 2012). To mitigate these declines, state agencies, zoos, and universities are participating in collaborative efforts to reintroduce hatchling horned lizards into parts of their historic range. Unfortunately, these initial reintroduction efforts have been met with low survival and recruitment, possibly in part due to both direct and indirect competition with RIFA.

Red imported fire ants exhibit direct competition on horned lizards via predation on hatchlings and possibly eggs (Barber, unpub. data). Reduced fitness and survival at these early life stages due to RIFA have also been reported for other herpetofauna (Braman et al., 2021; Buhlmann & Coffman, 2001; Diffie et al., 2010; Dziadzio, Chandler, et al., 2016; Dziadzio, Long, et al., 2016; Epperson et al., 2021; Holcomb & Carr, 2023). Fence lizards have altered their behaviors when exposed to RIFA in the long term, but they have not yet developed physiological defenses against RIFA venom (Boronow & Langkilde, 2010; Langkilde, 2009). It is unknown whether THL have had sufficient time to evolve adequate physiological or similar behavioral mechanisms to respond/survive fire ant attacks.

Red imported fire ants can also exhibit indirect competition with THL by displacing ant species that are vital for a horned lizard's diet. The primary component of an adult THL's diet is harvester ants (*Pogonomyrmex* spp.). In recent years there has been a decline in harvester ant populations possibly due to both destruction of harvester ant colonies by RIFA and haphazard fire ant poisoning (Hook & Porter, 1990). Hatchling horned lizards are too small to consume harvester ants and must rely on smaller species of ants (genera *Pheidole*, *Crematogaster*, *Tetramorium*, and *Dorymyrmex*) and surface foraging termites for food. Red imported fire ants may displace these small native ant species. Studies have reported that RIFA infestations alter the abundance and diversity of invertebrates (Hook & Porter, 1990; Porter & Savignano, 1990;

Stoker et al., 1995), however, we are lacking species-level resolution on how these invertebrate populations are changing and interacting with RIFA.

To mitigate the negative impact of RIFA, there have been many strategies employed to reduce their overall abundance. Some of the most common strategies are pumping boiling water into colonies, release of the microsporidium *Thelohania solenopsae*, introduction of *Pseudacteon* decapitating flies, and pesticide application. Pumping boiling water into fire ant colonies has shown significant reductions of fire ants (Middleton et al., 2023; Tschinkel & King, 2007). In addition, it is a highly specific way of targeting only RIFA and minimizing impact to non-target species. However, it has substantial equipment barriers, as it is often difficult to transport boiling water to field locations. *Thelohania solenopsae* is a common RIFA-specific pathogen found in Argentina and Brazil (Briano et al., 1995). It reduces fire ant populations by weakening the queen which in turn leads to reduced or halted offspring production (Williams et al., 1998). This pathogen was found in the United States in 1996 and could serve as a viable biocontrol agent (Williams et al., 1998). There have been ten states so far that have released this pathogen in RIFA communities as a biocontrol agent (Williams & deShazo, 2004). Another common biological control agent is the introduction of *Pseudacteon* decapitating flies, commonly known as phorid flies. These flies fly over worker RIFA and lay their eggs in the thorax of the fire ant. This egg develops and eventually hatches inside of the ant. After hatching, the fly larva migrates towards the head of the ant, where it eats the neck muscles and releases an enzyme that causes decapitation. The larva then completes development inside of the head (Porter et al., 1995). While these flies do cause direct death of fire ants, it is hypothesized that these flies reduce fire ant colony densities due to a behavioral shift: Fire ants reduce their foraging levels in the

presence of phorid flies. This reduction limits resource acquisition and results in smaller colony sizes (Orr et al., 1995).

Currently, the primary tool to control RIFA populations is pesticide use. The most common application method is broadcast baiting, due to its simplicity, reduced cost, and long-lasting effects. Even when using ant-specific poisons, this widespread treatment method has had unintended consequences for some non-target organisms in the landscape (Darracq et al., 2017; Li & Cui, 2022; McNaught et al., 2014; Sirsi et al., 2020). In the 1960s and 70s, Mirex was the dominant ant poison used to eradicate RIFA. However, Mirex was banned in 1978 due to its highly toxic attributes that significantly damaged wildlife communities and human health (Kaiser, 1978). Today, less toxic poisons such as Amdro<sup>®</sup> and Extinguish<sup>®</sup> are used for fire ant control (EPA, 1998; Glare & O'Callaghan, 1999). While these new poisons are better for human health, more research should be conducted to investigate their effect on wildlife. Current literature provides conflicting results on whether broadcast baiting provides net positive or negative effects to the ecosystem at large, with some studies finding that broadcast baiting is especially beneficial to organisms at higher trophic levels that experience direct predation by RIFA (Allen et al., 1997; Allen et al., 1995). On the other hand, broadcast baiting could be riskier to use if management goals require diverse and abundant native invertebrate communities. Studies have presented conflicting results on how invertebrates, especially native ants, are affected after broadcast baiting treatments. Table 1 summarizes a wide body of literature on how broadcast baiting affects RIFA and native invertebrates. Across all studies reviewed, broadcast baiting decreased RIFA abundance. In contrast, there is no clear pattern for how native invertebrate populations change in these studies, likely due to the following key differences in

study design: native species evaluated, frequency of poison application, type of poison applied, initial RIFA infestation level, sampling method, and data collection intervals.

Due to the uncertainties associated with broadcast baiting effects, a suite of studies have focused on designing specialized poisoning methods. These methods are designed with the focal environment and at-risk non-target species in mind. Strategies employed include using containerized bait to prevent residual pesticides from being left in the environment (Kramm et al., 2024; Taniguchi et al., 2003), specialized structures to physically block non-target species access to the poison (Gaigher et al., 2012), and usage of specialized bait that would be most attractive to the target species (Buczowski, 2017). These specialized bait stations have minimal effects to non-target invertebrates and show significant reductions in target ant species (Buczowski, 2017; Gaigher et al., 2012). To date, there are no known studies that assess how targeted baiting affects both RIFA and native ant communities in a scrubland ecosystem.

My study was conducted at Mason Mountain Wildlife Management Area (MMWMA), which is a state-owned, scrubland property within the historic range of the Texas horned lizard. This site has hosted active hatchling Texas horned lizard reintroductions since 2017 and has also had a noticeable increase in RIFA abundance in recent years (Mitchell, personal correspondence). I designed a controlled study to determine the effects of a targeted poison application of Amdro<sup>®</sup> on RIFA and hatchling horned lizard prey at MMWMA in the summers of 2022 and 2023. Using pitfall traps and bait stations, I assessed whether our targeted poisoning could decrease RIFA abundance without harming native ants that are important in a hatchling horned lizard's diet.

**TABLE 1.** Current literature examining effects of broadcast baiting on RIFA and other invertebrates

<b>Reference</b>	<b>Insecticide/ Active ingredient</b>	<b>Number of broadcast applications per year</b>	<b>Invertebrate sampling technique</b>	<b>Effect on RIFA<sup>±</sup></b>	<b>Effect on invertebrates<sup>±</sup></b>
Allen et al. (2001)	Amdro <sup>®</sup> / Hydramethylnon	1.5	a. UV light traps b. Circle sweeps	Decreased	Increased volume, richness, and diversity <sup>x</sup>
Apperson et al. (1984)	Amdro <sup>®</sup> / Hydramethylnon	2	a. Pitfall traps b. Food lures	Decreased	No significant effect
Calixto et al. (2007)	Extinguish <sup>®</sup> / s-methoprene	1.5	a. Pitfall traps b. Food lures c. Direct sampling d. Colony counts	Decreased	Increased diversity and densities of many species increased
Calixto (2008)	(i) Extinguish <sup>®</sup> / s-methoprene (ii) Advion <sup>®</sup> / indoxacarb	1	a. Pitfall traps b. Food lures	Decreased	No significant effect
Darracq et al. (2017)	Amdro <sup>®</sup> / Hydramethylnon	Site specific	a. Pitfall traps b. Food lures	Decreased	Increased diversity and evenness, but not abundance
Epperson and Allen (2010)	Logic <sup>®</sup> / Fenoxycarb	1.33	a. Food lure b. Colony counts c. Light traps	Decreased	a. Food lures: Increased richness and diversity b. Light traps: No significant effect
Epperson et al. (2021)	Logic <sup>®</sup> / Fenoxycarb	1.33	a. Food lure b. Gopher tortoise burrow sampling	Decreased	a. Food lure: No significant effect b. Burrow sampling: Increased diversity and abundance

Ipser and Gardner (2010)	(i) Amdro®/ Hydramethylnon (ii) Over-n-Out™/ Fipronil	1	a. Pitfall traps b. Food lures c. Colony counts	(i) Hydra-methylnon : Initial decrease then quick population rebound (ii) Fipronil: Decreased	No significant effect
Li and Cui (2022)	Indoxacarb	1	a. Pitfall traps b. Food lures	Decreased	Initial decrease then increase after 45 days
Markin et al. (1974)	Mirex/ Mirex	1	a. Colony counts b. Food lures	Initial decrease then quick population rebound	<i>Pheidole</i> spp. increased, sugar-feeding ants not affected, four other ant spp. significantly declined
McNaught et al. (2014)	(i) Mix of methoprene and pyriproxyfen (ii) Hydramethylnon	Minimum of four treatments per year over three years	(i) Pitfall traps (ii) Colony counts	Decreased	No significant effect on most ants. <i>Pheidole</i> spp. abundance significantly declined
Morrow et al. (2015)	Extinguish® Plus/ Hydramethylnon	1	(i) Food lures (ii) Vegetation sweeps	Decreased	Some invertebrates slightly increased, most not affected
Sirsi et al. (2020)	Extinguish® Plus/ s-methoprene and hydramethylnon	1	(i) Pitfall traps (ii) Food lures	Decreased	Decreased
Vander Meer et al. (2007)	Hydramethylnon and methoprene mixture	Site specific	(i) Pitfall traps (ii) Food lures (iii) Colony counts	Decreased	Arthropods not affected. Non-target ants usually declined (Only quantified with pitfall traps)



Vogt et al. (2005)	Seige Pro <sup>®</sup> / Hydramethylnon	1	a. Pitfall traps b. Food lures c. Colony counts	Decreased	Significant reduction in <i>Monomorium minimum</i> , species in subfamily Dolichoderinae increased, all other ants not affected
Zakharov and Thompson (1998)	(i) Logic <sup>®</sup> / Fenoxycarb (ii) Amdro <sup>®</sup> / Hydramethylnon	2	a. Food lures	Decreased	(i) Logic <sup>®</sup> : Increased (ii) Amdro <sup>®</sup> : Decreased

± refers to abundance if not specified

<sup>x</sup> did not include ant species

## METHODS

### *Study Area*

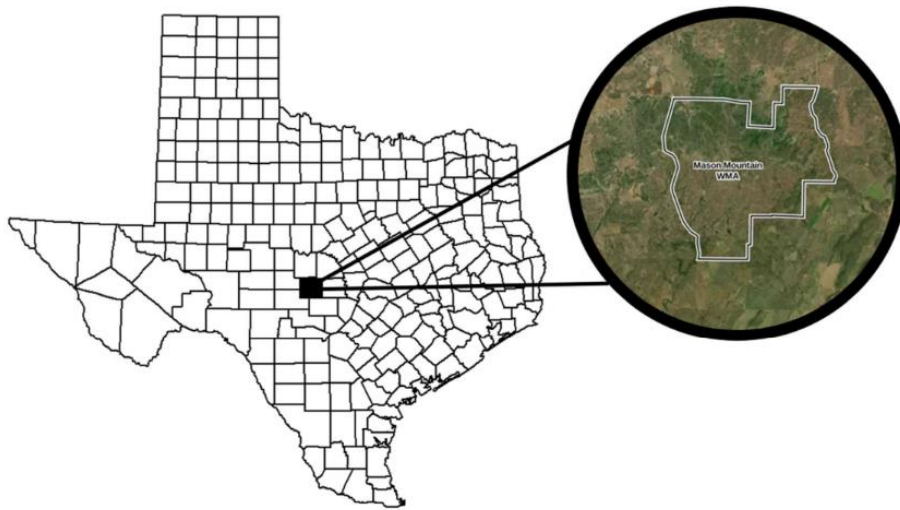
I conducted this fieldwork at Mason Mountain Wildlife Management Area (Fig. 1), located in the Edwards Plateau Ecological Region in central Texas (30.8398, -99.2178). MMWMA was historically used as a livestock ranch and exotic hunting preserve before being donated to the Texas Parks and Wildlife Department (TPWD) in 1997. Management at the site is now dedicated to preserving native species. Mason Mountain Wildlife Management Area is also part of an active Texas horned lizard reintroduction program that is a partnership between TPWD and Texas zoos. Captive breeding programs at the Fort Worth, Dallas, and Caldwell Zoos have raised over 1,400 hatchlings that have been released at this location since 2017.

The dominant vegetation at this location includes the Texas live oak (*Quercus fusiformes*), post oak (*Quercus stellata*), agarita (*Mahonia trifoliolata*), elbow bush (*Forestiera pubescens*), Texas white brush (*Aloysia gratissima*), bluestem (*Schizachyrium scoparium*), silver

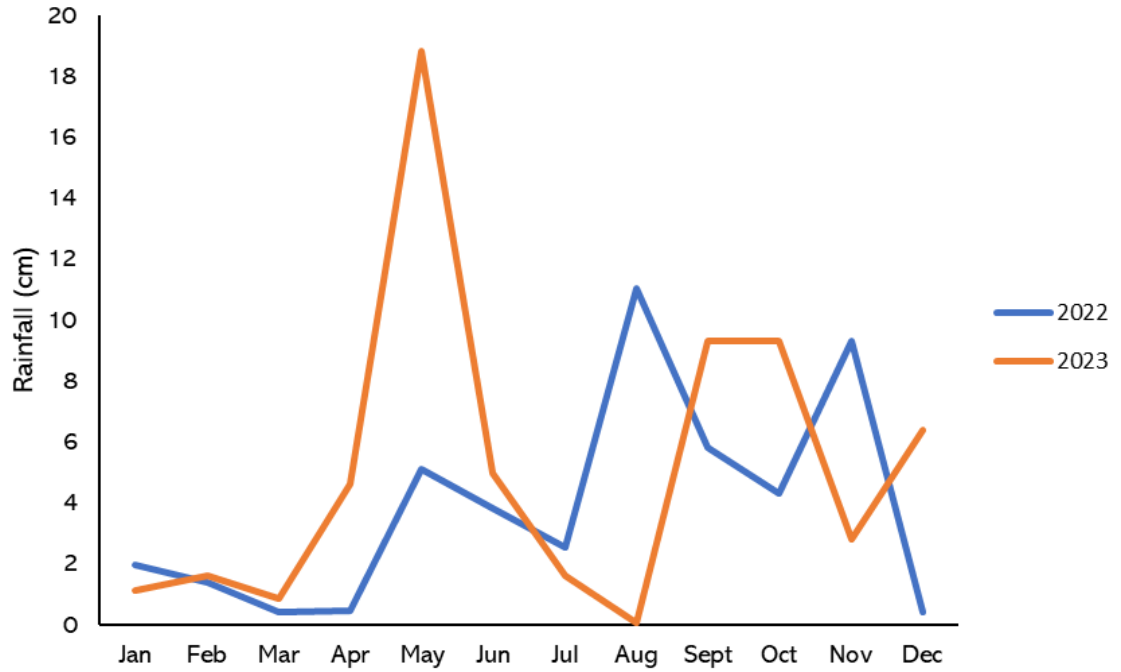
bluestem (*Bothriochloa saccharoides*), threeawns (*Aristida* spp.), grama grasses (*Bouteloua* spp.), and love grasses (*Eragrostis* spp.) (Hargrave, 2015; Mitchell, personal correspondence). Areas of MMWMA are prescribed burned which helps control overdominance of prickly pear cacti (*Opuntia* spp.) and prevents the encroachment of woody vegetation such as Ashe juniper (*Juniperus ashei*) and mesquite (*Prosopis glandulosa*). The climate for this county is humid and subtropical, with an average annual rainfall of 67.8 cm and average annual temperature of 18.5°C (NOAA 2024). The region has hot summers, mild dry winters, low rainfall, high evaporation rates, high temperature, and high wind speeds (Natural Resources Conservation Service, 2011). During this study, MMWMA experienced one dry year (2022) and one wet year (2023), with 46.6 cm of precipitation and 61.4 cm of precipitation in each year respectively (Fig. 2).

This study utilized four 50 m<sup>2</sup> plots. Preliminary analysis of these plots in May 2022 classified two of the plots as having moderate levels of RIFA and two plots as having no known RIFA present (Fig. 3a). Plots 2, 3, and 4 had similar abundances of native ants, while plot 1 had over twenty times the number of native ants with an especially high abundance of *Crematogaster punctulata* (Fig. 3b). These four study plots were assigned to one of two different treatment types, treated or untreated. The two plots that were classified as treated plots were poisoned with Amdro<sup>®</sup> (one of these plots had a moderate starting abundance of RIFA and one had no RIFA initially present). The other two plots were untreated plots and never had poison applied (one of these plots had a moderate starting abundance of RIFA and one had no RIFA initially present) (Table 2). Each plot was separated by a minimum of 100 m (Fig. 4) to ensure poison application at our treated plots did not affect the ants at our untreated plots.

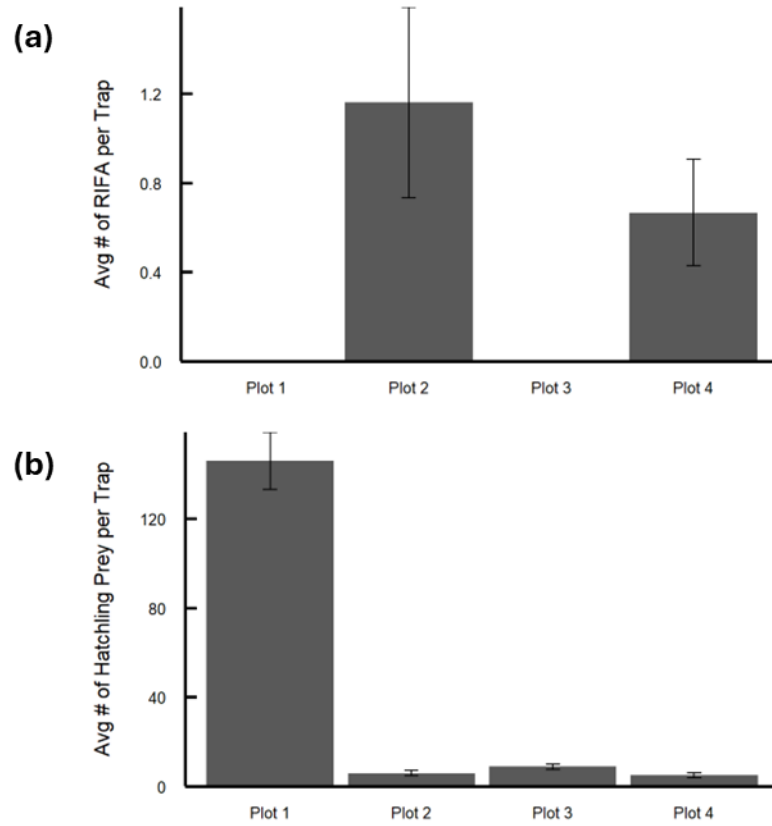
In July of 2022, we noticed that one of our untreated plots with a previously low abundance of RIFA had quickly become infested with RIFA (Fig. 5). This plot remained an untreated plot for the 2022 season. However, in 2023, this plot was used as a treated plot to try and protect hatchling horned lizards that were released there (Table 2).



**FIG. 1.** Study site located at Mason Mountain Wildlife Management Area in the Edwards Plateau Ecological Region, Mason County, Texas.



**FIG. 2.** Monthly precipitation (cm) for 2022 and 2023 at Mason Mountain Wildlife Management Area, Mason, Texas. Data obtained from Mason Mountain Wildlife Management Area weather station from TexMesonet (Texas Water Development Board, 2024).

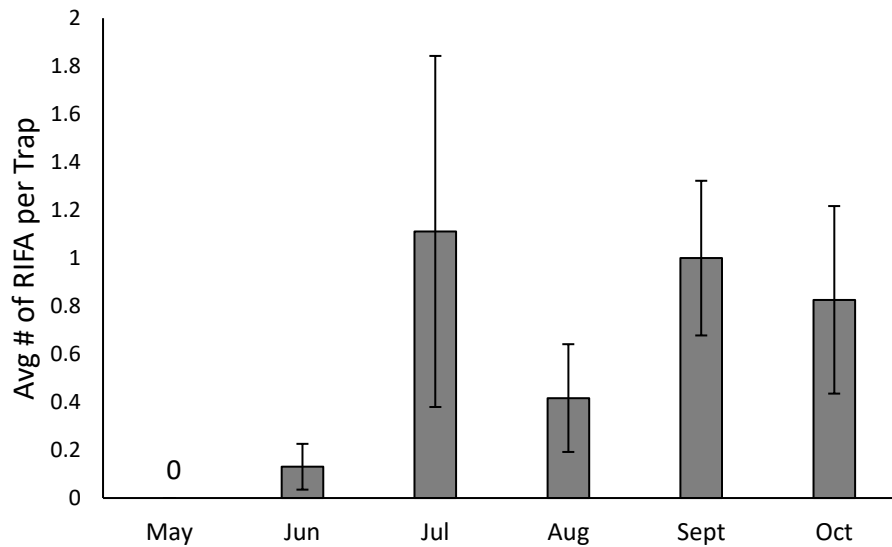


**FIG. 3.** Average number of RIFA (panel a) and hatchling prey (panel b) per pitfall trap for each plot in May 2022 ( $n = 20, 25, 22, 24$  for plots 1–4 respectively). Error bars represent standard error.



**FIG. 4.** Locations of ant baiting grids at MMWMA. Each tan dot represents an ant bait station.

Each plot is a minimum of 100 meters apart.



**FIG. 5.** Average number of RIFA in each pitfall trap at plot 3 for the 2022 season (n = 22, 23, 18, 24, 22, and 23 for May – Oct respectively). Error bars represent standard error.

**TABLE 2.** Treatment types assigned to each plot in 2022 and 2023. Treated indicates plots were poisoned with Amdro<sup>®</sup> and untreated indicates plots were not poisoned with Amdro<sup>®</sup>.

	<b>2022</b>	<b>2023</b>
<b>Plot 1</b>	No RIFA; Treated	Very low levels of RIFA; Treated
<b>Plot 2</b>	Moderate levels of RIFA; Treated	Moderate levels of RIFA; Treated
<b>Plot 3</b>	No RIFA; Untreated	Moderate levels of RIFA; Treated
<b>Plot 4</b>	Moderate levels of RIFA; Untreated	Moderate levels of RIFA; Untreated

### *Targeted Poisoning*

At each plot, slices of hot dogs were placed on top of deli cup lids five meters apart from one another in 10x10 grids for a total of 100 baits. After leaving baits for 30 minutes, we recorded whether RIFA, native ants, or no ants recruited to each bait (Fig. 6). At treated plots, approximately one teaspoon of Amdro<sup>®</sup> was applied to baits where RIFA were present. No poison was applied to untreated plots. Baits were then left for another 30 minutes (one hour after initial placement). After, we collected each sample by affixing a deli cup to the lid, ensuring that no residual Amdro<sup>®</sup> was left in the environment. All samples collected were later identified to genus and used for modeling. Targeted poisoning was repeated once per month between May and August, totaling four poisoning events per year. For 2022, baiting occurred May 26<sup>th</sup>, June 28<sup>th</sup>, July 26<sup>th</sup>, and August 31<sup>st</sup>. For 2023, baiting occurred May 26<sup>th</sup>, June 16<sup>th</sup>, July 21<sup>st</sup>, and August 25<sup>th</sup>.



**FIG. 6.** Bait station set-up: Slice of hot dog placed on top of an upside-down deli cup lid. Native ants seen recruiting to this bait station.

#### *Identification of Ant Communities at Treated and Untreated Plots*

We assessed ant communities in two ways: First, we evaluated the change in the frequency of RIFA at baits over time. Second, we evaluated the overall abundance of ants and small invertebrates at each plot by using 10 mL test tube pitfall traps (2 cm diameter x 8 cm length) placed five meters apart from one another in a 5x5 grid for a total of 25 traps. The pitfall trap grid was placed in the center of our baiting grid, creating a 15-20 meter buffer between unpoisoned areas and pitfall traps. Each test tube was filled with approximately seven mL of non-toxic propylene glycol, which serves as a preservative. All test tube contents were collected and sorted after being set in the field for four days. Two days is typically considered the minimum to accurately assess ant communities (Borgelt & New, 2006). During rain events, pitfall traps were capped to prevent overflowing of trap contents and to prevent data loss. Pitfall traps were reopened as soon as a rain event concluded and were left on the landscape until total time open equated to four days. In 2023, if rainfall caused a pitfall trap to overflow, the contents



of the trap were filtered using a kitchen strainer to recover any ants within traps, and a new trap was set out to continue sampling for the remainder of the sampling period ( $n = 6$ ). Ants were identified to genus, and all other taxa were identified to order.

Since Amdro<sup>®</sup> is a metabolic inhibitor with delayed toxicity release, we set pitfall traps no sooner than two weeks after a targeted poisoning. This allowed us to better quantify how the ant community changed following the effects of the poison. Pitfall arrays were set on May 14<sup>th</sup>, 2022, to determine starting ant abundance and were continued on the following dates to see the response of poisoning: June 18<sup>th</sup>, July 16<sup>th</sup>, August 13<sup>th</sup>, September 17<sup>th</sup>, and October 15<sup>th</sup> in 2022 and June 9<sup>th</sup>, July 1<sup>st</sup>, August 8<sup>th</sup>, and September 1<sup>st</sup> in 2023. Data could not be recovered for sixty-two pitfall traps in 2022 and six pitfall traps in 2023 due to rainfall, traps filling in with dirt, or animals digging up traps.

### *Data Analysis*

Data from 2022 and 2023 were analyzed separately due to the substantial precipitation differences between years. Precipitation significantly affects ant mating behavior and activity level, potentially leading to different population level assessments depending on rainfall (Morrill, 1974; Vogt & Smith, 2007). I constructed generalized linear mixed models (GLMMs) for each year. Plot was included as a random effect while treatment type and date of baiting/pitfall trapping were included as fixed effects.

To select the best distribution for each GLMM, I used the DHARMA package in R. Selected models had residuals that were uniformly distributed and had no strong pattern of over- or under-dispersion. Uniformity was tested using the Kolmogorov-Smirnov (KS) test. If multiple

distributions met the above assumptions, I opted to use the distribution that produced the lowest AIC value (Table 3).

These models were constructed and plotted in R (version 4.3.1) using `glmmTMB` and `ggeffects` packages. Using the bait station data, I constructed two binary GLMMs (one for each year) by coding each bait as 1 or 0 depending on if RIFA were present or not. This assessed how the frequency of RIFA at baits changed in response to the treatment type over time. Using the pitfall trap data, I constructed four GLMMs. First, I evaluated how RIFA abundance in pitfall traps changed in response to the treatment type over time in 2022 and 2023 separately. Secondly, I evaluated how hatchling prey abundance changed in response to the treatment type over time in 2022 and 2023 separately. I defined hatchling prey as the ant species that dominate a hatchling’s diet: *Crematogaster punctulata*, *Pheidole* spp., *Tetramorium* spp., and *Dorymyrmex flavus* (Alenius, unpub. data). Where relevant, I assumed significance at an alpha value of 0.05.

**TABLE 3.** Response distribution (family function) used in each GLMM

	2022	2023
<b>Pitfall traps, RIFA abundance</b>	Negative binomial 1	Zero-inflated generalized Poisson
<b>Pitfall traps, Hatchling prey abundance</b>	t-family	t-family
<b>Bait station, RIFA abundance</b>	Binomial	Zero-inflated binomial

Identification accuracy of field technicians was evaluated between years using a two-proportion test to determine if human error was consistent. Overall error rates at treated plots were determined by calculating the percentage of times native ants were poisoned as well as the percentage of times RIFA were not poisoned.

## RESULTS

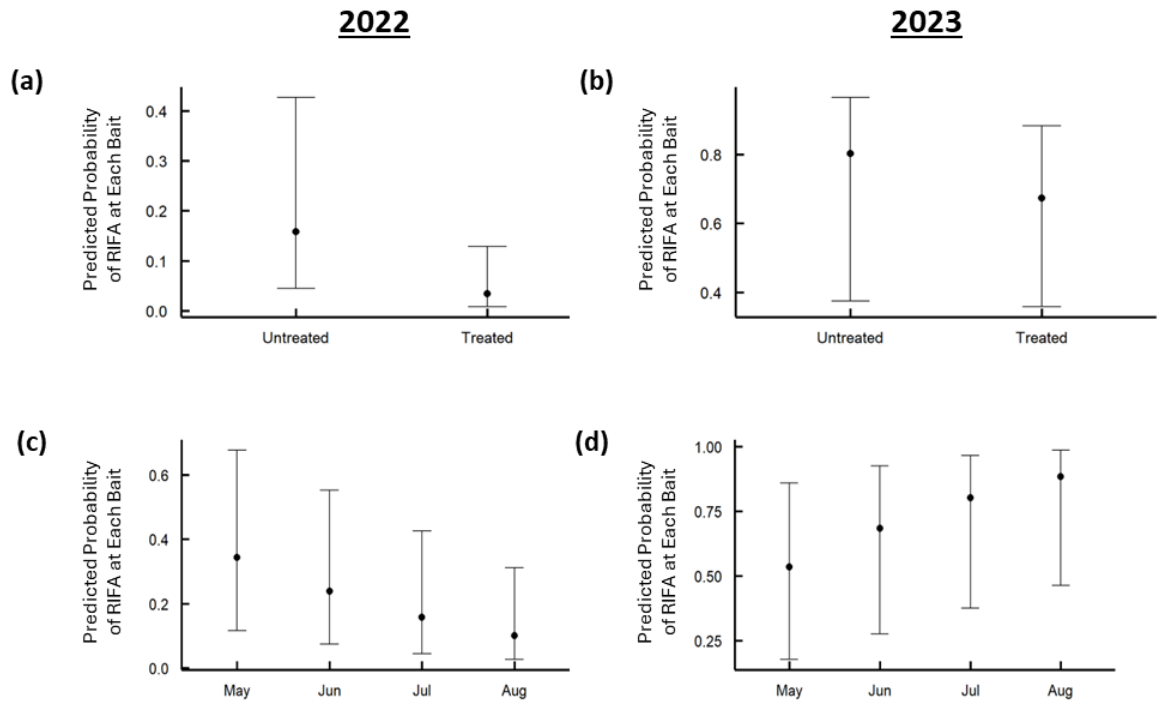
In 2022, ants were collected from 910 out of the 1,600 bait stations. Of the 910 baits, field technicians correctly identified whether ants were RIFA or native ants 85.6% of the time. In 2023, ants were collected from 1,059 out of the 1,600 bait stations. Of the 1,059 baits, field technicians correctly identified whether ants were RIFA or native ants 85.2% of the time. A two-proportion test revealed that there was no significant difference in identification success between years ( $z = 0.269$ ,  $p = 0.788$ ).

Of the 800 baits that were in the treated plots in 2022, twenty-five were lost in transport, skipped in the set-up process, or had their bait stolen by surrounding wildlife. Of the 775 that were recovered, 3.1% were misidentified as RIFA and 2.7% were misidentified as native ants. The best-fit baiting model for 2022 showed no significant difference in the number of RIFA dominated baits in treated plots compared to untreated plots ( $\beta = -1.66$ ,  $SE = 1.01$ ,  $Z = -1.65$ ,  $p = 0.099$ ; Fig. 7a) and a significant decrease in RIFA abundance as the season progressed ( $\beta = -0.514$ ,  $SE = 0.073$ ,  $Z = -6.99$ ,  $p < 0.001$ ; Fig. 7c).

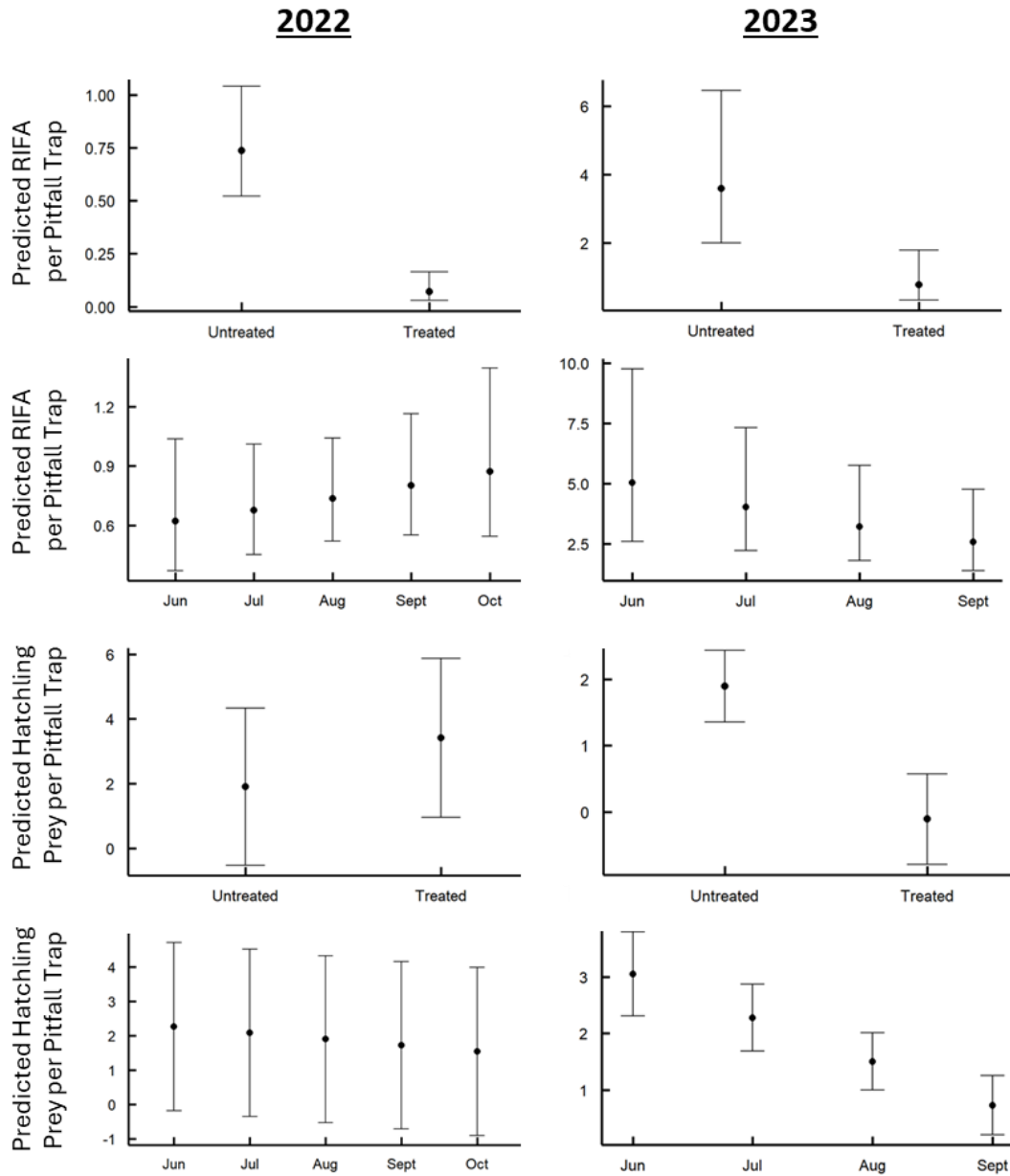
Of the 1,200 baits that were in the treated plots in 2023, sixty-five were lost in transport, skipped in the set-up process, or had their bait stolen by surrounding wildlife. Of the 1,135 that were recovered, 2.7% were misidentified as RIFA and 5.7% were misidentified as native ants. The best-fit baiting model for 2023 showed no significant difference in the number of RIFA dominated baits in treated plots compared to untreated plots ( $\beta = -0.680$ ,  $SE = 0.982$ ,  $Z = -0.692$ ,  $p = 0.489$ ; Fig. 7b) and a significant increase in RIFA abundance as the season progressed ( $\beta = 0.631$ ,  $SE = 0.239$ ,  $Z = 2.64$ ,  $p = 0.008$ ; Fig. 7d).

The best-fit RIFA abundance model using the 2022 pitfall trapping data showed a decrease in the number of RIFA in treated plots compared to untreated plots ( $\beta = -2.34$ ,  $SE = 0.430$ ,  $Z = -5.45$ ,  $p < 0.001$ ; Fig. 8a), with time of year having no significant effect on RIFA abundance ( $\beta = 0.084$ ,  $SE = 0.089$ ,  $Z = 0.950$ ,  $p = 0.342$ ; Fig. 8c). The best-fit RIFA abundance model for 2023 pitfall trapping data showed a decrease in the number of RIFA in treated plots compared to untreated plots ( $\beta = -1.53$ ,  $SE = 0.335$ ,  $Z = -4.57$ ,  $p < 0.001$ ; Fig. 8b) and a significant decrease in RIFA abundance as the season progressed ( $\beta = -0.225$ ,  $SE = 0.090$ ,  $Z = -2.50$ ,  $p = 0.012$ ; Fig. 8d).

In pitfall traps, the most common hatchling prey items were *Crematogaster punctulata* ( $n = 924$ ) in 2022 and *Pheidole* spp. ( $n = 541$ ) in 2023. The best-fit hatchling prey abundance model using the 2022 pitfall trapping data showed no significant difference in the number of hatchling prey in treated plots compared to untreated plots ( $\beta = 1.51$ ,  $SE = 1.76$ ,  $Z = 0.858$ ,  $p = 0.391$ ; Fig. 8e) and a significant decrease in hatchling prey abundance as the season progressed ( $\beta = -0.179$ ,  $SE = 0.070$ ,  $Z = -2.55$ ,  $p = 0.011$ ; Fig. 8g). The best-fit hatchling prey model using the 2023 pitfall trapping data showed a decrease in the number of hatchling prey in treated plots compared to untreated plots ( $\beta = -2.00$ ,  $SE = 0.349$ ,  $Z = -5.72$ ,  $p < 0.001$ , Fig. 8f) and a significant decrease in hatchling prey abundance as the season progressed ( $\beta = -0.772$ ,  $SE = 0.121$ ,  $Z = -6.40$ ,  $p < 0.001$ ; Fig. 8h).



**FIG. 7.** Predicted probability of RIFA occupying each bait station based on the best-fitting GLMM (see Table 3). Error bars indicate 95% confidence intervals around the mean. Panels a and b show the effect of treatment type on predicted probability when controlling for date. Panels c and d show the effect of date on predicted probability when controlling for treatment type.



**FIG. 8.** Predicted average number of ants per pitfall trap based on the best-fitting GLMM (see Table 3). Error bars indicate 95% confidence intervals around the mean. Panels a, b, e, and f show the effect of treatment type on predicted averages when controlling for date. Panels c, d, g, and h show the effect of date on predicted averages when controlling for treatment type.

## DISCUSSION

### *Ant Response to Targeted Poisoning*

Our targeted poisoning strategy likely reduced the abundance of RIFA at MMWMA. Pitfall trapping data revealed that our targeted poisoning significantly reduced the abundance of RIFA for both years. However, bait station data showed that treatment type had no significant effect on RIFA abundance for 2022 and 2023. For this study, pitfall trapping is likely a more accurate assessment of overall ant community structure. Baiting is heavily biased to more aggressive ant species (Gotelli et al., 2011; Hacala et al., 2021). Red imported fire ants, being one of the most aggressive ant species on the landscape, can competitively exclude native ants (Calcaterra et al., 2008; Hacala et al., 2021; Jones & Phillips, 1987). Pitfall trapping removes the effect of competition and instead evaluates community composition by relying on ants to randomly fall into traps (Stringer et al., 2011). In addition, this study utilized a protein rich bait which is highly attractant to RIFA, especially during the summer months (Stein et al., 1990). This protein-rich substance may not have been as attractive to native ants whose diets primarily consist of carbohydrates, and as such, these native ants may have been under-sampled using bait stations (Nyamukondiwa & Addison, 2014; Rahardjo et al., 2023). Furthermore, only recording presence/absence of RIFA at bait stations may have been too minimal to detect treatment level effects. Bait data could be more powerful by quantifying how many RIFA workers recruit to each bait. This strategy has been employed in another study that produced a better estimate of RIFA density on the landscape (Drees et al., 2011).

Pitfall trapping also revealed that hatchling prey could be affected by our targeted poisoning method. In 2022, the targeted poisoning had no significant effect on hatchling prey, initially confirming our goal of designing a method that would not affect non-target ant species.

In 2023, however, the pitfall trapping model indicated that targeted poisoning did significantly reduce hatchling prey abundance. The reason for these different results is unclear, as accidental poisoning rates between years were relatively similar (3.1% in 2022 and 2.7% in 2023). I hypothesize that hatchling prey were affected differently due to precipitation levels. Rainfall was much higher in 2023 compared to 2022, which seemed to lead to a much higher abundance of ants in 2023. The summed number of RIFA and hatchling prey was 4.8 ants per active trap in 2022 and 6.4 ants per active trap in 2023. In 2022, the lower level of RIFA coupled with our poisoning efforts could have reduced competitive pressures on the native ants, allowing their population to remain stable. However, in 2023, despite our poisoning significantly reducing RIFA, targeted poisoning alone may not have decreased the RIFA population enough to reduce the harsh competitive landscape the fire ants impose on the native ants. Another possibility is that with such low densities of RIFA and hatchling prey in 2022, we may have simply not been able to detect a treatment level effect.

With few exceptions, there was a significant decrease in ant abundance as the field season progressed. This suggests that ants were more active early in the summer compared to late summer. As such, future targeted poisoning efforts should be preferentially conducted earlier in the season. This will ensure there are more foragers on the landscape, increasing the chance that the poison will make it back to the colony.

### *Future Experiments*

A difficulty encountered during this study was rain during pitfall trapping periods. During the May 2023 trapping period, a rainfall event caused many traps to overflow or fill with sediment. Ants were still recovered from overflowing pitfall traps; however, it is unclear how this affected results. Future studies should consider alternative pitfall trap designs that would



prevent traps from overflowing, such as a shaded cover or funnel design. Closing traps during predicted rain events is one option, but rain can be unpredictable, and it is not always feasible to close all traps prior to a large rain event.

The small number of plots limited the statistical power of my analyses. Future studies should increase the number of plots to better detect treatment effects. Conducting a priori power analyses using preliminary data would provide important guidance about how many plots would be needed in a robust design. Using this, researchers could find a balance between sample units, expense, and time to carry out the most robust design possible. Calixto (2008) utilized a power analysis on a similar study on broadcast baiting in central Texas. This study suggested using six replicates, however, differences in ant communities and study design may render this suggestion insufficient in many settings.

To minimize spatial autocorrelation and ensure statistical independence of study areas, plots need to be appropriately spaced apart. In this study, each plot was spaced a minimum of 100 meters apart to ensure worker ants from one plot were unlikely to immigrate into another plot. Despite this, our study could have still been impacted by worker or alate ants from surrounding areas immigrating into our plots. Under ideal circumstances, a 100 meter buffer region between plots and surrounding areas should be implemented. Even with a large buffer, researchers should recognize potential noise in data from alates (winged reproductive ants), which can travel up to 5 km, recruiting in plots. Taking all these steps is easier in theory than in practice, especially when time, personnel, and budget constraints must be considered.

In addition, future studies should better quantify how harvester ants and termites change in response to these poisonings, as these are two groups that are important dietary components for horned lizards. Both harvester ants and surface-foraging termites are line foragers; thus, they

often do not fall into pitfall traps in a random manner like other invertebrates. Colony counts could be used as a supplementary method to quantify harvester ant abundance, but there is no equivalent method for surface-foraging termites. To make this poisoning method cost-effective and efficient, researchers should also conduct studies that quantify the ideal amount of poison per bait station and the total time poison should be left on the landscape.

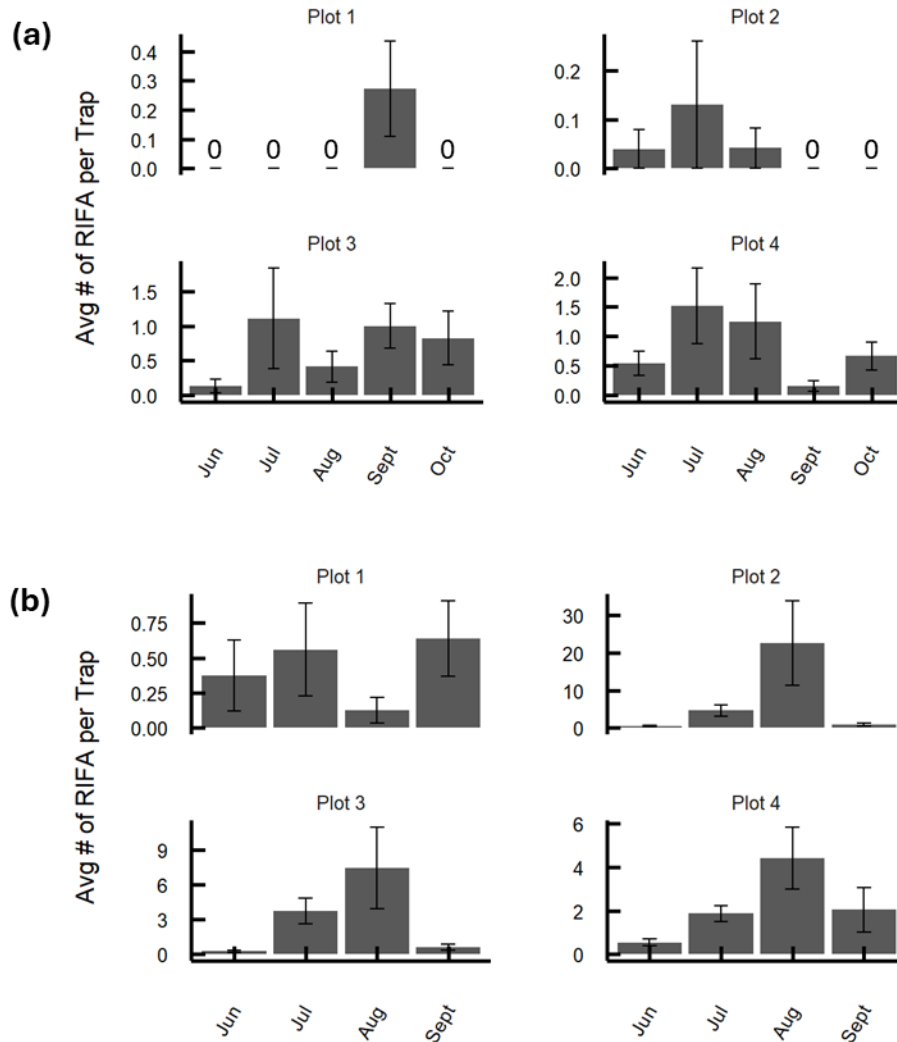
The ultimate test of these methods would be to evaluate how THL hatchling and nest survival vary in response to a targeted poison application. We did not feel comfortable making these comparisons with our data due to the lack of THL recruitment prior to this study (from 2017 – 2021) and due to the change in treated plots in 2023 (Table 2). It would be ideal for these analyses to be conducted on a well-established natural population of THL to see how they respond solely to targeted poisoning effects in the long-term.

### *Conservation Implications*

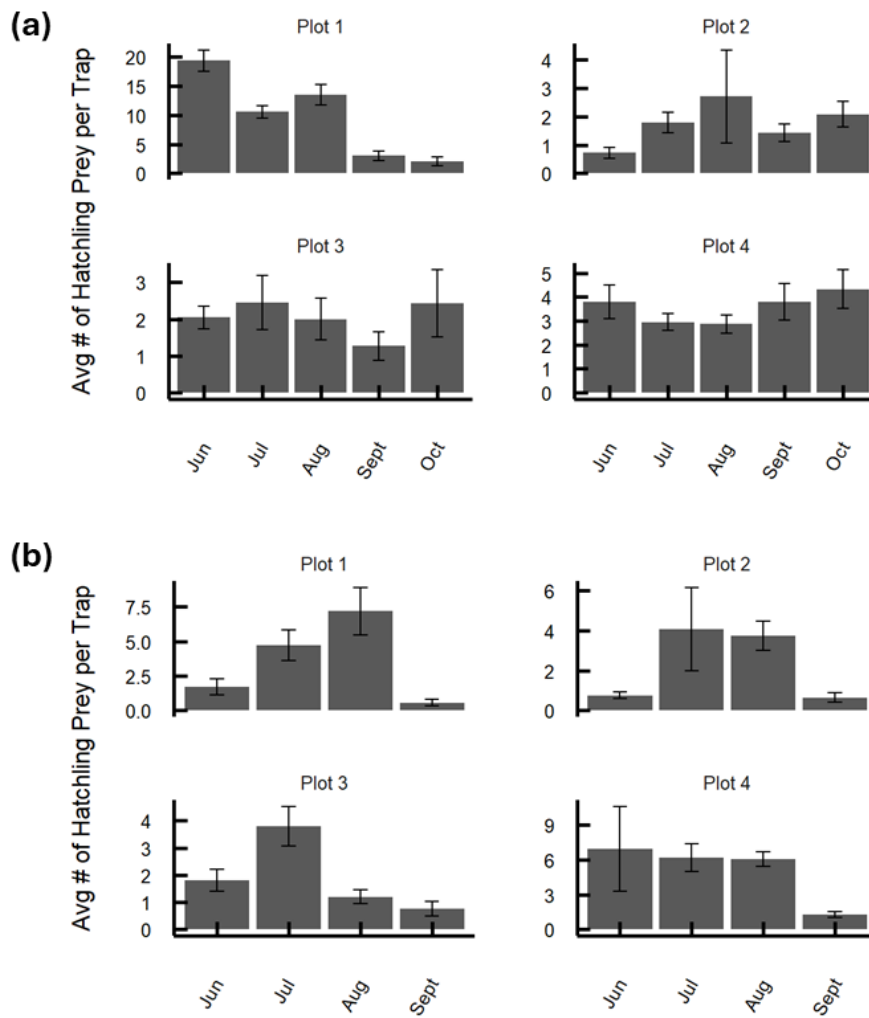
As red imported fire ants continue their range expansion, it is inevitable that more wildlife, including herpetofauna, will experience direct or indirect competition from them. Our study highlights one potential way to mitigate RIFA populations in the wake of this invasion. Our results support our hypothesis that our targeted poisoning method reduces RIFA abundance. While impacts on hatchling prey are not fully understood, it is predicted that targeted poisoning will have less of an impact on native invertebrates than broadcast baiting, especially in areas with low ambient levels of RIFA. We hope that implementation of this method will increase survivorship of herpetofauna in early life stages and increase nest success by reducing RIFA competition.

## APPENDICIES

Appendix I. Average number of RIFA per pitfall trap at each plot. Error bars represent standard error. Panels indicate average RIFA abundance per pitfall trap for 2022 (panel a; n = 13-25) and 2023 (panel b; n = 24-25). In 2022 (panel a), plots 3 and 4 were untreated while plots 1 and 2 were treated. In 2023 (panel b), plot 4 was untreated while plots 1, 2, and 3 were treated.



Appendix II. Average number of hatchling prey per pitfall trap at each plot. Error bars represent standard error. Panels indicate average hatchling prey abundance per pitfall trap for 2022 (panel a; n = 13-25) and 2023 (panel b; n = 24-25). In 2022 (panel a), plots 3 and 4 were untreated while plots 1 and 2 were treated. In 2023 (panel b), plot 4 was untreated while plots 1, 2, and 3 were treated.



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## ABSTRACT

### USING TARGETED POISONING OF RED IMPORTED FIRE ANTS TO IMPROVE TEXAS HORNED LIZARD HABITAT

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The spread of red imported fire ants (*Solenopsis invicta*; RIFA) is often cited as a factor contributing to the decline of the Texas horned lizard (*Phrynosoma cornutum*; THL). Many studies have attempted broadcast poison application to eradicate RIFA; however, this could have unintended consequences for non-target invertebrates that THL need for food. Using a targeted application method, we sought to reduce RIFA abundance over the summers of 2022 and 2023 at Mason Mountain WMA in central Texas, a locality with an ongoing THL reintroduction program. At treated sites, one teaspoon of ant poison (Amdro<sup>®</sup>) was applied to bait stations with RIFA present thirty minutes after placement. Effects of each targeted poisoning were evaluated using pitfall traps and bait stations. Pitfall trapping was likely a more accurate estimate of ant abundance and revealed that targeted poisoning decreased RIFA abundance for both years and had variable effects on hatchling THL prey abundance.