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Use of Extinguish Plus™ to Reduce Red Imported Fire Ants and Increase Northern Bobwhite Abundance

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USE OF *EXTINGUISH PLUS*™ TO REDUCE RED IMPORTED FIRE ANTS AND INCREASE NORTHERN BOBWHITE ABUNDANCE

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ABSTRACT

Populations of northern bobwhite (*Colinus virginianus*) have been declining throughout Texas since at least the 1970s. The red imported fire ant (RIFA, *Solenopsis invicta*) was introduced from South America and reached Texas by the 1950s. Previous studies have documented the negative effects of RIFA on northern bobwhite populations through both direct predation and indirect reduction of small invertebrates. In 2013, 2014, and 2015, large areas (1,490 ha in 2013 and 2,380 ha in 2014 and 2015) of the 3,744-ha portion of the Attwater Prairie Chicken National Wildlife Refuge (APCNR) in Colorado County, Texas, were aerielly treated with *Extinguish Plus*™, a chemical pesticide and reproductive inhibitor that targets ants. Our study took place on APCNR and our objectives included evaluation of the impacts of RIFA treatment on 1) RIFA abundance, and 2) northern bobwhite nest success and brood survival. We trapped, banded, and radiocollared northern bobwhites in areas treated and not treated with *Extinguish Plus* from May 2014 through August 2016. We also collected RIFA abundance data using baited Petri dishes on areas treated and nontreated areas during these periods. Treatment reduced RIFA presence on the refuge by 73.4% during 2014 and 2015, but did not reduce RIFA presence during 2016 possibly because of displacement of RIFA colonies due to heavy flooding on the refuge. We detected a difference ($\chi^2_1 = 11,009$, $P = 0.0009$) in the number of females sighted with and without broods between treated (19.5% F with broods) and nontreated areas (35.7% F with broods). We also found a difference ($t_{12} = 2.51$, $P = 0.027$) in mean number of chicks per brood sighted within treated (4.7) versus nontreated (9.3) areas for 2014 and 2014; but in 2016, we found no difference ($t_{12} = 0.32$, $P = 0.754$) in mean number of chicks per brood sighted within treated (7.7) and nontreated (8.4) areas, which was possibly due to extensive flooding in the nontreated area in April that destroyed most first nests. Our data suggested treatment with *Extinguish Plus* did not increase northern bobwhite abundance on the APCNR during the 2014, 2015, and 2016 bobwhite nesting seasons. Our results differ from previous studies, possibly because of time since treatment and differences in environmental factors between treated and nontreated areas such as ecological site, vegetation composition, and predator abundance.

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Key words: brood survival, *Colinus virginianus*, northern bobwhite, red imported fire ant, *Solenopsis invicta*

Northern bobwhite (*Colinus virginianus*; hereafter, bobwhite) numbers have been on the decline throughout Texas since at least the 1970s (Bridges et al. 2001). Red imported fire ants (RIFA; *Solenopsis invicta*) were introduced to the United States from South America at

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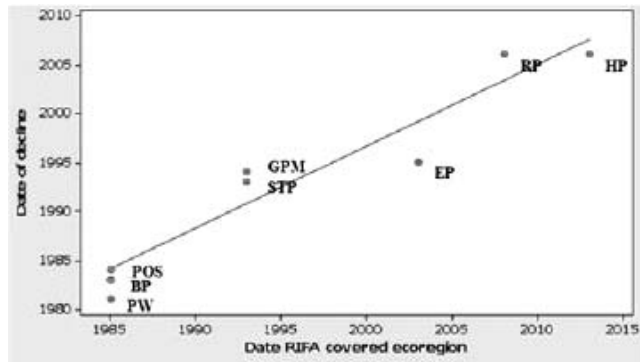


Fig. 1. Correlation of spread of red imported fire ants across ecoregions by date with northern bobwhite declines across ecoregions (PW = Pinyonwoods, BP = Blackland Prairies, POS = Postoak Savannas, STP = South Texas Plains, GPM = Gulf Coast Prairies and Marshes, EP = Edwards Plateau, RP = Rolling Plains, and HP = High Plains) of Texas, USA (from Caldwell 2015).

Mobile, Alabama, around the 1920s (Drees and Vinson 1993). Red imported fire ants began a steady spread through the southern United States, reached Texas in the 1950s, and spanned the state by 2013 (Caldwell 2015). Caldwell (2015) found a direct correlation between the spread of RIFA and the decline of bobwhite in Texas (Fig. 1).

A number of studies have reported that RIFA will prey on young birds, including bobwhites (Drees 1994, Allen et al. 1995, Mueller et al. 1999, Campomizzi et al. 2009). Red imported fire ants are known to kill both hatchling bobwhite chicks and older chicks (Mueller et al. 1999). In experimental trials, captive-reared bobwhites spent time and effort responding to RIFA exposure, which decreased time and effort devoted to other activities, thereby reducing fitness (Pedersen et al. 1996). According to Giuliano et al. (1996) bobwhite chicks experienced reduced survival when exposed to as few as 50 RIFA for 60 seconds compared with chicks not exposed to RIFA. Red imported fire ants are known to negatively impact bobwhites by preying on invertebrates, which are a major food source for bobwhites (Savory 1989, Wojcik et al. 2001). Porter and Savignano (1990) found that arthropods were reduced by 75% following RIFA invasion. If a lack of small insects and other invertebrates exists to feed young bobwhite chicks, malnutrition, dehydration, and death may follow hatching. Morrow et al. (2015) documented the importance of invertebrate abundance to the Attwater's prairie-chicken (*Tympanuchus cupido attwateri*; APC). They concluded that strong invertebrate populations were essential to APC brood survival, and to the long-term recovery of this critically endangered species. They also documented that RIFA had a clear negative impact on invertebrates during their study.

The decline of bobwhites has been concurrent with the westward spread of RIFA (Allen et al. 1995). This correlation may represent a long-term negative impact through direct predation or indirectly through insect reduction. Chemical reduction of RIFA may contribute

to the recovery of bobwhite populations (Allen et al. 1995, Mueller et al. 1999).

Our research hypotheses were the following: 1) treatment of areas with *Extinguish Plus*™ would reduce the abundance of RIFA; 2) bobwhite nest success would be greater in areas treated with *Extinguish Plus*; and 3) bobwhite brood survival would be greater in areas treated with *Extinguish Plus*. If significantly more bobwhite chicks survived to fledgling age and more fledglings survived to adulthood in the treated areas of the refuge than in the nontreated areas, it could be inferred that chemical reduction of RIFA was successful at increasing bobwhite nest success and brood survival.

STUDY AREA

We conducted research on the 3,744-ha portion of the Attwater Prairie Chicken National Wildlife Refuge (APCNWR) located approximately 97 km west of Houston, Texas, in Colorado County. The APCNWR was dedicated to management of the critically endangered APC. The refuge was located on the border between the Gulf Prairies and Marshes and the Post Oak Savannah ecoregions. The refuge was bordered primarily by agricultural fields and properties infested with woody vegetation. Common ecological sites on the refuge included loamy prairie, coarse sand, and claypan prairie (Fig. 2). Little bluestem (*Schizachyrium scoparium*) was the dominant grass in the climax community of the APCNWR, accompanied by a wide diversity of primarily native grasses and forbs. Grasslands were managed for a heterogeneous landscape by burning 16–28-ha patches on a 4-year rotation (Fuhlendorf and Engel 2001, Fuhlendorf et al. 2006). Light to moderate grazing was used at the refuge. Herbicide treatment of individual plants and limited spot-treatment of brush and nonnative plant species occurred on the refuge.

Control of potential APC nest predators also was done on APCNWR. During 2014, 32 feral hogs (*Sus scrofa*), 4 Virginia opossum (*Didelphis virginiana*), 7 raccoons (*Procyon lotor*), and 21 striped skunks (*Mephitis mephitis*) were removed by Wildlife Services from the refuge; and during 2015, 5 feral hogs, 7 Virginia opossum, 5 raccoons, and 18 striped skunks were removed. During 2016, 122 feral hogs, 8 Virginia opossum, 11 raccoons, and 28 striped skunks were removed. Bobcats (*Lynx rufus*) and coyotes (*Canis latrans*) were not controlled on the refuge; however, they are known to prey on northern bobwhite nests (Staller et al. 2005).

In Columbus, Texas (16 km W of the refuge), rainfall totaled 105.2 cm in 2014 (Fig. 3), of which 33.6 cm fell in May (National Centers for Environmental Information; www.ncdc.noaa.gov). The highest rainfall recorded in a 24-hour period during May 2014 was 21.1 cm (National Centers for Environmental Information; www.ncdc.noaa.gov). During January–June 2015, 107.3 cm of rainfall was recorded (Fig. 4). The highest rainfall recorded in a 24-hour period (28 cm) during 2015 occurred when Tropical Storm Bill hit the Texas Gulf Coast on 16 June 2015. A

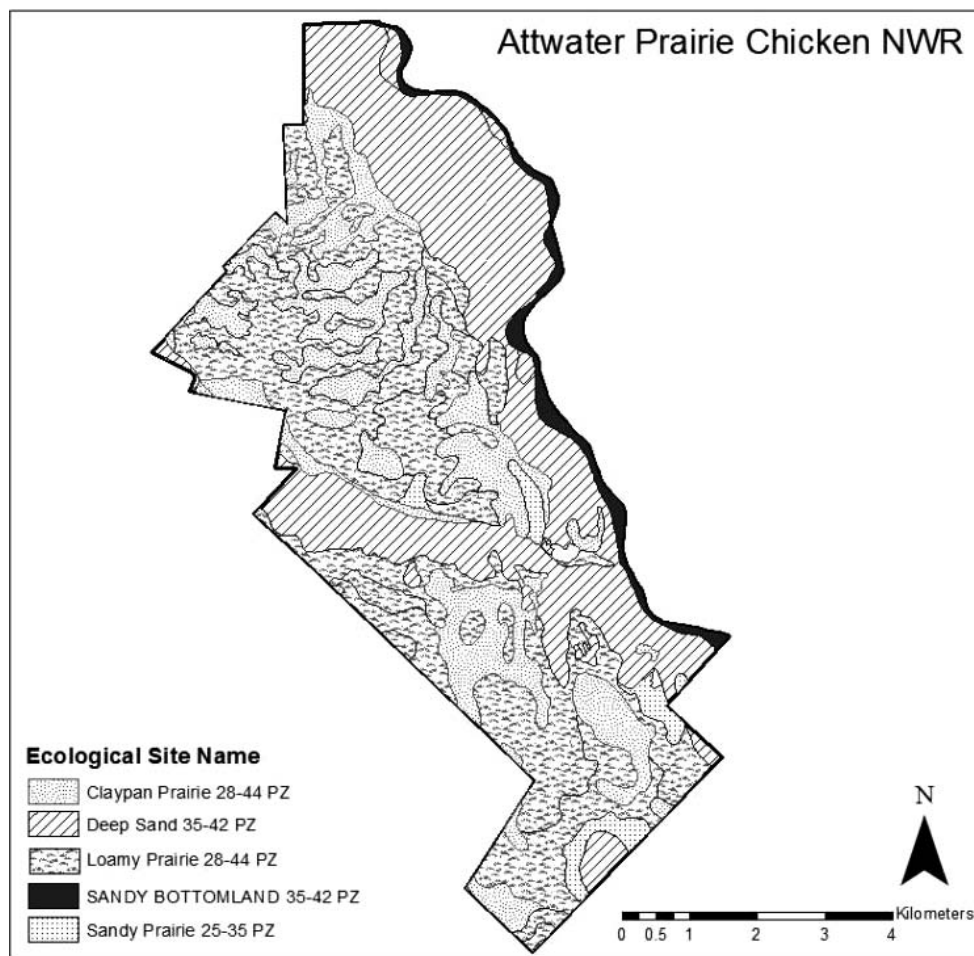


Fig. 2. Ecological sites, Attwater Prairie Chicken National Wildlife Refuge, Colorado County, Texas, USA (<http://sdmdataaccess.nrcs.usda.gov/>).

typical year's amount of rainfall had occurred by the end of June 2015 and below-average rain then was received in August 2015 (Fig. 4). From January to August 2016, 109.4 cm of rain was received at the refuge (U.S. Climate Data; www.usclimatedata.com). The greatest rainfall recorded in a 24-hour period (24 cm) during 2016 occurred on 18 April; however, upstream from Couchatta Creek (which ran through the refuge) and the San Bernard River (the east boundary of the Colorado County portion of the refuge) up to 41 cm of rainfall was recorded on 18 April 2016, which caused major flooding of the refuge (Fig. 5).

In October 2013, in September–October 2014, and again in October 2015, portions of the APCNWR were aerially treated with *Extinguish Plus* (Wellmark International, Schaumburg, IL, USA; Fig. 6), a chemical pesticide and reproductive inhibitor that targets ants. *Extinguish Plus*™, approved for rangelands in 2007, contains both an adulticide (Hydramethylnon) and an insect growth regulator (S-Methoprene), which allows for the sterilization of queens and the killing of worker ants ([Extinguishfireants.com](http://www.extinguishfireants.com); <http://www.extinguishfireants.com/products.php?type=nursery>). According to the manufacturer's website, *Extinguish Plus* is known to be toxic only to ants and fish. The bait is taken up quickly (within

<2 hr) by ants (Calixto et al. 2007), is slow to act, and requires 3–6 months to take full effect when applied in the autumn (Calixto et al. 2007, Nester 2013). Although all ants are susceptible to this product, fire ants dominate bait products such as *Extinguish Plus* because of their aggressive foraging behavior (Calixto et al. 2007). According to Nester (2013), full effect of the product varies with reinvasion pressure; however, 1 application/year is usually sufficient. *Extinguish Plus* was applied at 1.7 kg/ha (1.5 lbs/ac) to 1,490 ha (40% of area in 2013) and 2,380 ha (64% of area in 2014 and 2015) of the 3,744 ha-portion of the refuge in Colorado County. Although the treatment was applied by the U.S. Fish and Wildlife Service to promote APC recruitment, it allowed us an opportunity to determine the effects of large-scale chemical treatment of fire ants on bobwhite abundance, nest success, and brood survival as well as food invertebrate abundance. The effects of such large-scale treatment of RIFA on bobwhites and their food invertebrates have not been studied extensively. The purpose of our research was to determine whether large-scale RIFA treatment is an effective method of increasing bobwhite abundance.

We conducted research on treated and nontreated areas of APCNWR. Locations for treatment were selected

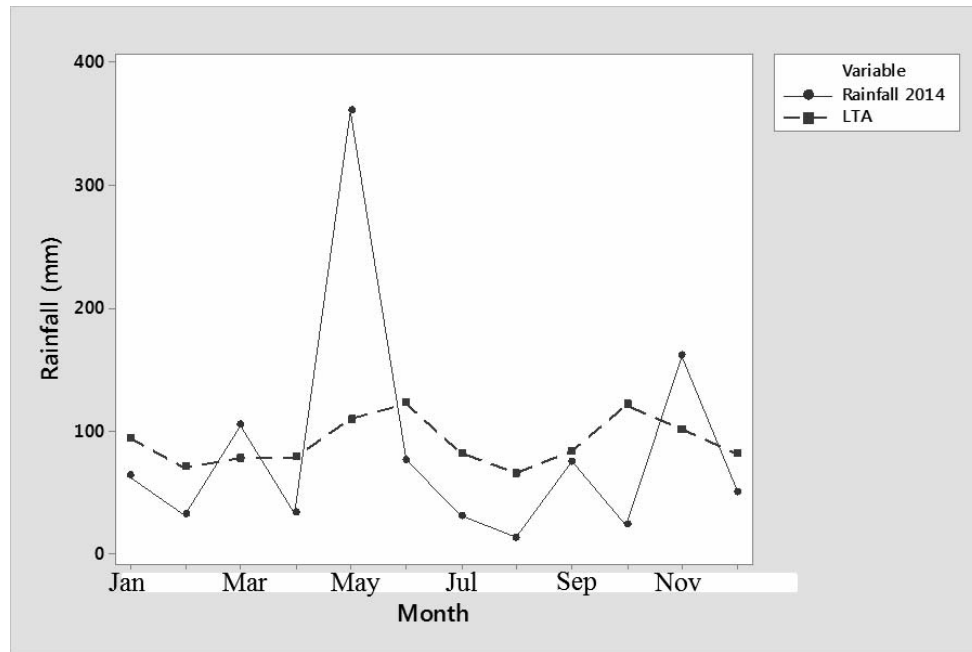


Fig. 3. Rainfall by month in 2014 and 30-year average (LTA) at Columbus, Texas, 16 km west of Attwater Prairie Chicken National Wildlife Refuge in Colorado County, Texas, USA.

by U.S. Fish and Wildlife Service personnel to maximize benefit to APC. This resulted in a number of environmental differences or biases between treated and nontreated areas of the refuge including ecological sites, vegetation composition, rainfall, and predator abundance. For example, in 2014 treated areas consisted largely of claypan prairie and loamy prairie ecological sites, while 4 pastures in the nontreated areas contained large amounts of coarse sand and corresponding vegetation. However, in

2015, 2 of the coarse sand areas were treated for RIFA leaving only 2 others in the nontreated. In addition, 3 pastures in the nontreated area and 1 pasture in the treated area had been leveled and were former rice fields under restoration to prairie plant communities. These leveled pastures were more likely to retain water from rainfall longer and were in generally lower successional states than other nonleveled pastures. Differences such as these resulted in a level of bias that may have influenced RIFA

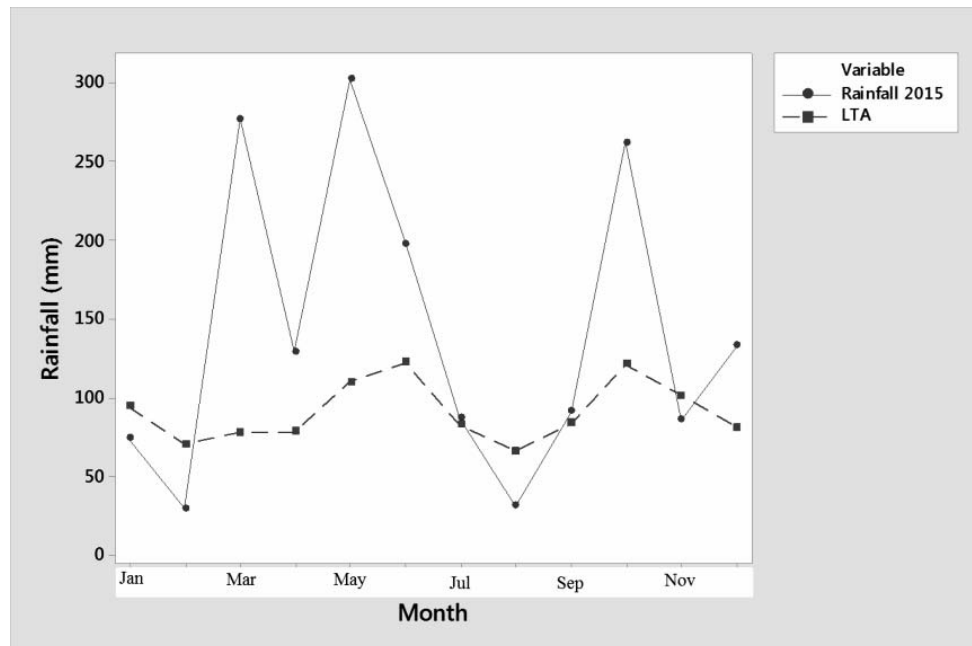


Fig. 4. Rainfall from by month in 2015 and 30-year average (LTA) at Columbus, Texas, 16 km west of Attwater Prairie Chicken National Wildlife Refuge in Colorado County, Texas, USA.

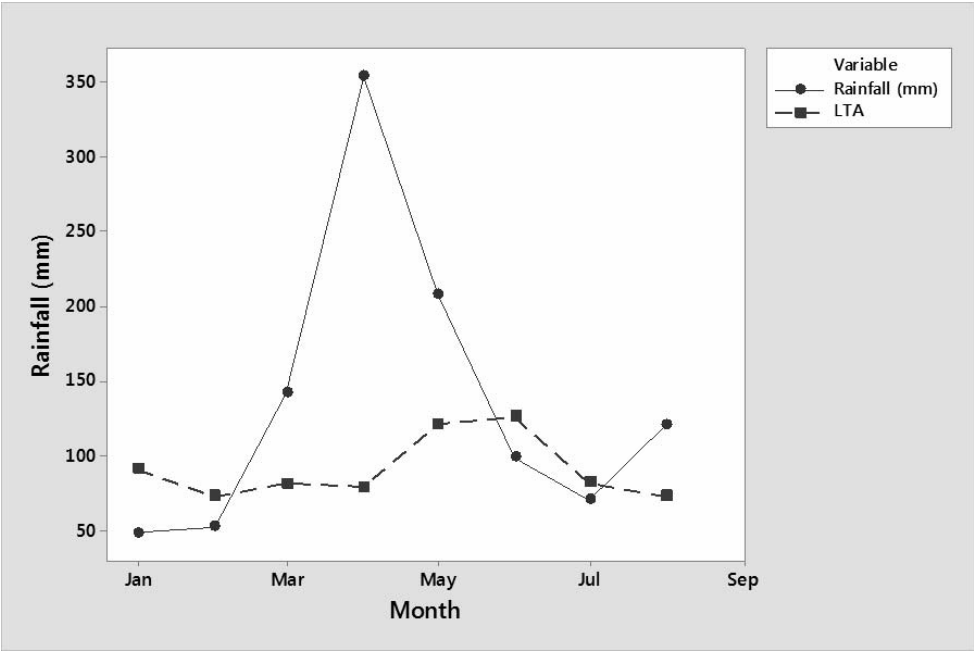


Fig. 5. Rainfall from by month in 2016 and 30 year average (LTA) at Columbus, Texas, 16 km west of Attwater Prairie Chicken National Wildlife Refuge in Colorado County, Texas, USA.

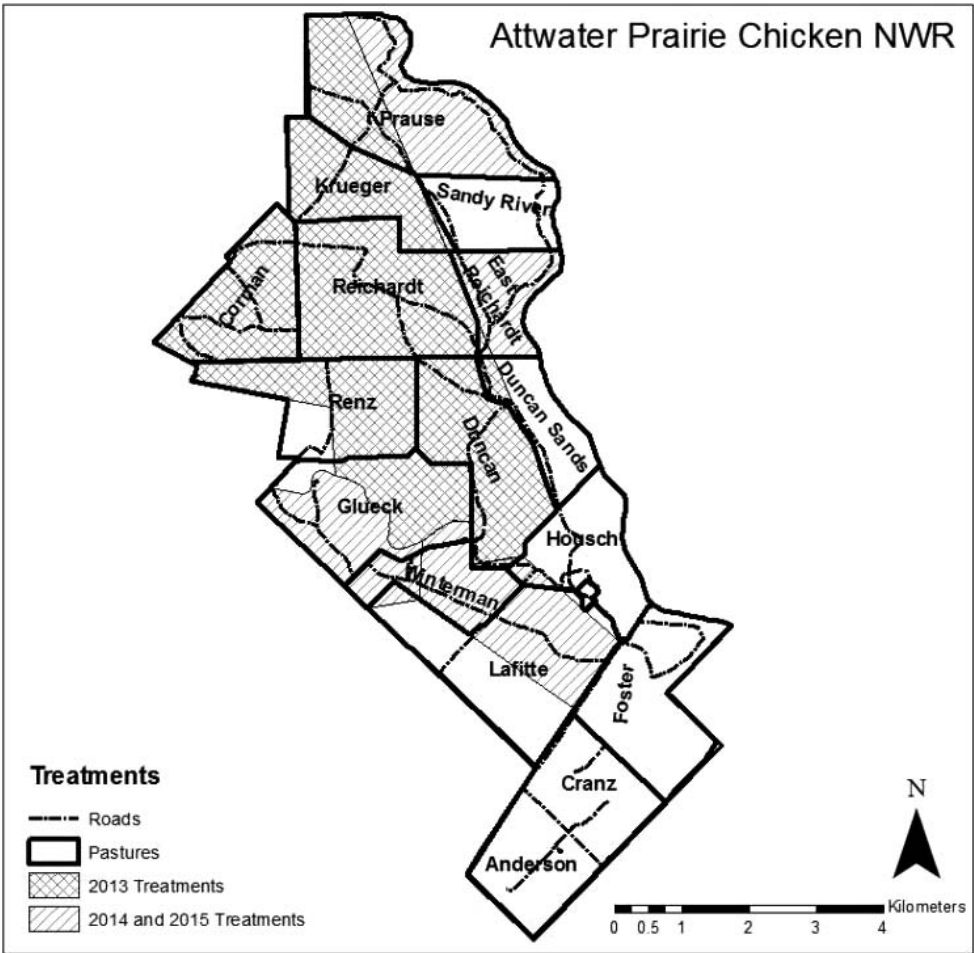


Fig. 6. Areas of Attwater Prairie Chicken National Wildlife Refuge (Colorado County, TX, USA) treated with *Extinguish Plus™* in 2013, 2014, and 2015 to control for red imported fire ants (map provided by Refuge personnel).

or bobwhite abundance in treated and nontreated areas of APCNWR during our study.

METHODS

Fire Ant Abundance

We estimated relative abundance of red imported fire ants once monthly during May–August 2014 and April–August 2015 and 2016 during the bobwhite nesting seasons at 26 locations randomized each month across the treated and nontreated areas of the refuge. We placed 2 Petri dishes baited with hot dog slices (commonly used for RIFA sampling; Morrow et al. 2015) within 3 m at each site and left them exposed to surrounding ants for 20 minutes. We collected dishes, sealed them with tape to capture any ants inside, and then froze them. We later keyed ants to species (Cook et al. 2014) and counted individuals of each species. We compared total numbers of RIFA collected for both the treated and nontreated areas of the refuge to determine an estimated percent reduction of RIFA following treatment. We used Chi-square tests to determine whether there were differences in the number of ant samples with RIFA between treated and nontreated areas (Ott and Longnecker 2008).

Trapping and Marking

Beginning in April 2014, the start of the bobwhite nesting season and 6 months after treatment with *Extinguish Plus*, we trapped bobwhites according to Texas A&M University Animal Use Permit (AUP) Institutional Animal Care and Use protocol 2014-0012 using funnel traps (Kuvlesky et al. 1989). We selected trap locations based on the following criteria: near heavy escape cover, visible to the investigators from a vehicle on refuge roads, relatively hidden from public view, and near epicenters of audible bobwhite mating calls or bobwhite sightings.

We prebaited potential trap locations weekly with commercially purchased grain mixes that included cracked corn, milo, sunflower, millet, and wheat seeds. Prebaiting continued until final trap locations were selected based on grain disappearance week-to-week. In 2014, we placed bait stations along 13.0 km of roads in both treated and nontreated areas. However, in 2015 and 2016, we placed bait stations along 15.1 km of roads in the treated area and along 10.2 km of roads in the nontreated area.

Once we chose promising locations and following ≥ 1 week of prebaiting, we began trapping. Twice-weekly trips to the refuge consisted of baiting all chosen sites, placing a funnel trap at each site, and checking all sites hourly for trapped bobwhites. We aged bobwhites by primary covert color, sexed each by head color (Lyons et al. 2012), weighed each, banded each with a size-7, blue colored band (National Band and Tag Company, Newport, KY, USA) on the right leg, and fitted each with an 8.0-g ($\sim 4\%$ body wt) neckless-style radiotransmitter (150 MHz; Wildlife Materials, Carbondale, IL, USA). We fitted approximately half of the available radiotransmitters

on females in the treated area, and approximately half on females in the nontreated area.

Bobwhite Relative Abundance

We used mark–recapture methods to calculate estimates of adult bobwhite relative abundance near our traps in 2014, 2016, and 2016 (Pierce et al. 2012). We used a modified Schnabel method using only known (recaptured or observed after each estimate) adult birds alive at the time of each estimate as the total number marked to obtain conservative relative abundance estimates of bobwhite using our trap sites during June of each year (Silvy et al. 1977).

Nest Success and Brood Survival

Initially, we were going to determine nest success. However, during 2014, we made no attempts to locate active nests because Mueller et al. (1999) observed high abandonment rates (7% [6 of 79] in 1997 and 15% [13 of 87] in 1998) while attempting to locate active nests. Additionally, we had fitted only 5 females with radiotransmitters by 1 June 2014, which provided a very small sample size of potentially nesting females. However, in 2015 we made attempts to locate all female bobwhites with radiotransmitters (by triangulation) twice weekly. We located females suspected of nesting by using a handheld 3-element yagi antenna (Wildlife Materials). We did not attempt to flush a setting female off her nest, but if a female did flush, we noted the number of eggs within the nest. We determined a Global Positioning System location for each nest so we could locate it later. We checked females on confirmed nests (by triangulation) 3–4 times/week during midday (assuming the female would not be off-nest feeding during the heat of the day) for signs they had left their nests. If the female was off the nest, we checked to determine whether the nest had hatched or been lost to predation.

To estimate brood survival in treated and nontreated areas without influencing brood survival by flushing radiotagged female s with broods during 2014, we recorded all females sighted or trapped with and without broods in treated and nontreated areas and recorded the number of chicks per brood. We collected these data once we sighted the first brood on 10 June 2014 and continued until 31 August 2014. During 2015 and 2016, we first observed broods in June and again collected data until 31 August of each year. We sighted broods while driving refuge roads while collecting data for other aspects of this study, but we made most sightings while trapping (trapping conducted a min. of 2 days/week from Mar through Aug each year). To avoid pseudoreplication, we only used the first observation of a female with a brood for each of the 16 pastures (Fig. 6) of APCNWR because radiotagged females with broods had small (11.5 ha, SD = 5.24 ha) monthly ranges and we never observed them to move between pastures. However, different broods within a given pasture could be determined by location, chick size, or date of sighting thereby providing data on different brood sightings within a given pasture over

Table 1. Total ants and total red imported fire ants (RIFA; sample size in parentheses) collected in each month's sampling by treatment versus nontreatment on Attwater Prairie Chicken National Wildlife Refuge, Colorado County, Texas, USA, 2014–2016.

Month and year	Treated total (n)	Nontreated total (n)	Treated RIFA (n)	Nontreated RIFA (n)
May 2014	130 (26)	1,924 (26)	1 (26)	1,054 (26)
Jun 2014	320 (42)	2,441 (36)	134 (42)	2,318 (36)
Jul 2014	1,257 (33)	2,613 (25)	906 (33)	2,379 (25)
Aug 2014	2,418 (24)	1,085 (28)	274 (24)	208 (28)
Apr 2015	292 (26)	998 (26)	5 (26)	768 (26)
May 2015	211 (32)	410 (20)	103 (32)	58 (20)
Jun 2015	826 (34)	322 (18)	313 (34)	64 (18)
Jul 2015	128 (26)	569 (26)	95 (26)	231 (26)
Aug 2015	1,157 (26)	446 (26)	104 (26)	182 (26)
Total 2014–2015	6,739 (269)	10,808 (231)	1,935 (269)	7,262 (231)
Apr 2016	454 (26)	530 (26)	299 (26)	158 (26)
May 2016	138 (26)	766 (26)	64 (26)	286 (26)
Jun 2016	517 (26)	157 (26)	280 (26)	65 (26)
Jul 2016	246 (26)	166 (26)	169 (26)	12 (26)
Aug 2016	3,971 (26)	1,254 (26)	2,256 (26)	1,151 (26)
Total 2016	5,326 (130)	2,873 (130)	3,068 (130)	1,672 (130)
Grand total	12,065 (399)	13,681 (361)	5,003 (399)	8,934 (361)

time. For example, if we sighted a female with bumble bee–size chicks (1–3 days after hatch) in a given area and the next day we sighted a female with flying-size chicks (>7 days after hatch) in the same area, we counted this as 2 females with broods in the area.

To determine the number of females without broods, we used our relative abundance estimates of females in treated and nontreated areas as total females available for these areas. To obtain an estimate of females without broods, we subtracted the number of females with broods from the total number of females available from our relative abundance estimates. We used a Chi-square goodness-of-fit test to determine differences in number of females with and without broods located in treated and nontreated areas. We used a 2-sample *t*-test to determine differences in the mean number of chicks per brood between treated and nontreated areas.

RESULTS

Fire Ant Abundance

Across the project's first season (May–Aug 2014), 1,315 RIFA were found in 17 (13.6%) of 125 samples from areas treated with *Extinguish Plus* and 5,959 RIFA were found in 37 (32.2%) of 115 samples from areas not treated (Table 1). During the project's second season (Apr–Aug 2015), 620 RIFA were found in 20 (13.9%) of 144 samples in treated areas and 1,303 RIFA were found in 31 (26.7%) of 116 samples in nontreated areas. During April–August 2016, 3,068 RIFA were found in 36 (27.7%) of 130 samples in treated areas and 1,672 RIFA were found in 42 (32.3%) of 130 samples in nontreated areas (Table 1). Overall, 5,003 RIFA were collected in 73 (18.3%) of 399 samples from treated areas and 8,934 RIFA were collected in 110 (30.5%) of 361 samples from nontreated areas (Table 1). Lower numbers of samples with RIFA were found in treated areas in 2014 ($\chi^2_1 =$

11.850, $P < 0.001$), 2015 ($\chi^2_1 = 6.713$, $P < 0.01$), and overall ($\chi^2_1 = 15,368$, $P < 0.00009$), but not for 2016 ($\chi^2_1 = 0.6593$, $P = 0.6593$). Other ant species collected at bait sites included crazy ants (*Nylanderia teretica*), leaf cutter ants (*Atta* or *Acromyrmex* spp.), pyramid ants (*Dorymyrmex pyramicus*), and harvester ants (*Pogonomyrmex* spp.).

Trapping and Marking

In 2014, 11 traps were established in the nontreated area and 16 traps in the treated area; and in 2015 and 2016, 12 traps were established in nontreated area and 20 traps were established in treated areas. Because 4 trap sites that were in nontreated areas in 2014 were treated in 2015 and 2016, they subsequently became treated trap sites. In 2015 and 2016, 5 additional trapping sites were added to the nontreated areas to increase the sample size in the nontreated areas. From March 2014 through August 2016, 433 bobwhites (205 M, 136 F, and 92 chicks too young to be sexed) were trapped, banded, and released at trap site. Over the course of the project, 437 recaptures took place. During this time, 124 bobwhites were recaptured up to 3 times and 84 were recaptured ≥ 4 times. One bobwhite male was recaptured 8 times.

During 2014, 68 bobwhites (41 M and 27 F) were radiotagged. Of the 27 radiotagged females, 14 were in the treated area and 13 in the nontreated areas; however, only 5 females were radiotagged before 1 June 2014. In 2015, 43 bobwhites (all F) were radiotagged (28 in the treated area and 15 in nontreated area); and in 2016, 31 females (21 in the treated area and 10 in the nontreated area) were radiotagged.

Bobwhite Relative Abundance

June 2014 had an estimated bobwhite relative abundance of 83 (95% CI = 71–95) bobwhites (54 treated and 29 nontreated), June 2015 had an estimated relative

abundance of 82 (95% CI = 64–100) bobwhites (49 treated and 33 nontreated), and June 2016 had an estimated relative abundance of 87 (95% CI = 47–108) bobwhites (60 treated and 27 nontreated) in the areas influenced by our traps. No banded or radiotagged bobwhites captured in the treated areas were recaptured in or observed to move into a nontreated area. The same held for birds captured in nontreated areas.

Nests Success and Brood Survival

Only 1 nest was found (incidentally) during 2014 and all eggs were predated shortly after the nest's discovery in a treated area. During 2015, 17 nests were located and only 1 nest hatched (in a nontreated area), 3 nests were destroyed as a result of flooding (nontreated area), and 13 were predated (7 in treated area and 6 in nontreated area). During 2016, 12 nests (9 in treated area and 3 in nontreated area) were located. Only 1 (33%) nest hatched (2 females were killed on the nest) in the nontreated area and 6 (67%) nests hatched in the treated area. In 2016, 2 females in the treated area nested twice (1 female lost her first nest and was successful with the second nest, but she was then killed shortly after leaving her second nest; the second female was successful with both nests, but was killed shortly after leaving her second nest).

Pooled data from 2014, 2015, and 2016 demonstrated a difference ($\chi^2_1 = 11,009$, $P = 0.0009$) in the number of females sighted with broods versus without broods between nontreated ($n = 56$ females, $n = 20$ females with broods) and treated areas ($n = 77$ females, $n = 15$ females with broods). During 2014 and 2015, the treated area yielded a mean of 6.1 chicks/brood sighted compared with 8.9 chicks/brood in nontreated areas. Nontreated areas had a larger ($t_{12} = 2.51$, $P = 0.027$) mean brood size, which is a trend opposite that which we had hypothesized. However in 2016, the mean brood size for treated (7.7) and nontreated (8.4) areas was nonsignificant ($t_{12} = 0.32$, $P = 0.754$). These data suggest more quail chicks were produced per adult female in nontreated areas. Further support of this was the fact that more ($n = 28$) hatch-year chicks were trapped in 11 traps in the nontreated site traps than hatch-year chicks ($n = 23$) were trapped in the 16 treated site traps in 2014. Trapping was discontinued on 31 July 2015 prior to any hatch-year chicks being captured because the initial 2 years of funding had ended and a final report was due on 31 August 2015. In 2015, 1 radiotagged female in the non-treated area produced a brood with 10 chicks. This female still had chicks when the study ended on 31 August 2015. However, trapping results (Jun–Aug) in 2016 also indicated there were more hatch-year chicks produced in the nontreated area (34 chicks trapped in 15 traps) than in the treated area (19 chicks trapped in 16 traps).

DISCUSSION

Fire Ant Abundance

In 2014 and 2015, with a few exceptions, monthly samples within the nontreated areas contained more RIFA

than treated areas during both collection seasons. These data suggest that treatment with *Extinguish Plus* was successful in reducing RIFA on treated areas during the 2014 and 2015 bobwhite nesting seasons. This result is consistent with that of a similar study on the APCNWR by Morrow et al. (2015), which concluded that treatment significantly reduced RIFA. Our result also is consistent with that of Aubuchon et al. (2006), who found decreased abundance of RIFA on 2 different 8-ha grazed pastures in Alabama that had been treated with *Extinguish Plus*—an earlier form of *Extinguish Plus* containing methoprene, but not hydramethylnon. Mitchell and Knutson (2004), as well, found RIFA presence in 2 peanut fields reduced by 85–98% after treatment with *Extinguish*.

However in 2016, except for the May collection, we found more RIFA in the treated area than the nontreated area. This was probably the result of creek and river flooding during 17–18 April 2016 when much of the nontreated area was flooded, which caused RIFA colonies to raft and float out of the area. In addition, river flooding along the eastern boundary of the treated area probably brought RIFA colonies into the treated area from areas north of the refuge. If a colony is flooded during a rainstorm or other high-water situation, the ants cling together and form a living raft that floats on the flood waters (Adams et al. 2011). Once the raft hits dry ground or a tree, rock, or other dry object, the ants can leave the water (Adams et al. 2011).

Bobwhite Relative Abundance

Schnabel estimates of adult bobwhite relative abundance provided an approximate number of individuals within the vicinity of our bait sites. Adult bobwhite estimates were larger in treated sites (66% of total in Jun 2014, 60% in Jun 2015, and 70% in Jun 2016) than nontreated sites. However, the area influenced by our traps was less in the nontreated site because fewer traps were used in nontreated area than treated areas during all 3 years of our study.

Another estimate of bobwhite relative abundance is catch per trap site. The mean number of adult bobwhites captured per trap site was greater in nontreated (2014 = 7.5, 2015 = 4.5, and 2016 = 10.5) than treated (2014 = 5.6, 2015 = 2.9, and 2016 = 8.3) areas during both years, suggesting that bobwhite relative abundance was greater in nontreated areas during both years. The fewer number of quail captured per trap site in 2015 was because data were collected from March to July, whereas in 2014 and 2016 data were collected from March to August. Allen et al. (1995) found, where bobwhite densities (call counts) were monitored for 2 years after treatment on 5 treated and 5 nontreated areas, that only in the second year after treatment were autumn bobwhite densities greater ($P = 0.028$) on treated areas.

Nest Success and Brood Survival

Data on bobwhite nest success (only 8 successful nests in 3 yr) were limited to the point that we could not determine whether treatment influenced nest success.

However, Mueller et al. (1999) found no difference in bobwhite nest success between areas treated for RIFA with *Amdro*™ (AMBRANDS, Atlanta, GA, USA) and areas not treated.

Extreme rainfall in May 2014 (Fig. 3), 2015 (Fig. 4), and 2016 (Fig. 5), which led to major flooding on the refuge, probably destroyed most first nests attempted by female bobwhites. Flooding was extensive in 2015 because heavy rainfall occurred from April to June. On 16 June 2015, Tropical Storm Bill dumped >28 cm of rainfall on APCNR. Several bobwhite and APC nests were flooded and 2 female bobwhites and some APC females died due to the flooding. After the June flooding event, second bobwhite nesting attempts were usually placed on small sandy mounds surrounded by water, which led to smaller search areas for predators. This in turn led to high rates of nest depredation. In 2015, only 1 of our radiotagged bobwhite nests hatched and it was placed on the edge of a raised gravel road in the nontreated area of the refuge.

The heavy rains during the nesting seasons of 2014, 2015, and 2016 likely flooded (Fig. 7) many first nesting attempts by bobwhites; however, of nests we observed, the 3 flooded nests in 2015 were in the nontreated area. In 2014, much of the nontreated areas consisted of a coarse-sand ecological site and much of the treated areas consisted of claypan prairie and loamy prairie. However, this was not the case in 2015 and 2016 when the treated area was expanded to include more of the coarse-sand ecological sites of the refuge. It is possible that during May 2014, sandy soils in the nontreated site allowed for better drainage of rainfall and, thus, better first-nest survival, which typically results in larger broods than subsequent nesting attempts (N. Silvy, Texas A&M University, unpublished data). The differential flooding of the nontreated site in 2016 probably eliminated all first nests in the nontreated area; however, this was not the case in the treated site where we found 2 nests with 18 and 17, respectively (date and clutch size indicating first nests). Both nests located in the nontreated site contained 11 and 9 eggs, respectively, indicating a second nesting attempt. Heavy rainfall during the flood in April 2016 also affected first nests in the treated area as most nests located in the treated area contained 7–13 eggs, indicating they were second or third nesting attempts. Also in 2016, we observed 1 female that died in the treated area due to flooding of a low area by the heavy rainfall. In addition, we saw fewer females with broods in treated areas.

During our study, we found more bobwhite chicks survived to fledgling age per female in the nontreated area of the refuge. These results are not consistent with those of Mueller et al. (1999), who observed bobwhite brood survival to 3 weeks was greater for broods that hatched in treated areas. Morrow et al. (2015) concluded that strong invertebrate populations were essential to APC brood survival, and to the long-term recovery of this critically endangered species. Also, Morrow et al. (2015) observed that APC broods on APCNWR located in areas with the greatest median invertebrate count (338 invertebrates/25 sweeps) had a survival probability of 0.83 at 2 weeks posthatch compared with 0.07 for broods located in areas

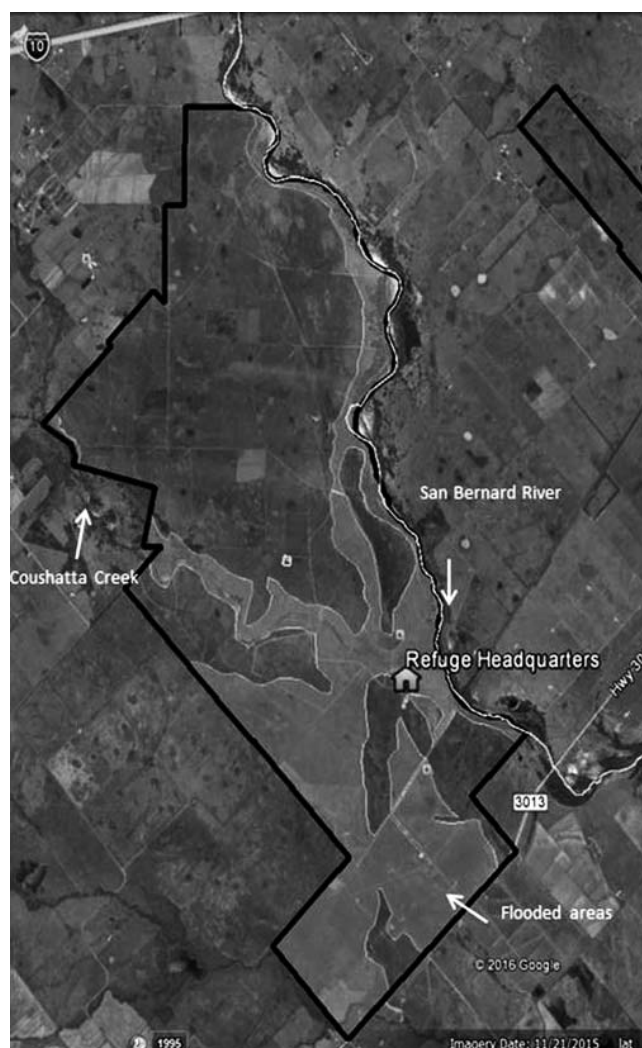


Fig. 7. Areas of Attwater Prairie Chicken National Wildlife Refuge, Colorado County, Texas, USA, flooded by heavy rains on 18 April 2016. (Map generated by J. Magera, Deputy Refuge Manager, Attwater Prairie Chicken National Wildlife Refuge, based on his personal observations of the flooding).

with the smallest median invertebrate count (18 invertebrates/25 sweeps). Allen et al. (2001) also noted that loggerhead shrike (*Lanius ludovicianus*) and native insect abundance were reduced in areas associated with RIFA. It is possible that our results are related to factors other than those that we researched, such as predator abundance, vegetation succession, or rainfall differences between the treated and nontreated areas of the APCNWR in 2014, 2015, and 2016. Grasslands in the treated area were managed for APCs in a higher successional state with less woody cover than may have been optimal for bobwhites (USFWS 2010). Extreme amounts of rainfall received during all 3 years of our study (Figs. 3, 4, and 5) not only affected bobwhites directly, but also likely adversely affected invertebrates as well, irrespective of RIFA treatment (Uvarov 1977). Given that availability of insects is an important driver of bobwhite chick survival (Savory 1989, Wojcik et al. 2001), these extreme precipitation patterns may have negated any potential

benefits to insect abundance resulting from RIFA suppression. It also is possible that ecological site and associated vegetation composition differences between treated and nontreated areas influenced our results.

CONCLUSIONS AND MANAGEMENT IMPLICATIONS

The bobwhite is an ecologically and economically important game bird species that is experiencing a decline. Although large-scale treatment of ants with *Extinguish Plus* on the APCNWR was initiated as a management action for the critically endangered APC, it also provided us an opportunity to evaluate the effectiveness of large-scale RIFA reduction on bobwhite abundance.

Based on our study, we drew the following conclusions:

1. Treatment with *Extinguish Plus* reduced RIFA relative abundance in 2014 and 2015, but not in 2016.
2. Because of small sample size, we could not conclude whether treatment with *Extinguish Plus* improved bobwhite nest success in 2014, 2015, or 2016.
3. Treatment with *Extinguish Plus* did not improve the percent of female bobwhites with broods or the mean brood size per female.

Based on the results of our research, the use of *Extinguish Plus* to reduce RIFA did not lead to greater bobwhite relative abundance. It is possible that our results are related to factors other than those that we researched, such as predator abundance and vegetation succession differences between the treated and nontreated areas of the APCNWR during our study.

Additional research or a longer term study of the effects of large-scale RIFA treatment on bobwhites would be beneficial to either strengthen or oppose the conclusions of our study. Unlike other proposed remedies to the quail decline (e.g., habitat restoration), aerial RIFA treatment is relatively inexpensive and easy to implement. If such treatment proves effective at increasing bobwhite abundance, these methods will provide wildlife managers a tool that could increase their chances of slowing, stopping, or reversing the quail decline.

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