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NORTHERN BOBWHITE RESPONSE TO CONTROL OF RED IMPORTED FIRE ANTS IN THE GULF COAST PRAIRIE OF TEXAS

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ABSTRACT

The population decline of northern bobwhite (*Colinus virginianus*; hereafter, bobwhite) on the Texas Gulf Coast Prairie, USA is largely attributed to habitat loss. However, red imported fire ants (*Solenopsis invicta*) occur throughout the region and are considered a possible contributing factor to the bobwhite decline. The objectives of our study were to determine the influence of red imported fire ants on bobwhites by comparing bobwhite nest success, survival, and density between sites treated with fire ant bait (treatment) and reference (control) sites. Our study was conducted on 3 private ranches in Goliad and Refugio counties, Texas. Each ranch contained 2 paired experimental units that consisted of a treatment and control site (500 ha each). The treatment sites received an aerial application of fire ant bait (Extinguish® Plus) during April 2018, whereas the control sites were not treated. We estimated mound density by counting fire ant mounds using distance sampling. We used radio-telemetry to monitor bobwhite nest success and survival, and we estimated bobwhite densities using distance sampling via helicopter surveys. Fire ant mound density decreased through time on both treatment and control sites. However, fire ant mound density was lower on treatment sites than control sites, indicating the insecticide was effective at decreasing fire ant mound density. Bobwhite survival, nest success, and density did not statistically differ between control and treated sites either pre-treatment (2017) or post-treatment (2018), but survival and nest success metrics were numerically higher in treated units. Bobwhite survival remained relatively stable in the treatment units 4 weeks after application but decreased in the control units. Following treatment, apparent nest success in the treated units increased by 37.4% while nest success in the control units decreased by 35.2%. Bobwhite populations were low in this ecoregion, which influenced our ability to trap and monitor many bobwhites or monitor many nests. In addition, it may be possible that repeated, annual treatments for fire ants are necessary for a benefit to accrue and be observed in bobwhites. Our results indicate that there may be potential benefits to bobwhites from fire ant reduction that deserve further research attention.

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Key words: *Colinus virginianus*, Extinguish® Plus, hydramethylnon, northern bobwhite, red imported fire ant, *Solenopsis invicta*, Texas

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The northern bobwhite (*Colinus virginianus*; hereafter, bobwhite) is an important gamebird that has declined throughout its geographic distribution (Brennan 1991). Bobwhites are a significant economic and recreational species for hunting, birding, and photography (Johnson et al. 2012). Over the last 30 years, bobwhite populations have exhibited a steady decline, which is of particular concern to property owners, land managers, and sportspeople (Brennan 1991, Johnson et al. 2012). Although bobwhite populations in some regions of Texas, USA generally are considered relatively stable (Brennan 2007), the Gulf Coast Prairies and Marshes (hereafter, Gulf Coast Prairie) is an ecoregion of Texas (Gould 1969) where bobwhite populations have experienced population declines (Perez 2007).

There are 2 leading hypotheses concerning why bobwhites have declined in the Gulf Coast Prairie: habitat loss, and impacts from the nonnative red imported fire ant (*Solenopsis invicta*; hereafter, fire ant). Most ecologists and managers agree that the most significant reason for the decline of bobwhites has been the loss, degradation, and fragmentation of their habitat (Perez 2007, Hernández et al. 2013). In the Gulf Coast Prairie, 3.6 million ha have been lost to development from presettlement times to 1999, while the remaining land has succumbed to increasingly intensified agriculture and nonnative plant invasions (Lehmann 1941, Allain et al. 1999). Although habitat loss may be the ultimate factor, it is possible that invasion by fire ants could be a contributing factor in the population declines given that this ant is widespread throughout the region and known to cause bobwhite mortality (Allen et al. 1993, 1995, 2000).

Fire ants were accidentally introduced from South America to Mobile, Alabama, USA in the 1930s and have successfully invaded one-quarter of the U.S. mainland (Vinson and Sorenson 1986). When fire ants invade an area, these ants have the potential to cause both direct and indirect impacts on avian species (Allen et al. 1994, 1995). For example, depredation by fire ants can be a source of direct nest loss for ground- and shrub-nesting birds (Mueller et al. 1999, Allen et al. 2004). Nests are especially vulnerable to fire ants when chicks start to pip out of their shells (Johnson 1961, Mitchell 1989). If chicks are not stung to the point of mortality, fire ants can affect body mass and overall health of the bird. Giuliano et al. (1996) documented that chick body mass was negatively affected when the birds were exposed to 200 ants for 60 seconds. Mueller et al. (1999) linked increased fire ant activity in the nest to decreased probability of bobwhite chick survival to 21 days. In addition, several studies have documented adult bobwhite disturbance (e.g., ant bites) and mortality as a result of fire ants (Travis 1938, Johnson 1961, Dewberry 1962).

Fire ants also can have indirect effects on bobwhites (Vinson 2013). Both bobwhites and fire ants consume insects as a food source throughout the year, and insects are especially critical food items during the first few weeks of life for gamebirds (Jones 1963, Hurst 1972, Rumble et al. 1988, Savory 1989, Hagen et al. 2005). Several studies have demonstrated the ability of fire ants to displace and outcompete other invertebrates (Glancey et al. 1976, Hooper 1976, Burns and Melancon 1977, Morril 1978, Lopez 1982, Summerlin et al. 1984, Vinson and Scarborough 1991, Porter 1992). Porter and Savignano (1990) documented that native ant species richness was 70% lower and total number of native individuals declined by 90% in fire ant infested areas. Morrow et al. (2015) suggested that the reduction of invertebrate abundance by fire ants negatively affected brood survival of Attwater's prairie-chickens (*Tympanuchus cupido attwateri*), an endangered galliform species whose chicks have similar food habits to those of bobwhite chicks (TPWD 2017*a*). They reported that Attwater's prairie-chicken broods located in areas with higher invertebrate abundance had a greater probability of survival than broods located in areas with lower invertebrate abundance. Probability of brood survival was >2 times higher for broods that spent all of their time in fields treated for fire ants compared to those that spent no time in these areas (Morrow et al. 2015).

Research on the effects of fire ants on bobwhite populations is ambiguous. For example, Allen et al. (2000, 2004) documented that bobwhite abundance decreased following fire ant invasion into an area although these results may have been confounded by the study area habitat, which was dominated by bermudagrass (*Cynodon dactylon*). Johnson (1961) suggested that fire ants did not affect bobwhite production. Brennan et al. (1991) documented a 10-fold increase in bobwhite coveys found per day following 13 years of habitat management on a study site in Mississippi, USA, even though fire ant mound density was high (200/ha). This finding suggests that even in areas infested with fire ants, bobwhite populations can persist and have the potential to increase if habitat is managed.

Despite the number of studies that have been conducted attempting to link declines of upland gamebirds with fire ants, the impact that fire ants can have on galliform populations remains uncertain. There are 2 potential reasons for this. First, studies have occurred during different seasons and employed different methodologies, which may explain why studies fail to yield comparable results (Travis 1938, Johnson 1961, Dewberry 1962, Mitchell 1989, Allen et al. 1995, Giuliano et al. 1996, Pedersen et al. 1996, Mueller et al. 1999, Allen et al. 2000, Morrow et al. 2015). Another reason could be that the effects of fire ants on bobwhites could differ by scale of observation. For example, at the individual (point-of-use or point-in-time) scale, bobwhites (especially chicks) may be negatively affected, particularly when in close proximity to fire ants (Mueller et al. 1999). At the population (pasturewide) scale, however, the negative effects experienced by individuals may be canceled given the compensatory nature of this *r*-selected species. Such scale-dependent phenomena (i.e., detrimental effects at the individual scale but neutral effects at the population scale) have been documented regarding bobwhite survival and nest success. For example, depredation is the primary cause of nest failure for bobwhites (Stoddard 1931, Newton 1998, Rollins and Carroll 2001) and a major source of mortality at all life stages for bobwhites (Rollins and

Carroll 2001). However, several studies indicate that predator control to increase bobwhite populations has had little to no population effect (Beasom 1974, Guthery and Beasom 1977, Lehmann 1984, Palmer et al. 2005, Rader et al. 2007, Ellis-Felege et al. 2012). One reason why bobwhite populations are able to persist despite such high mortality is their tendency to renest, which increases the likelihood of nest success (Hernández and Peterson 2007). Another reason may be the compensatory nature of mortality in bobwhites. Guthery (2002) suggested that reducing one source of mortality was likely to increase the probability of another, resulting in the cumulative effect of predators even when the depredation sources change spatially and temporally. Thus, although reducing predator numbers could increase survival of a nest or individual, it does not appear to translate into a population-level effect. This same phenomenon also could apply to the relationship between fire ants and bobwhites. Other studies have not examined the effects on bobwhites at both the point-of-use and pasture scales. There is continued controversy among scientists as to whether fire ants are the ultimate cause of the bobwhite decline, particularly in the Gulf Coast Prairie.

The objectives of our study were to 1) determine the effect of fire ant density on nest success and bobwhite survival (point-of-use scale) and 2) document changes in bobwhite density following application of an aerially applied insecticide to reduce fire ant densities (pasture scale). Both sets of results were examined as a test for scale-dependent effects of fire ants on bobwhites. We hypothesized that 1) fire ants would negatively affect nest success and bobwhite survival because fire ants can invade bobwhite nests and cause direct mortality of adults and 2) aerially applied insecticide would reduce fire ant densities, but bobwhite densities would remain unaffected because of the compensatory nature of mortality factors in bobwhite populations.

STUDY AREA

Our study was conducted on 3 spatially independent (≥ 11 km apart) privately owned ranches in the Refugio-Goliad Prairie of southeastern Texas. The Refugio-Goliad Prairie complex was the largest parcel (approximately 105,000 ha) of native midgrass/tallgrass prairie remaining along the coast of the Gulf of Mexico. This area was located in the Gulf Coast Prairies and Marshes ecoregion (TPWD 2017*b*). The ecoregion was nearly level (0–5% slopes: NRCS 2017*a*) and experienced mild winter temperatures (mean: 14º C) and hotter and humid summer temperatures (mean: 28.8º C). Annual rainfall averaged 88.7 cm (Goliad County, 1912–2010; WRCC 2017) and occurred in a bimodal pattern, peaking in April–May and September–October. Historically, this region was maintained by frequent, anthropogenic and natural fires that rendered it nearly devoid of woody vegetation (with the exception of bisecting water courses and mottes). Vast areas were dominated by midgrasses and tallgrasses existing in a matrix of mima and pimple mounds and prairie potholes on Vertisol clays. Eolian sand hills and marshes, with their own unique vegetation assemblages, also dotted the landscape (TPWD 2017*c*). In this system, Attwater’s prairie-chicken and other prairie-obligate and facultative wildlife species thrived (Morrow et al. 1996). However, beginning about 200 years ago, coinciding with settlement by Europeans, this landscape began to change as row-crop farming, overgrazing by livestock, and the suppression of grassland fires altered its vegetation composition (NRCS 2017*a*). Today, much of the Gulf Coast Prairies ecoregion is considerably more wooded, farmed, or overgrazed by cattle (or combination thereof). In contrast to the general condition of the ecoregion, our study ranches still contain parcels of native grassland.

Ranch 1 (3,240 ha) was located in southwestern Goliad County, Texas (N28º31’4.2” W97º30’46.0”; Figure 1). Our study areas on this ranch were dominated by Weesatche sandy clay loam and Goliad sandy clay loam soils, which support clay loam ecological sites (NRCS n.d.). This ranch was dominated by mid-grass and tallgrass herbaceous species, intermixed with frequent post oak (*Quercus stellata*) and live oak (*Q. virginiana*), and various Tamaulipan shrubs. Primary land management activities included the feeding and harvest of white-tailed deer (*Odocoileus virginianus*) and bobwhite, brush management via roller-drum chopper and prescribed fire, and conservative grazing by cattle (5.66 ha/animal unit rotated every 35–40 days).

Ranch 2 (10,117 ha) was located in northern Refugio County, Texas (N28°26’31.7” W97°09’54.3”; Figure 1). Our study areas on this ranch were dominated by Victoria clay and pockets of unclassified sandy soil supporting the blackland ecological site (NRCS 2017*b*). These areas were dominated by mid-grass and tallgrass herbaceous species, intermixed with honey mesquite (*Prosopis glandulosa*) and huisache (*Acacia farnesiana*). Primary land management activities included conservative grazing by cattle (6 ha/animal unit rotated every 30–40 days), the spraying of mesquite and huisache

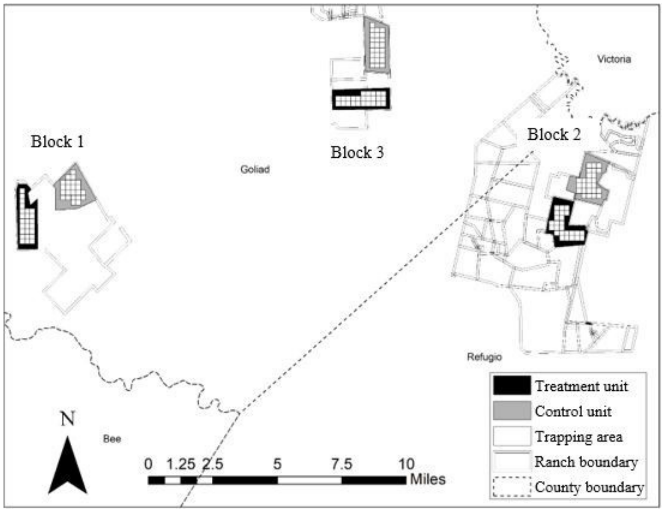

Fig. 1. Locations of ranches (blocks), experimental units, and internal 10-ha sampling cells, Goliad and Refugio counties, Texas, USA, 2017–2018.

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via fixed-winged aircraft and individual plant treatments using GrazonNext® (51.06% 2,4-D triisopropanolamine salt; Corteva Agrisciences™), Tordon™ (20.9% 2,4-D and 5.4% picloram; Corteva Agrisciences), Grazon P+D® (39.6% 2,4-D triisopropanolamine salt and 10.2% picloram; Corteva Agrisciences), MSM 60™ (60% metsulfuron methyl; Alligare, LLC, Opelika, AL, USA), triclopyr, and Sendero™ (30.82% monoethanolamine salt; Corteva Agrisciences) herbicide, and wildlife harvest of bobwhite, white-tailed deer, and wild turkey (*Meleagris gallopavo*).

Ranch 3 (1,780 ha) was located in southern Goliad County, Texas (N 28°35’50.5” W 97°17’04.0”; Figure 1). Our study areas on this ranch were dominated by Greta fine sandy loam, Wyick fine sandy loam, and Vidauri fine sandy loam soils, which support claypan prairie, clay loam, and blackland ecological sites (NRCS n.d.). This ranch was almost exclusively open midgrass prairie (<5% woody cover) with only 6 small mottes (<6 ha in size) of coastal live oak (a distinctly more rhizomatous form of *Quercus virginianus*) scattered about the property. Primary land management activities included conservative grazing by cattle (10.07 ha/animal unit rotated every 30–40 days), relatively frequent prescribed fire (5-year return interval on average), weed spraying in strips using 2, 4-D (2,4-Dichlorophenoxyacetic acid), and brush control using spot application of herbicides such as GrazonNext.

Woody vegetation communities differed on the 3 ranches. Ranch 3 contained the least amount of woody cover, and habitat represented treeless midgrass prairie. Ranch 2 contained greater amounts of woody cover than ranch 3 and represented midgrass/tallgrass/mesquite/huisache savanna. Ranch 1 contained the highest woody cover of the 3 ranches and habitat generally represented mid-grass/tallgrass/post oak savanna.

Herbaceous vegetation communities on all 3 ranches were similar and represented by native, coastal midgrass and tallgrass prairie species. Specifically, these communities were dominated by little bluestem (*Schizachyrium scoparium*), brownseed paspalum (*Paspalum plicatulum*), rosettegrasses (*Dichanthelium* spp.), plains lovegrass (*Eragrostis intermedia*), longtom paspalum (*Paspalum lividum*), longspike threeawn (*Aristida longispica*), yellow indiangrass (*Sorghastrum nutans*), big bluestem (*Andropogon gerardii*), wooly croton (*Croton capitatus*), western ragweed (*Ambrosia psilostachya*), eastern dewberry (*Rubus trivialis*), spike rushes (*Elocharis* spp.), and a gallery of various other grasses, forbs, sedges, and rushes (Gould 1969, NRCS 2017*b*). To a lesser degree, each ranch had monotypic pockets of noxious nonnative grasses such as bahiagrass (*Paspalum notatum*), bermudagrass (*Cynodon dactylon*), Kleberg bluestem (*Dichanthium annulatum*), and Angleton bluestem (*Dichanthium aristatum*). Scientific plant names are standardized according to the U.S. Department of Agriculture’s PLANTS Database (NRCS 2017*b*).

Hurricane Harvey was a destructive weather event that passed over all 3 of our study areas on 25 August 2017, delivering maximum sustained winds between 177–209 km/hr and rainfall exceeding 40 cm (NOAA 2017).

METHODS

Experimental Design

Our study design was a repeated measures, randomized complete block design where ranches served as the blocking factor and 2 experimental units (500 ha each) occurred within each block (Figure 1). We designated ranch 1 as block 1, ranch 2 as block 2, and ranch 3 as block 3 (Figure 1). We had 6 experimental units (3 ranches × 2 experimental units/block). The experimental units within a given block were nearly identical in vegetation composition and received the same management. Experimental units on block 1 represented midgrass-tallgrass/post oak savanna, block 2 represented midgrass/tallgrass/mesquite/huisache savanna, and block 3 represented treeless midgrass prairie. These plant communities were selected in order to encompass the greatest amount of variation in communities represented in this region. Fire ants were found in all pastures.

The repeated measures component of our study involved a pretreatment and posttreatment period. The first year of our study (Mar 2017–Mar 2018) served as the pretreatment period. After this period, we randomly chose experimental units on blocks 1 and 2 to receive a treatment of Extinguish® Plus (0.25% methoprene and 0.36% hydramethylnon; Central Garden and Pet, Walnut Creek, CA, USA). Extinguish Plus is a bait that contains an insect growth regulator specific to ants and a slow-acting insecticide (TAMU 2017). These chemicals disrupt the production of energy in the insect’s cells, affecting the queen ant’s reproduction. The bait is gathered by foragers and shared among the colony, ultimately leading to the demise of the colony. There are few risks to other ant species during application because fire ants are more dominant and aggressive at retrieving the bait before other species (Barr et al. 2005, Knutson and Campos 2008). The ranch manager for block 3 had already aerially applied Extinguish Plus at the recommended label rate of 1.7 kg product/ha to a 1,619-ha portion of the property (6 April 2016), a year prior to the start of our study (March 2017). Thus, we could not collect any true pretreatment data nor randomly assign treatments on block 3. For block 3, we randomly designated a treatment site (500 ha) within the already treated area and randomly selected an untreated unit (500 ha) to serve as the experimental control. The insecticide was applied to the treatment units of all 3 blocks via a fixed-winged aircraft at the recommended label rate of 1.7 kg/ha to designated treatment experimental units during 5–6 April 2018. Thus, block 3 had 2 treatment applications: April 2016 and April 2018. Treatment on block 1 was applied from approximately 0900–1300 under cloudy conditions and there was moderate rainfall starting around 1300. Treatment was applied to block 2 on April 6 approximately 0900–1300 under sunny, clear conditions. Treatment on block 3 was applied approximately 1400–1800 under cloudy conditions, but there was no precipitation during or after treatment. Experimental units within each block where insecticide was not applied served as experimental controls.

We chose the March–April time for insecticide application because this has been recommended as the best time of application based on preliminary data collected by the Attwater Prairie Chicken National Wildlife Refuge (M. Morrow, Attwater Prairie Chicken National Wildlife Refuge, personal communication). The 6 months following treatment application served as our posttreatment period (15 Mar 2018–31 Aug 2018). We documented fire ant density and bobwhite demographics during the breeding season on each experimental unit during pretreatment (15 Mar 2017–31 Aug 2017) and posttreatment (15 Mar 2018–31 Aug 2018). We monitored bobwhite response at 2 spatial scales: individual bobwhites (point-of-use) and population density (pasture scale).

Fire Ant Abundance

Point-of-use scale.—We collected data on fire ant abundance at bird locations, at nest locations, and at paired random points. Because of time constraints, we obtained fire ant abundance only at a subsample of bird locations, but we were able to obtain fire ant abundance at all nests. For bird locations, we randomly selected twice per week 4 bobwhites in each of the 6 experimental units to sample (6 experimental units × 4 randomly selected hens/unit × 2 days/week = 48 points sampled weekly). When a randomly selected bird was located, we placed a marking flag in the exact location where the bird was observed so the point could be sampled the following day. If 2 randomly selected birds were found at identical locations, we randomly selected another bird location to sample. We also established paired, random points for bird locations and nests by randomly selecting a direction and distance between 20–50 m from the original location (i.e., bird location or nest; Collins et al. 2009).

At each sample point (i.e., bird location, nest, or random point), we sampled fire ant density using the point center quarter method (Cottam and Curtis 1949, 1956). We delineated the area surrounding a sample point into 4 quarters using the 4 cardinal directions as delineations and the sample point as the center. We measured the distance to the nearest active fire ant mound (up to 10 m) in each quarter and calculated the mean distance. We documented measurements to active mounds only. We confirmed mounds to be active by poking a hole into the center of the mound, waiting a moment, and observing fire ants escaping. We then used this information to calculate fire ant mound density at the bird location as per Cottam and Curtis (1956) using the equation:

$$\text{Mound density} = \frac{1}{(\text{Mean distance to nearest mound})^2}$$

We used the known-fate and nest survival platforms in Program MARK, version 6.2 to model the influence of fire ants on bobwhite survival and nest success (White and Garrot 1990, White and Burnham 1999).

Pasture scale.—We quantified fire ant abundance on each experimental unit during pretreatment (Sep 2017) and posttreatment (Sep 2018) using 2 methods: 1) distance sampling to estimate density of fire ant mounds (Burnham et al. 1980, Buckland et al. 2004), and 2) baited cup method to estimate relative abundance of foragers (Porter and Tschunkel 1987, Mueller et al. 1999).

For distance sampling, we established 10 100-m transects in each experimental unit. The starting points and orientation of the transects were randomly chosen within the experimental unit boundaries using ArcGIS 10.3 (Esri Inc., Redlands, CA, USA). We walked transects during 0800–1800 and measured the perpendicular distance (0.1-m increments) to each fire ant mound detected using a Nikon® ProStaff® 3i laser range finder (Nikon Instruments, Melville, NY, USA). We did not leave the transect line in order to avoid finding other mounds that would not have been detected otherwise. Thus, we could not determine whether fire ant mounds were active. In our study area, we rarely observed mounds that were inactive, so we assumed all mounds to be active if visually detected. We walked all transects in one experimental unit before moving on to the next, and all experimental units were sampled within a 7-day period. We sampled the same transects during pretreatment (May and Sep 2017) and posttreatment (May and Sep 2018).

We calculated fire ant mound densities for each experimental unit using Program DISTANCE (Thomas et al. 2010). Assumptions of distance sampling include the following: 1) animals were distributed independently of the transect, 2) pertinent objects on the line were detected with 100% certainty, 3) points did not move before detection, 4) points did not move once located, 5) distances were measured accurately, and 6) each sighting was independent of one another (Burnham et al. 1980, Buckland et al. 2004). To determine the effectiveness of the insecticide, we compared fire ant density between treatment and control sites by block during pretreatment and posttreatment using 95% confidence intervals (CIs). We also pooled data across blocks 1 and 2 (the two blocks with true pretreatment and posttreatment data) and compared fire ant density between treatment and control sites using 95% CIs.

We used the baited cup method (Porter and Tschunkel 1987, Mueller et al. 1999) to estimate the relative abundance of foragers during pretreatment (Sep 2017) and posttreatment (Sep 2018). To designate sampling sites, we created a grid (10-ha cells) in the internal 250-ha area of each 500-ha experimental unit using the fishnet tool in ArcGIS 10.3. This process resulted in 25 grid cells in each experimental unit, of which we randomly selected 10 for sampling using their centroid as the sampling point. At each sampling point, we placed 1 30-ml plastic cup baited with a 0.5-g piece of hot dog on its side following the general protocol of Mueller et al. (1999). After 30 minutes, we securely capped the cup and placed it in a freezer at -20º C. Once the cup was frozen, we counted the number of fire ants in each cup (Mueller et al. 1999). Surveys were conducted only during periods of maximum fire ant foraging (22–32º C; Porter and Tschinkel 1987), and all sampling points in an experimental unit were completed within a 3-hour period before moving on to the next experimental unit. We used the same points for sampling during both pretreatment and posttreatment.

We compared relative abundance of foragers between treatment and control sites by block during pretreatment and posttreatment using analysis of variance (ANOVA). As was the case for prior analyses, we pooled data across blocks 1 and 2—the two blocks with true pretreatment and posttreatment data—and compared relative abundance of foragers between treatment and control sites using a repeated measures ANOVA. We determined statistical significance using an alpha level of $P \leq 0.05$. We report all findings as mean ± standard error unless otherwise noted.

BOBWHITE DEMOGRAPHY

Survival.—We monitored survival of bobwhites using radio-telemetry during the breeding seasons (Apr–Aug 2017 and 2018). To designate trap sites, we created a grid (10-ha cells) in the internal 250-ha area of each 500-ha experimental unit using the fishnet tool in ArcGIS 10.3. One funnel-style trap was placed in the centroid of each grid cell in order to ensure equal trapping effort across each experimental unit. We placed traps along roadways (in order to check all traps within a 3-hour session) and under dense-canopied shrubs to reduce risk of predation and sun exposure (Stoddard 1931). Traps in open prairie or in areas lacking woody cover were covered with limbs of woody plants and other natural vegetation. Each trap site was prebaited with 1.5 L of milo (*Sorghum bicolor*) every 4 days, 12 days before trapping commenced. We set traps before sunrise, checked traps every 3 hours during the day, and closed the traps before twilight (Abbott et al. 2005). Upon capture of each bird, we documented its mass, age (hatch year or after hatch year), and sex. We banded all birds using size 7 aluminum bands (Rosene 1969), and bobwhite hens weighing ≥150 g were fitted with a 6-g necklace-style very high frequency radio-transmitter (American Wildlife Enterprises, Monticello, FL, USA). The radio-transmitters operated at frequencies 150.000–151.999 MHz. We collared only 3 hens/trap/covey to maintain even sampling distribution of bobwhites throughout the experimental units. Our goal was to maintain at least 15 birds in each experimental unit (6 experimental units × 15 hens/unit = 90 hens) throughout the nesting season (Apr–Aug 2017 and 2018).

We located bobwhites via homing 2–3 days/week during 15 March–31 August 2017 and 2018. We alternated days in which birds were located. Tracking times were stratified across 4 time periods (sunrise–0900, 0901–1200, 1201–1500, and 1501–sunset) to collect an equal number of locations for every radio-marked bird during each time period throughout the field season. We recorded bobwhite locations using a Trimble® Juno™ Global Positioning System (GPS) unit (Trimble Navigation Ltd., Sunnyvale, CA, USA). At each bird location, we recorded the date, time, physical location, association with other birds, and number of chicks in the group. If a bobwhite traveled outside of the ranch boundary, we made every effort to obtain access to the property to continue collecting data. If the bird was not located, it was used in analyses until the date it went missing, at which point it was censored. When a mortality signal was detected, we immediately located the transmitter and classified the suspected cause of death as 1) avian predation (skeleton intact, curled antenna, stripped tendons), 2) mammalian predation (feathers only, bite marks on transmitter), 3) snake predation (in a snake or its feces), 4) unknown (carcass intact), or 5) other reasons (Carter et al. 2002). Trapping, handling, and general research were conducted under the Texas A&M University-Kingsville Institutional Animal Care and Use Protocol #1384.

We estimated survival of radio-marked bobwhites during the breeding season (15 Mar–31 Aug 2017 and 2018) using the Kaplan-Meier estimator and staggered-entry approach (Kaplan and Meier 1958, Pollock et al. 1989). It is possible that the stress of handling and marking bobwhites may influence survival probabilities, so some studies have removed from analysis birds that survived ≤7 days (Pollock et al. 1989) or ≤14 days (Cox et al. 2004). Our study had a low sample size of bobwhites, so to keep as many birds as possible for analysis, we assumed that trapping, handling, and radio-collaring did not affect bobwhite survival. We removed from analysis only bobwhites which did not survive from the initial trapping day to the first tracking day. Birds that went missing were kept in the analysis through the last known day of survival, after which point, they were censored. Because block 3 did not have a true pretreatment data collection period, we excluded it from the Kaplan-Meier survival analyses. In addition, because of low sample sizes resulting from low bobwhite abundance in the ecoregion, we had to pool radio-marked bobwhites across blocks 1 and 2 by treatment. We compared survival curves between treatment and control during pretreatment and posttreatment for the full bobwhite breeding season (Mar–Aug) using a log-rank Chi-squared test (Pollock et al. 1989). Because the treatment takes up to 4 weeks to reach full efficacy (TAMU 2017), we also compared bobwhite survival starting 4 weeks after treatment with survival from the same pretreatment timeframe (5 May–31 Aug).

We modeled the influence of fire ants and other covariates on bobwhite survival using the known-fate platform in Program MARK, version 6.2 (White and Garrot 1990, White and Burnham 1999). This type of analysis is similar to a regression framework; thus, we used radio-marked bobwhites from all blocks for this analysis given that each bobwhite had an encounter history (i.e., "response variable") and corresponding estimates of fire ant abundance and other covariates of interest (i.e., "predictor variables"). We developed an encounter history for each bird using a 7-day interval. We modeled bobwhite survival based on time trend, age, sex, year, nearest fire ant mound, and fire ant mound density. For a given bird, we averaged nearest fire ant mound and fire ant mound density across its sampled locations. We developed 12 *a priori* models for evaluation (Table 1) and selected the best model using Akaike's Information Criterion corrected for small sample size (AIC_c) and Akaike weights (w_i) (Burnham and Anderson 2002).

Reproduction.—We considered radio-marked bobwhites to be nesting when we documented an individual in the same location for ≥2 consecutive tracking days. We obtained GPS locations of every nest location using a Trimble Juno and placed flagging tape 10 m from the nest in the 4 cardinal directions

Table 1. List of 12 *a priori* models used to evaluate the influence of time, demographic, and red imported fire ant (*Solenopsis invicta*) covariates on survival of radio-marked northern bobwhites (*Colinus virginianus*), Goliad and Refugio counties, Texas, USA, April–August 2017 and April–August 2018.

Hypothesis category model no.	Model	Explanation
Null		
1	S(.)	No effect of any covariate
Demographic		
2	S(Trend)	Survival varies by week
3	S(Age)	Survival varies by age
4	S(Sex)	Survival varies by sex
5	S(Year)	Survival varies between years
6	S(Age + Sex + Year)	Survival varies by age, sex, and year
Fire ant		
7	S(Nearest mound)	Survival varies by nearest fire ant mound
8	S(Mound density)	Survival varies by fire ant density
9	S(Nearest mound + Mound density)	Survival varies by nearest fire ant mound and fire ant density
Demographic and fire ant effects		
10	S(Age + Nearest mound + Mound density)	Survival varies by age, nearest fire ant mound, and fire ant density
11	S(Sex + Nearest mound + Mound density)	Survival varies by sex, nearest fire ant mound, and fire ant density
12	S(Year + Nearest mound + Mound density)	Survival varies by year, nearest fire ant mound, and fire ant density

surrounding the nest (i.e., flagged shrubs formed a "cross" with the nest in the center). We monitored each nest ≥10 m away every other day until nest fate was determined. Hens were not flushed from their nests. If the signal strength was strong towards nest location, we assumed that the hen was still incubating. The first time the incubating hen was away from the nest, we visually confirmed the nest, documented clutch size, and recorded more precise GPS coordinates. We classified nests as 1) successful (≥1 egg hatched), 2) depredated (eggs missing, eggs crushed, or surrounding vegetation trampled, or combination thereof), 3) abandoned (eggs present but hen off nest ≥7 days), or 4) other (Terhune et al. 2006, Scott et al. 2012).

We compared apparent nest success between control and treatment units during pretreatment and posttreatment using Fisher's exact test (Zar 1999) in SAS 9.2 (SAS Institute Inc., Cary, NC, USA). As was the case for survival, we analyzed only blocks 1 and 2 in this type of analysis (because these blocks possessed true pretreatment and posttreatment data) and pooled across blocks by treatment due to low sample sizes.

We modeled the influence of 5 covariates (trend, age, year, distance to nearest mound, and mound density) on nest survival using the nest survival platform in Program MARK, version 6.2. This software uses a maximum-likelihood estimator (MLE) with a logit link function to provide estimates of survival probability based on the mean and variance of the daily survival rate as influenced by covariates in a given model. The model assumed that daily survival rate was the same for all nests on all dates and for all nest ages and that nest fates were independently and identically distributed within a sample (Johnson 1979, Bart and Robson 1982). We created an encounter history for each nest based on the day the nest was found, the last day the nest was known to be alive, the last day the nest was checked, and the fate of the nest (successful or failed). We standardized the nesting-season days using day 1 as the date the first nest was detected for each year. We developed 10 *a priori* models (Table 2) and selected the best model based on AIC$_c$ and w_i (Burnham and Anderson 2002).

Bobwhite density.—We measured bobwhite density using helicopter surveys with a distance-sampling framework during December 2017 and December 2018 following the general protocol of Rusk et al. (2007), Schnupp et al. (2013), and Edwards (2019). We recorded data on bobwhite detections using the Modified System for Electronic Surveys (MSES) as defined by Schnupp et al. (2013) and modified by Edwards (2019). The equipment consisted of a MDL LaserAce 300™ laser range finder (Measurement Devices Ltd., Aberdeen, Scotland, UK), 2 Ironix DuoTouch™ tablets (General Dynamics, St. Petersburg, FL) equipped with ArcPad 7 (Esri Inc., Redlands, CA), a Raven Cruizer™ guidance and differential GPS (Raven Industries, Sioux Falls, SD, USA), and 2 17-key keypads (Edwards 2019).

We established linear transects (n = 6–20/experimental unit), spaced 200 m apart within each experimental unit, using the fishnet tool in ArcGIS 10.3. Transects were oriented either east-west or north-south to allow flexibility in flight direction on the day of survey depending on the prevailing winds. Surveys were conducted during daylight hours (0800–1800) using a Robinson R44 helicopter (Robinson Helicopter Co.,

Table 2. List of 10 *a priori* models to evaluate the influence of time, demographic, and red imported fire ant (*Solenopsis invicta*) covariates on nest survival of radio-marked northern bobwhites (*Colinus virginianus*), Goliad and Refugio counties, Texas, USA, April–August 2017 and April–August 2018.

Hypothesis category model no.	Model	Explanation
Null		
1	S(.)	No effect of any covariate
Demographic		
2	S(Trend)	Survival varies by day
3	S(Age)	Survival varies by age
4	S(Year)	Survival varies between years
5	S(Age + Year)	Survival varies by age and year
Fire ant		
6	S(Nearest mound)	Survival varies by nearest fire ant mound
7	S(Mound density)	Survival varies by fire ant density
8	S(Nearest mound + Mound density)	Survival varies by nearest fire ant mound and fire ant density
Demographic and fire ant effects		
9	S(Age + Nearest mound + Mound density)	Survival varies by age, nearest fire ant mound, and fire ant density
10	S(Year + Nearest mound + Mound density)	Survival varies by year, nearest fire ant mound, and fire ant density

Torrance, CA) and were flown at an average air speed of 23–40 km/hr and average height of 8–11 m. Surveys were conducted using 4 observers: the pilot, 1 front-seat observer, and 2 back-seat observers. The pilot and the front-seat observer surveyed the 90° area in the front of the helicopter, which encompassed 45° on each side of the transect line (Edwards 2019). Once bobwhites were detected, the pilot would bring the helicopter to a hover while the observer recorded covey size and obtained a GPS location of the detection using MSES. After a covey flushed, the birds scattered and if they flew near an adjacent transect, we made note of the location to avoid double counting.

We attempted to calculate bobwhite density for each experimental unit using Program DISTANCE, but we did not have enough quail detections to obtain reliable estimates. Thus, we instead calculated relative abundance (number of bobwhites/km) to estimate bobwhite abundance pooled across blocks 1 and 2 by treatment. We compared bobwhite abundance between treatments using a generalized linear model with repeated measures (PROC GLM; SAS 9.2). We also conducted simple linear regression to evaluate at the pasture scale the relationship between bobwhite relative abundance and fire ant mound density.

RESULTS

Fire Ant Abundance

Pasture scale.—In general, fire ant mound density decreased through time on both treatment and control sites on all blocks (Figure 2). However, 95% CIs of fire ant mound density did not overlap between treatment and control sites during posttreatment in block 2 (May and Sep sampling) or block 3 (May sampling), indicating that the insecticide was effective at decreasing fire ant mound density in these blocks (Table 3). For data pooled across blocks 1 and 2, we observed the same general trend of decreasing fire ant mound density through time on both treatment and control sites (Figure 3). Regarding these pooled data, the 95% CI of fire ant mound density for the pooled dataset did not overlap between treatment and control sites during the last survey of the posttreatment period (Sep sampling), again indicating that the insecticide was effective at decreasing fire ant mound density (Table 3).

Fire ant forager abundance increased in both units of block 2 regardless of treatment but decreased to 0 foragers in the treatment units of blocks 1 and 3 (Table 4). Of the 3 blocks, only block 1 exhibited a statistical difference in forager abundance between control (n = 770 foragers) and treatment (n = 0 foragers, P = 0.05) following insecticide application (Figure 4). For pooled data across blocks 1 and 2, we observed a trend of increasing fire ant abundance through time regardless of treatment (Figure 5). However, for the pooled data, we documented no difference in fire ant forager abundance between control (n = 1,235 foragers) and treatment (n = 1,044 foragers; P = 0.76) following insecticide application (Figure 5).

Bobwhite Demography

Survival.—We captured and radio-marked 93 bobwhites in 2017 and 100 bobwhites in 2018. We were able to use survival information from 83 bobwhites in 2017 and 81

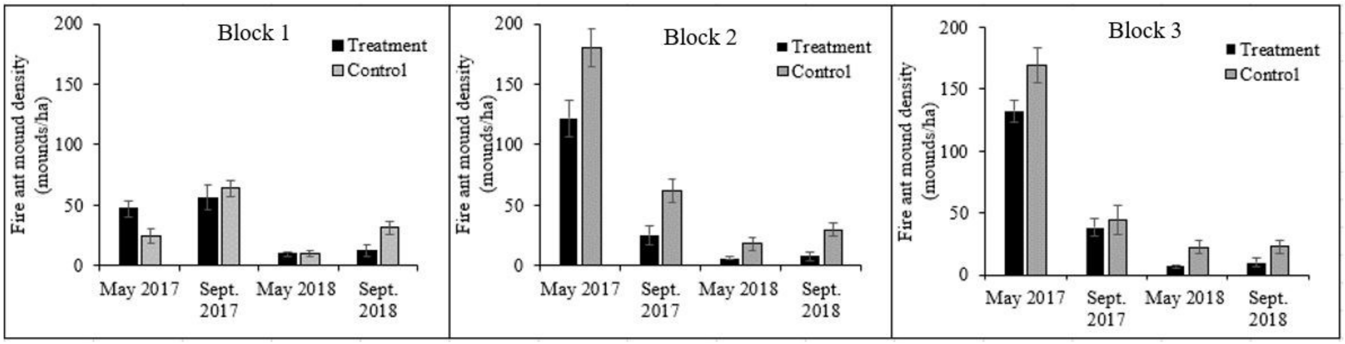

Fig. 2. Red imported fire ant (*Solenopsis invicta*) densities (mounds/ha) ($\hat{D}$ ± 95% CI) of treatment and control units estimated using distance sampling before and after application of Extinguish® Plus (5–6 Apr 2018) for block 1, block 2, and block 3 in Goliad and Refugio counties, Texas, USA. Block 3 was treated with the insecticide during April 2016 and April 2018; thus, fire ant mound density is after treatment for both years.

Table 3. Red imported fire ant (*Solenopsis invicta*) densities (mounds/ha), sample sizes, 95% confidence intervals (CIs), and coefficient of variation (CV; %) of treatment and control units estimated using distance sampling before and after application of Extinguish® Plus (5–6 Apr 2018) for blocks 1, 2, 3 and blocks 1 and 2 pooled in Goliad and Refugio counties, Texas, USA. Block 3 was treated with the insecticide during April 2016 and April 2018; thus, fire ant mound density is after treatment for both years.

Block	Date	Unit	n	$\hat{D}$ [a]	95% CI Lower	95% CI Upper	% CV
1	May 2017	Treatment	78	46.951	34.519	63.860	14.38
		Control	63	24.370	13.995	42.435	25.17
	Sep 2017	Treatment	49	56.084	38.192	82.358	18.57
		Control	50	64.171	51.785	79.519	10.56
	May 2018	Treatment	46	9.723	6.731	14.045	16.51
		Control	31	10.123	5.485	18.684	28.4
	Sep 2018	Treatment	12	12.250	5.765	26.033	37.28
		Control	38	31.589	22.257	44.835	17.46
2	May 2017	Treatment	159	121.620	93.222	158.660	12.64
		Control	217	179.880	149.820	215.970	8.91
	Sep 2017	Treatment	16	25.351	12.961	49.583	31.07
		Control	33	61.969	44.423	89.444	16.28
	May 2018	Treatment	18	5.891	3.599	9.642	22.61
		Control	22	18.212	10.123	32.765	29.18
	Sep 2018	Treatment	11	7.515	2.775	20.353	50.37
		Control	36	29.607	20.499	42.762	18.38
3	May 2017	Treatment	194	132.510	115.140	152.510	6.89
		Control	226	169.080	142.290	200.920	8.38
	Sep 2017	Treatment	30	38.373	26.955	54.628	17.49
		Control	47	44.176	24.340	80.151	27.18
	May 2018	Treatment	26	6.549	3.676	11.668	26.23
		Control	29	22.316	14.263	34.915	22.18
	Sep 2018	Treatment	17	10.000	4.705	21.254	37.72
		Control	31	22.490	13.988	36.157	23.29
1 and 2 pooled	May 2017	Treatment	237	88.69	69.39	113.35	11.91
		Control	280	86.40	63.58	117.43	14.77
	Sep 2017	Treatment	65	40.10	27.11	59.32	19.27
		Control	83	60.90	50.15	73.94	9.63
	May 2018	Treatment	64	12.88	9.13	18.17	16.63
		Control	53	10.97	7.49	16.08	18.60
	Sep 2018	Treatment	23	9.94	5.48	18.04	30.14
		Control	74	31.19	24.32	40.00	12.56

[a] $\hat{D}$: Density

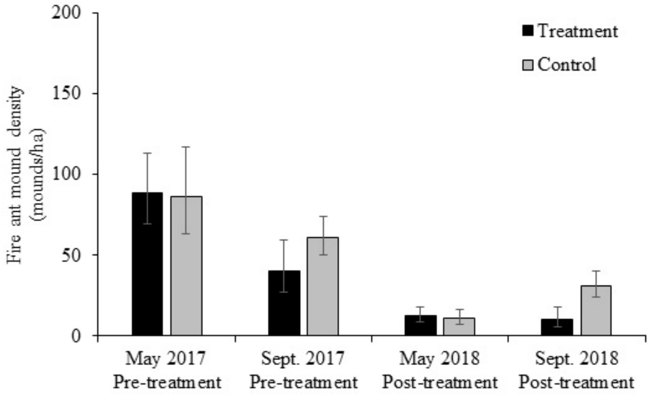

Fig. 3. Red imported fire ant (*Solenopsis invicta*) densities (mounds/ha) ($\hat{D}$ ± 95% confidence interval) of treatment and control units estimated using distance sampling before and after application of Extinguish® Plus (5–6 Apr 2018) for blocks 1 and 2 pooled in Goliad and Refugio counties, Texas, USA, 2017–2018.

Table 4. Red imported fire ant (*Solenopsis invicta*) forager abundance and standard errors (SE) of bait cup traps in treatment and control units before and after application of Extinguish® Plus (5–6 Apr 2018) for blocks 1, 2, 3 and blocks 1 and 2 pooled in Goliad and Refugio counties, Texas, USA. Block 3 was treated with the insecticide during April 2016 and April 2018; thus, fire ant mound density is after treatment for both years.

Block	Date	Unit	Forager abundance	SE	P
1	Sep 2017	Treatment	291	34.37	0.712
		Control	231	37.09	
	Sep 2018	Treatment	0	0.00	0.047
		Control	770	114.32	
2	Sep 2017	Treatment	140	20.03	0.266
		Control	270	29.68	
	Sep 2018	Treatment	1044	112.60	0.227
		Control	465	93.51	
3	Sep 2017	Treatment	205	25.75	0.272
		Control	90	19.17	
	Sep 2018	Treatment	0	0.00	0.087
		Control	283	49.44	
1 and 2 pooled	Sep 2017	Treatment	431	28.45	0.720
		Control	501	32.75	
	Sep 2018	Treatment	1044	94.20	0.761
		Control	1235	102.85	

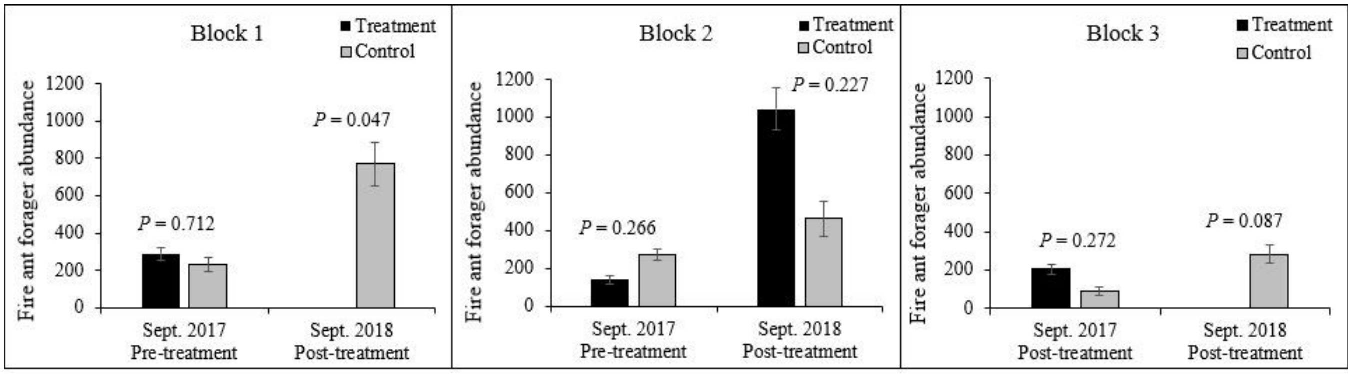

Fig. 4. Red imported fire ant (*Solenopsis invicta*) forager abundance and standard errors of treatment and control units before and after application of Extinguish® Plus (5–6 Apr 2018) for block 1, block 2, and block 3, Goliad and Refugio counties, Texas, USA, September 2017 and September 2018. Block 3 was treated with the insecticide during April 2016 and April 2018; thus, fire ant forager abundance is after treatment for both years.

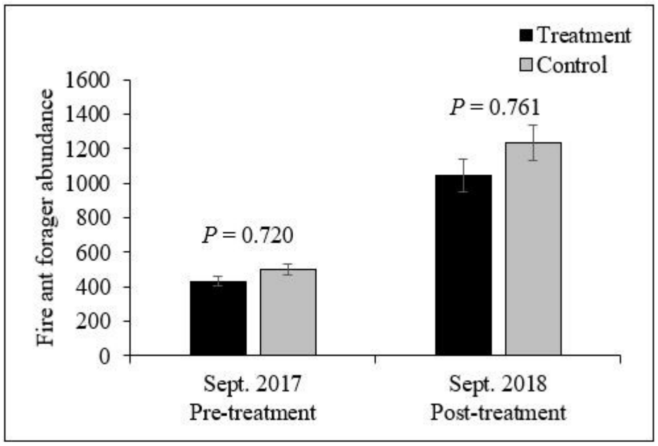

Fig. 5. Red imported fire ant (*Solenopsis invicta*) forager abundance and standard errors of treatment and control units before and after application of Extinguish® Plus (5–6 Apr 2018) for blocks 1 and 2 pooled, Goliad and Refugio counties, Texas, USA, September 2017 and 2018.

bobwhites in 2018. There was no difference in seasonal bobwhite survival between control (0.12 ± 0.02; *n* = 45 bobwhites) and treatment (0.16 ± 0.02; *n* = 38 bobwhites) during the pretreatment period (*P* = 0.91) (Figure 6). We also documented no difference in bobwhite survival between control (0.33 ± 0.02; *n* = 52) and treatment (0.46 ± 0.01; *n* = 29) during posttreatment (*P* = 0.21) during the full breeding season (15 March–31 Aug; Figure 6). Bobwhite survival was lower in the control (0.50 ± 0.01) than the treatment (0.69 ± 0.01) beginning 4 weeks after treatment took full effect until the end of the bobwhite breeding season (5 May–31 Aug 2018), but this difference was not significant (*P* = 0.14). However, there was more of a divergence in bobwhite survival between the control and treatment units 4 weeks following treatment application compared to pretreatment, which provides evidence for biological significance. Predation accounted for most mortalities in 2017 (59%; *n* = 49 total mortalities) and 2018 (70%; *n* = 58 total mortalities). We did not document any confirmed mortalities caused by fire ants.

Regarding evaluation of the influence of covariates on bobwhite survival, we were able to use 72 bobwhites for analyses from 2017 and 50 bobwhites from 2018. Of the 12 *a priori* models evaluated, the most parsimonious model

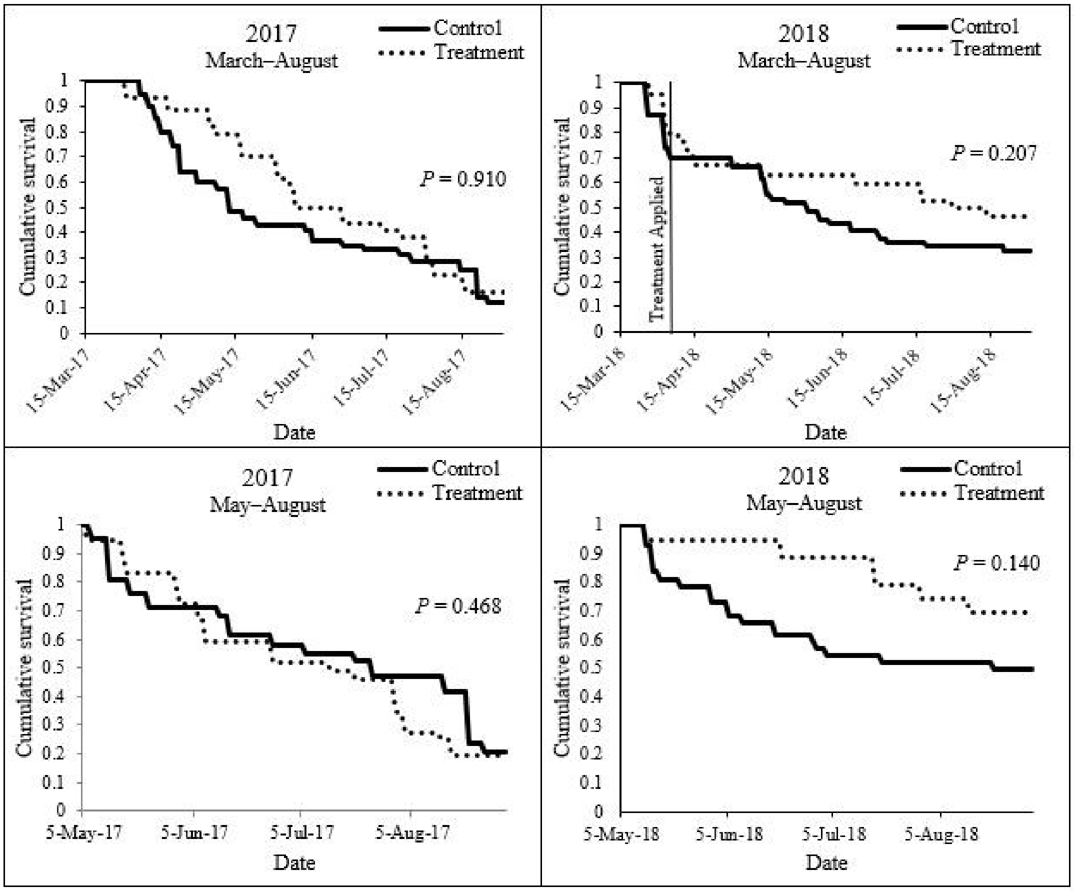

Fig. 6. Survival curves of radio-marked bobwhites (*Colinus virginianus*) before and after application of Extinguish® Plus (5–6 Apr 2018) during full breeding season (15 Mar–31 Aug) and starting 4 weeks after treatment (5 May–31 Aug), Goliad and Refugio Counties, Texas, USA, 2017–2018. Data were pooled across blocks 1 and 2 by treatment.

was the null model (Table 5). There were 9 other competing models (i.e., within 2Δ AIC_cs); however, the CIs of the beta estimates for these competing models overlapped zero, indicating they contained uninformative parameters. For example, the second-best model included an additive effect of fire ant mound density (β = 0.204, 95% CI = -0.087 to 0.494) and nearest fire ant mound (β = 0.231, 95% CI = -0.068 to 0.530), but both betas for these parameters included 0. In addition, the Akaike model weights for the top 4 models were similar (0.10–0.12), indicating no strong statistical support for any particular model (Table 5). Collectively, this information suggests that none of the competing models was superior to the null model, thereby indicating that none of the covariates (trend, age, sex, year, nearest mound and mound density) that we measured in our study influenced adult survival of our radio-collared bobwhites (Table 5).

Reproduction.—We found 26 bobwhite nests in 2017 and 25 bobwhite nests in 2018. We documented no statistical difference in apparent nest success between control (58.3%; *n* = 12 nests) and treatment (18.2%; *n* = 11 nests) during pretreatment (*P* = 0.09; Figure 7). We also documented no statistical difference in apparent nest success between control (23.1%; *n* = 13 nests) and treatment (55.6%; *n* = 9 nests) during posttreatment (*P* = 0.19; Figure 7). However, following treatment, apparent nest success in the treated units increased by 37.4% while nest success in the control units decreased by 35.2%. This result provides evidence that even though we did not detect a significant difference between the treated and controlled areas, the treatment may have been effective at improving nest success.

We observed a similar finding regarding clutch size. We documented no difference in clutch size between control (11.4 ± 3.3 eggs, *n* = 10 nests) and treatment (13.0 ± 2.2 eggs, *n* = 7 nests) during pretreatment (*P* = 0.28; Table 6), nor a difference in clutch size between control (11.1 ± 3.3 eggs, *n* = 12 nests) and treatment (12.4 ± 2.8 eggs, *n* = 9 nests) during posttreatment (*P* = 0.38; Table 6).

Of the 10 *a priori* models evaluating the influence of covariates on bobwhite nest survival, the most parsimonious model was the null model (Table 7). There were 4 other competing models (i.e., within 2Δ AIC_cs); however, the CIs for these competing models overlapped zero, indicating they contained uninformative parameters. For example, the second-best model included an additive effect of time trend (β = -0.008, 95% CI = -0.0238 to 0.008), but the beta estimate included 0. In addition, the Akaike model weights for the top 5 models after the null model were similar (0.10–0.17), indicating no strong support for any particular model (Table 7). Collectively, this information suggests that none of the competing models was superior to the null model, thereby indicating none of the covariates (trend, nearest mound, year, mound density, and age) influenced adult survival of our radio-collared bobwhite nest survival (Table 7).

Bobwhite density.—We could not obtain reliable estimates of bobwhite density using distance sampling because of low detections (*n* = 2–14 covey detections/experimental unit in 2017 and *n* = 0–5 covey detections/experimental unit in 2018). Thus, we compared relative bobwhite abundance (no. individuals/km) between treatments across time. There was no difference between treatment type ($F_{1,5}$ = 0.15, *P* = 0.74), year ($F_{1,5}$ = 1.03, *P* = 0.60), or treatment × year ($F_{1,5}$ = 1.35, *P* = 0.37). Although the lack of treatment × year interaction permitted pooling across time, we compared the treatment and control units by year to evaluate potential differences

Table 5. Model selection results of analysis evaluating factors affecting breeding season (Apr–Aug) survival of radio-marked northern bobwhites (*Colinus virginianus*) in Goliad and Refugio counties, Texas, USA, 2017–2018.

Rank	Model	K [a]	AIC_c [b]	ΔAIC_c [c]	*w* [d]
1	S(.)	1	585.6297	0.000	0.1501
2	S(Mound density + Nearest mound)	3	586.0509	0.421	0.1216
3	S(Year)	2	586.1589	0.529	0.1152
4	S(Mound density)	2	586.3527	0.723	0.1046
5	S(Nearest mound)	2	586.4300	0.800	0.1006
6	S(Sex)	2	586.7268	1.097	0.0867
7	S(Year + Mound density + Nearest mound)	4	586.8613	1.232	0.0811
8	S(Sex + Mound density + Nearest mound)	4	587.2241	1.594	0.0677
9	S(Age)	2	587.6326	2.003	0.0552
10	S(Time Trend)	19	587.8838	2.254	0.0486
11	S(Age + Mound density + Nearest mound)	4	588.0194	2.390	0.0455
12	S(Year + Age + Sex)	4	589.3758	3.746	0.0231

[a] K: number of parameters.

[b] AIC_c: Akaike's Information Criterion corrected for small sample size.

[c] ΔAIC_c: difference between a model and the best performing model.

[d] *w*: Akaike model weight.

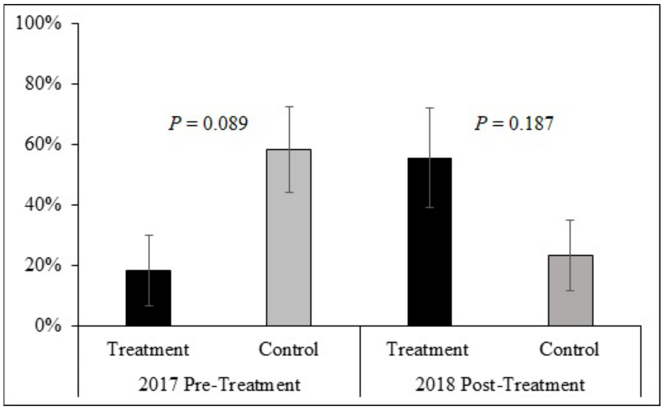

Fig. 7. Comparison of apparent nest success of northern bobwhite (*Colinus virginianus*) between treatment and control units before and after application of Extinguish® Plus (5–6 Apr 2018) in Goliad and Refugio counties, Texas, USA, December 2017 and December 2018. Data were pooled across blocks 1 and 2 by treatment.

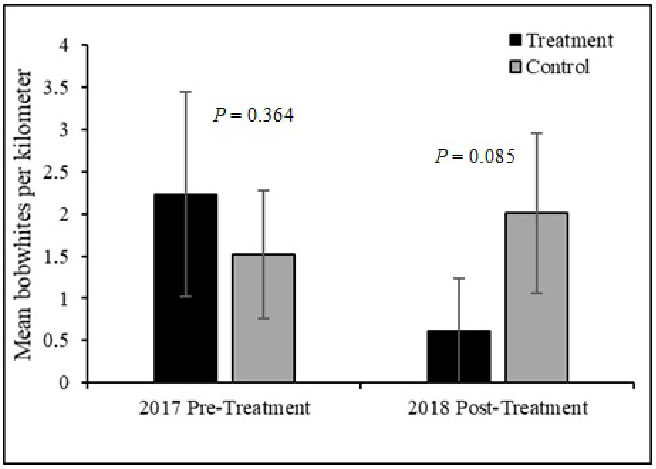

Fig. 8. Relative abundance (mean ± standard error; bobwhites/km) of northern bobwhites (*Colinus virginianus*) at treatment and control units of blocks 1 and 2 pooled before and after application of Extinguish® Plus (5–6 Apr 2018) in Goliad and Refugio counties, Texas, USA, December 2017 and December 2018.

Table 6. Mean clutch size of northern bobwhite (*Colinus virginianus*) before (2017) and after (2018) application of Extinguish® Plus (5–6 Apr 2018) in 2 sites (blocks 1 and 2), Goliad and Refugio counties, Texas, USA, 2017-2018. Block 3 was treated with the insecticide during April 2016 and April 2018; thus, mean clutch size is after treatment for both years.

	2017						
	Control			Treatment			
Site	*n*	Mean clutch size	SE	*n*	Mean clutch size	SE	*P*-value
Block 1	8	11.75	1.28	5	13.2	0.86	0.43
Block 2	2	10	1	2	12.5	2.5	0.45
Block 3	0			1	11		
Pooled (blocks 1 and 2)	10	11.4	1.05	7	13	0.82	0.28
Total (blocks 1, 2, and 3)	10	11.4	1.05	8	12.75	0.75	0.33
	2018						
	Control			Treatment			
Site	*n*	Mean clutch size	SE	*n*	Mean clutch size	SE	*P*-value
Block 1	5	11.8	1.66	4	13.25	1.31	0.53
Block 2	7	10.57	1.23	4	11.5	1.55	0.66
Block 3	0			1	15		
Pooled (blocks 1 and 2)	12	11.08	0.96	8	12.38	1	0.38
Total (blocks 1, 2, and 3)	12	11.08	0.96	9	12.67	0.93	0.26

specifically during the pretreatment and posttreatment periods. We documented no difference between the control (1.5 ± 0.8 bobwhites/km) and treatment (0.6 ± 0.6 bobwhites/km) units during pretreatment (*P* = 0.36; Figure 8). Similarly, we documented no difference between the control (2.0 ± 1.0 bobwhites/km) and treatment (0.6 ± 0.6 bobwhites/km) units during posttreatment (*P* = 0.09; Figure 8). This suggests that statistically, the insecticide treatment and year did not alter bobwhite population at the pasture scale during our study. Last, we did not document a linear relationship between pasturewide fire ant mound density and bobwhite relative abundance during either 2017 (*P* = 0.44) or 2018 (*P* = 0.30; Figure 9).

DISCUSSION

Overall, statistically, we did not document negative effects of fire ants on bobwhites at either the point-of-use or pasture scale. Our hypothesis that fire ants would negatively affect nest success and bobwhite survival was not statistically supported, but nest success and survival were both numerically higher in treated areas. Our hypothesis that the aerially applied

Table 7. Model selection results of analysis evaluating factors affecting breeding season (Apr–Aug) nest survival of radio-marked northern bobwhite (*Colinus virginianus*) in Goliad and Refugio counties, Texas, USA, 2017–2018.

Rank	Model	K [a]	AIC_c [b]	$\Delta\mathrm{AIC}_c$ [c]	*w* [d]
1	S(.)	1	214.8018	0.000	0.2833
2	S(Trend)	2	215.8013	1.000	0.1719
3	S(Nearest Mound)	2	216.7076	1.906	0.1092
4	S(Year)	2	216.7085	1.907	0.1092
5	S(Density)	2	216.7697	1.968	0.1059
6	S(Age)	2	216.8099	2.008	0.1038
7	S(Mound Density + Nearest Mound)	3	218.5380	3.736	0.0437
8	S(Year + Age)	3	218.7115	3.910	0.0401
9	S(Year + Mound Density + Nearest Mound)	4	220.4378	5.636	0.0169
10	S(Age + Mound Density + Nearest Mound)	4	220.5478	5.746	0.0160

[a] K: number of parameters.
[b] AIC_c: Akaike's Information Criterion corrected for small sample size.
[c] $\Delta\mathrm{AIC}_c$: difference between a model and the best performing model.
[d] *w*: Akaike model weight.

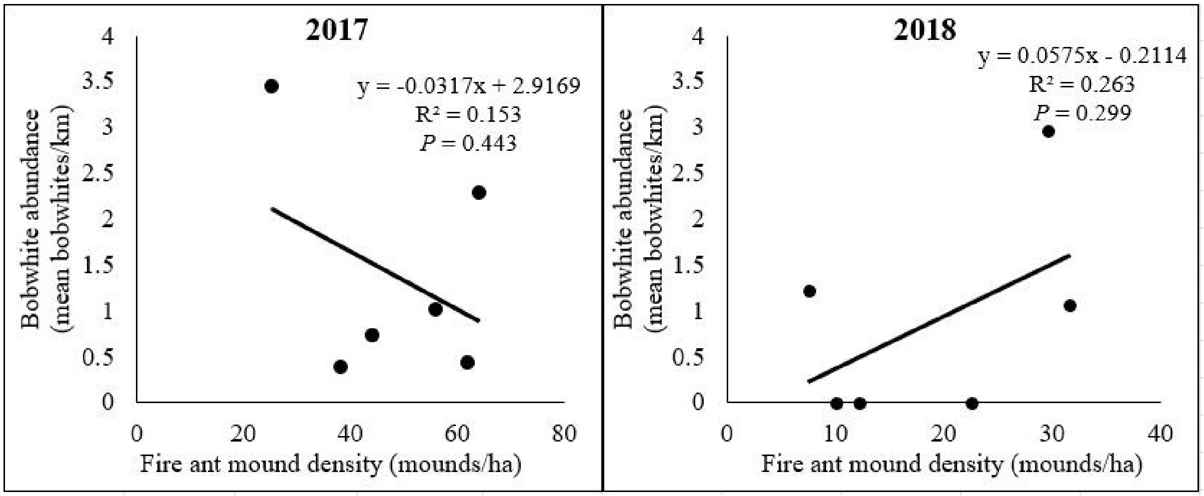

Fig. 9. Northern bobwhite (*Colinus virginianus*) relative abundance (bobwhites/km) as a function of red imported fire ant (*Solenopsis invicta*) mound density (mounds/ha) in Goliad and Refugio counties, Texas, USA, December 2017 and December 2018.

insecticide would reduce fire ant densities, but that bobwhite densities would remain unaffected, was partially supported. We did find evidence that the insecticide application reduced fire ant mound densities and forager abundances, but results were not consistent across blocks. In addition, bobwhite relative abundance was similar between control and treatment sites despite a potential reduction in fire ants. Overall, our study yielded small sample sizes which contributed to very low power in the statistical tests. Given the low power, it was unlikely for us to statistically show a difference if one existed.

Fire Ant Abundance

Pasture scale.—In general, we observed a decreasing trend in fire ant mound density. This finding is consistent with the Extinguish Plus user guide, which documented fewer active mounds in treated plots compared to untreated control plots (Central Life Sciences 2019). However, the effectiveness of the insecticide varied among blocks, which could be related to environmental conditions following insecticide application. It is recommended that the fire ant treatment be applied during times of no precipitation or dew for at least 8 hours so that the granules do not wash away or stick to the wet vegetation, making them impossible for the ants to pick up. In our study, there was brief, heavy rainfall directly following application of the treatment in block 1. This rainfall led to poor conditions and may have influenced the efficacy of treatment. However, in block 1, we still documented reduced mound density. One restriction of our methodology was that observers could not leave the pasturewide transects to verify whether mounds were active; instead, mounds were assumed active if detected. We assumed that due to weather and rainfall in the area the mounds would disintegrate quickly, but that is not always the case and we did not test this assumption. This restriction in methodology also may have obscured results. However, even though we may have included inactive mounds in our mound density analysis, we still documented a decrease in mound density after treatment.

Regarding overall fire ant forager abundance, in general, there was only a statistical difference in fire ant forager abundance between the control and treatment units on block 1. However, forager abundance decreased to 0 on both blocks 1 and 3, which indicates biological significance of the treatment efficacy. It is unknown why forager abundance decreased on 2 blocks but increased on 1 block after treatment. Forager abundance decreased on block 1, which may have received a compromised treatment due to rainfall, but foragers increased on block 2, which received an ideal treatment with respect to rainfall. Therefore, it is unclear whether these differences in fire ant abundances are a product of the fire ant treatment or other causes. We placed only 1 bait cup per sample cell (10 ha each), which may have been an inadequate sampling effort and could explain the difference between mound densities and foraging ants relative to treatment efficacy. It is also possible that our study areas contained polygyne populations of fire ants, that is, in which a single mound harbored multiple queens instead of a single queen. When polygene populations exist, forager densities are higher and more difficult to effectively treat (Porter et al. 1991). The presence of polygene populations may explain why we did not document decreased forager abundances on all sites. We did not take exact weather measurements at each block during treatment and were unable to gather information in our study areas afterwards. Taking weather measurements at application sites during treatment is something to consider for future studies. After pooling blocks 1 and 2 to account for variation between blocks, we did not detect a decrease in forager abundance, but still documented a decrease in mound density. These results could be due to restrictions of density dependence; when there are too many mounds, resources to produce foragers are limited. With a reduction in fire ant mounds from treatment, more resources could be available for a single mound to produce more foragers. Our results differed from Caldwell et al. (2017), who treated a 3,744-ha area with Extinguish Plus and found that fire ant abundance was reduced 2 out of the 3 years following treatment. They implicated flooding as a reason why there was not a decrease in fire ants for 1 of the 3 study years and this is similar to flooding in our study. In June 2018, our study sites received approximately 36 cm of rainfall, which was higher than the average of 10 cm for the area (NOAA 2019) and flooded areas of our study sites for approximately 2 weeks. Fire ants have been observed using their combined bodies to form floating rafts and drift to different locations, which likely happened during these flooding events (Adams et al. 2011). Collectively, these results indicate that the insecticide efficacy varies based on environmental conditions following application.

Bobwhite Demography

Survival.— We used both Kaplan Meir and Program MARK to analyze bobwhite survival since Program MARK selected the best model, but there was no assessment of how well the model fit the data. Kaplan Meir allowed us to compare the survival distributions with a corresponding p-value. Bobwhite survival was not influenced by fire ant mound density or distance to nearest fire ant mound. In addition, bobwhite survival was similar in the treatment and control units before and after insecticide application. However, 4 weeks following application, when the treatment became fully effective, bobwhite survival remained relatively stable in the treatment units but decreased in the control units. This result provides evidence for possible biological significance that the fire ant treatment benefits bobwhite survival even though the difference between treatment and control was not statistically significant in our study. Fire ants do have the potential to sting and harm full-grown bobwhites, but bobwhites in our study did not appear to be directly affected by fire ants. We never observed any confirmed bobwhite mortalities caused by fire ants although given the warm ambient temperatures during much of our study and abundant scavengers, it would be difficult to document deaths caused by fire ants. Rather, predation appeared to be the most common cause of mortality in this study, although fire ants could have indirectly caused some of these predation events. Pedersen et al. (1996) observed that pen-raised bobwhites exposed to fire ants had less time for pecking, loafing, and sleeping, which could lead to weakening and increased vulnerability of the individual, resulting in increased predation risk. Despite this possibility of direct mortality, our results are similar to those of Johnson (1961), Brennan (1993), and Brennan et al. (1991), who suggested that fire ants do not substantially affect demographic performance of bobwhites.

Reproduction.—Nest survival also was not influenced by fire ant mound density or distance of nearest fire ant mound to nest. Similar to survival, we never observed a nest failure caused by fire ants. We did observe an instance where a nesting hen was found dead on top of the nest covered in fire ants. It is possible that the fire ants overpowered the bird and caused the mortality and nest failure, but this could not be confirmed. Even if this nest failure is assumed to be caused by fire ants, our overall results were similar to a study by Simpson (1976), who found that only 1 of 1,072 bobwhite nests was lost to fire ants. Our results differed from Rader et al. (2007), who documented failure of 5 out of 43 (12%) bobwhite nests caused by a similar fire ant species, the native southern fire ant (*Solenopsis xyloni*), in South Texas. Our findings were similar to the results of Mueller et al. (1999), who hand-treated individual bobwhite nests with Amdro® (Ambrands, Atlanta, GA, USA) fire ant insecticide and did not document a difference in nest success when compared to untreated control nests. Mueller et al. (1999) did not report any bobwhite nests lost to fire ants but observed fire ants feeding on unsuccessful eggs in nests that were not treated with fire ant insecticide. Furthermore, no fire ants were found feeding on unsuccessful eggs in nests hand treated with fire ant insecticide. Mueller et al. (1999) treated individual nests with insecticide whereas we applied insecticide to pastures, but neither study reported significant differences in treatment effects on nest success.

Though we did not detect a statistical difference in bobwhite nest success between control and treated areas, apparent nest success did increase in treated areas, which provides evidence of possible biological significance. Because there was low statistical power, it was unlikely for us to demonstrate a statistical difference if one existed. The indirect effects of the treatment such as increased invertebrate richness and biomass may be beneficial for adult bobwhites and bobwhite chicks. Morrow et al. (2015) assessed the impacts of using a fire ant treatment to increase insect abundance. They treated fields with the same insecticidal ant bait used in this study and discovered 27% more individual invertebrates and 26% higher invertebrate biomass compared to control fields (Morrow et al. 2015). We did not sample invertebrates, but this is something to consider for future studies. Another point of consideration would be whether application of a fire ant treatment affects bobwhite brood survival. Mueller et al. (1999) documented an increase in proportions of brood surviving to 21 days of broods from treated nests ($n = 25$ broods, 53.5 ± 8.6%) compared to control nests ($n = 25$ broods, 24.7 ± 6.6%). Morrow et al. (2015) observed higher survival of Attwater's prairie-chicken broods that spent all of their time in areas treated to reduce fire ants compared to broods in untreated areas. We were unable to calculate brood survival due to the small sample sizes and the inability to count chicks in the tall prairie grass; we also suspected that some broods of chicks left radio-marked parents to join other broods, which would affect results.

Density.—Similar to our results for survival and nest success, we documented no difference in bobwhite relative abundance between treatments. One possible reason why we did not see an increase in bobwhite density after treatment is that with an already low density of bobwhites in the area, treatment may be less effective than if bobwhite density were high. For example, if there are fewer bobwhites in the area, there is greater opportunity to select high quality habitat and areas with already low fire ant abundances. In addition, there was no relationship between bobwhite relative abundance and fire ant mound density. These results are similar to Caldwell et al. (2017), who treated areas for fire ants; despite a documented decrease in fire ants, there was no increase in the number of female bobwhites during the nesting season, the number of females with broods, or the mean brood size per female.

Another possible alternative explanation for the lack of a population response to the fire ant treatment in our study may be the strong influence that environmental factors such as rainfall have on bobwhites. In the semiarid portions of the bobwhite geographic range, populations have been linked to precipitation, where populations can drastically increase during wet periods and decrease during drought (Hernández et al. 2005, Parent et al. 2016). However, in the Gulf Coast Prairie, too much precipitation can lead to flooding. Large areas of our experimental units were flooded during December 2018, possibly influencing our helicopter surveys and thus comparisons between control and treatment sites. Hurricane Harvey passed over all 3 of our study areas on August 25, 2017, causing damage and flooding at the end of our first field season. Maximum sustained winds ranged between 177–209 km/hr and rainfall exceeded 40 cm (NOAA 2017). We monitored 25 adult bobwhites before Hurricane Harvey hit and when we could re-enter our study areas 2 weeks later, there was 25% mortality ($n = 7$ bobwhites), of which 12% ($n = 3$ bobwhites) were located underwater. Hurricane Harvey was a powerful natural disaster that struck our study sites and may have impacted both bobwhite and fire ant populations in the area.

Collectively, there are a few possible explanations why we did not statistically detect influences of the fire ants or fire ant treatment on bobwhites in our study. We had low statistical power throughout our study which made it difficult for us to demonstrate a statistical difference if one existed. One biological reason is that fire ants would have to be the limiting factor for bobwhite populations in our study in order for a fire ant treatment to result in a population response. In our study, it is possible that the amount of habitat was a more influential factor than fire ants in bobwhite survival, nest success, and density. Although Allen et al. (2004) documented that bobwhite populations decreased over time following fire ant invasion in the Texas Coastal Bend, other researchers have highlighted that bobwhite habitat also has declined considerably in this area during the same timeframe (Allain 1999, Perez 2007). Another possible explanation for our results is that our sample sizes of radio-marked bobwhites, nests, and covey detections were low both years due to low bobwhite populations in the ecoregion (Perez 2007) and especially in our study area after the passage of Hurricane Harvey. As a result of these low sample sizes, our analyses may not have had the statistical power necessary to detect differences in survival, nest success, and bobwhite relative abundance between treatments. Additionally, because treatment efficacy as indicated by the number of foraging fire ants was poor on 1 of the 2 study blocks, we pooled data from the 2 blocks to evaluate treatment effects on bobwhites. This unfortunately may have diluted any effects that may have resulted from successful reduction in foraging fire numbers. Last, bobwhite response to fire ant treatment may require more than single applications or may exhibit a lag response, with effects appearing in future years. Thus, with repeated annual treatment, it is possible that fire ant densities would be reduced with a corresponding bobwhite response.

MANAGEMENT IMPLICATIONS

Even though fire ants have the potential to directly impact bobwhites, we did not statistically detect an influence of fire ants on bobwhites at the point-of-use or pasture scales in this study. However, after application of a fire ant treatment (Extinguish Plus), bobwhite survival and apparent nest success were higher than untreated control units. These trends indicate potential effects of treatment. Even though demographic performance of bobwhites was better in the treatment than control units, we did not detect a difference in overall bobwhite relative abundance 8 months post-treatment. Based on these findings, the following key points may be useful in managing bobwhites:

1. Fire ant treatment is expensive and may be impractical at a large scale.
2. Time, money, and effort may be better spent on creating habitat or increasing overall usable space to benefit bobwhites.
3. Given the relatively small sample sizes and potentially confounding effects of weather on our study, some results of our study (e.g., adult survival, apparent nest success) suggest that additional research on this issue is warranted.

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