

NORTHERN BOBWHITE CHICK MORTALITY CAUSED BY RED IMPORTED FIRE ANTS

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Abstract: Northern bobwhite (*Colinus virginianus*) populations have declined throughout their range during the last 30 years. Change in land management is thought to be a primary cause of this decline, but the invasion of northern bobwhite habitats by the red imported fire ant (*Solenopsis invicta*) also may be involved. We compared hatching success and subsequent survival of wild northern bobwhite chicks on the Coastal Prairie of Texas in 1997 and 1998 between broods that hatched under natural conditions or following fire ant suppression treatments. In 1997, the fire ant suppression treatment resulted in a 70% reduction in the number of red imported fire ants on baits placed in treated nests on the day after hatch. Using 2 fire ant suppression treatments in 1998 reduced the number of red imported fire ants on baits by >99%. No year or treatment×year interaction effects were detected for hatching success or survival, and no treatment effect was detected for hatching success ($P > 0.10$). However, the proportion of chicks surviving to 21 days was higher ($P = 0.010$) for treated nests ($n = 18$) than control nests ($n = 25$; proportions of broods surviving: $53.5 \pm 8.6\%$ [\pm SE] vs. $24.7 \pm 6.6\%$; chick survival: $60.1 \pm 7.6\%$ vs. $22.0 \pm 6.2\%$). The probability of chick survival decreased ($P < 0.001$) as our index of red imported fire ant activity in the nest increased. These results indicate northern bobwhite chicks can suffer high levels of mortality due to red imported fire ants, which could explain declines in some northern bobwhite populations following infestations by red imported fire ants. Current methods for controlling red imported fire ants are expensive and may last <3 months. Thus, strategies for mitigating the effect of red imported fire ants to northern bobwhite populations in this area should probably focus on reducing other mortality factors or increasing productivity.

Northern bobwhite populations have declined throughout their range during the last 30 years (Sauer et al. 1997). The major cause of these declines is thought to be loss of habitat due to changes in land use (Brennan 1991). Recently, Allen et al. (1995) presented associative and experimental results implicating the red imported fire ant as another factor that may be causing declines in northern bobwhite populations in areas where their ranges overlap. They found that the beginnings of population declines of northern bobwhites in Texas counties were correlated with the years the counties became infested with red imported fire ants; also, multiple treatments with a fire ant insecticide during 2 years resulted in areas having more northern bobwhites in the second year than untreated areas (Allen et al. 1995). Thus, it appears that red imported fire ants can limit northern bobwhite densities in some way.

Researchers have expressed strongly divergent opinions on the role red imported fire ants may play in northern bobwhite demographics (Brennan 1991, Allen et al. 1993, Brennan 1993). Some of these differences may stem from confusion about the different species of fire ants (*Solenopsis* spp.; Allen et al. 1993). Older studies (e.g., Stoddard 1931, Travis 1938, Johnson 1961, Lehmann 1984) that reported negligible effects of fire ants on northern bobwhites were conducted before colonization by red imported fire ants (Allen et al. 1993). However, recent studies that experimentally addressed the effect of red imported fire ants on northern bobwhites reported adverse effects (Allen et al. 1995, Giuliano et al. 1996, Pedersen et al. 1996). Red imported fire ants can directly affect individual northern bobwhites by killing chicks (Mitchell 1989, Giuliano et al. 1996) and altering their activity (Pedersen et al. 1996). In addition, red imported fire ant colonization may decrease the abundance of other invertebrates (Porter and Savignano 1990), an important component of northern bobwhite diets (Hurst 1972, Lehmann 1984:170-172). However, previous studies have not provided evidence that these particular factors affect northern bobwhite populations.

We hypothesized that the time of hatching was a period in the life history of the northern bobwhite when it was particularly vulnerable to red imported fire ants. We based our hypothesis on 2 facts. First, other species of fire ants commonly depredate or scavenge northern bobwhite chicks in the nest (Stoddard 1931, Travis 1938, Johnson 1961, Lehmann 1984). Second, in a laboratory study with red imported fire ants, survival of northern bobwhite chicks decreased following exposure to ≥ 50 ants for 60 sec or 200 ants for 15 sec (Giuliano et al. 1996). In a wild

northern bobwhite nest, it probably takes ≥ 2 hr from the time the first egg in a clutch begins to hatch until the entire brood has hatched (Stoddard 1931:36-37), and occasionally much longer (Lehmann 1984:89). During this period, the chicks may be restricted to the nest and vulnerable to stings by red imported fire ants. We designed this study to measure the effect of red imported fire ants on hatching success and subsequent survival of northern bobwhite chicks. We tested the null hypothesis that decreasing the number of red imported fire ants around northern bobwhite nests on the Texas Coastal Prairie would not affect northern bobwhite hatching success or subsequent survival.

STUDY AREA

We conducted this study on 8,000 ha of rangeland in Refugio County, Texas. Climate was subtropical with hot summers, mild winters, and average annual rainfall of 98 cm (Guckian 1988). Weather data were obtained from National Oceanic and Atmospheric Administration weather stations approximately 13 km southwest and 44 km southeast of the study area (National Oceanic and Atmospheric Administration 1997, 1998). Soils were primarily fine sandy loam or clay with slopes $< 1\%$ (Guckian 1988). Habitat was Coastal Prairie grassland (Gould 1975) interspersed with live oak (*Quercus virginiana*) motts, honey mesquite (*Prosopis glandulosa*), and huisache (*Acacia smallii*). In 1991--92, red imported fire ants occurred on the study area at a density of 205 mounds/ha and were predominately polygyne (Allen 1993, Allen et al. 1995).

METHODS

Study Design

Experimental units for the study were clutches of eggs in nests we found by tracking radiomarked birds. We assigned treatments to each clutch completely at random by using a coin toss as each nest was found. The treatment for each nest was either suppression of fire ants or no suppression. The model for the experiment was

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_{ijk}$$

The response variable (Y_{ijk}) was either the proportion of eggs that hatched successfully or the proportion of chicks that survived to 3 weeks of age. Two main effects, year and treatment, and their interaction were assessed. The 2

years of the study were 1997 and 1998.

Field Techniques

Field protocols were approved by the Texas Tech University Animal Care and Use Committee (approval numbers 96610 and 97710). We captured female northern bobwhites from 1 February through 23 April in 1997 and 1998 primarily with walk-in funnel traps (Smith et al. 1981) baited with milo. Each captured hen was radiomarked with a 6.5-g necklace-style radio transmitter without a body loop (American Wildlife Enterprises, Montacello, Florida, USA).

From late April until mid-July, we located most radiomarked hens at least once every other day to find nests. We approached each bird on foot until it flushed or moved away, we observed it, or we had circled it and determined that it might be on a nest. We never intentionally flushed hens from nests and rarely did so inadvertently. When each nest was found, we randomly assigned it a treatment. For the fire ant suppression treatment, we used a handheld broadcast spreader to apply Amdro® (American Cyanamid Company, Wayne, New Jersey, USA), a fire ant insecticide bait, at a rate of 1.7 kg/ha. We treated a 60- × 60-m area centered on the nest. The "no suppression" treatment included the same activities as the fire ant suppression treatment, but without application of the fire ant insecticide bait. Any nest found <100 m from another nest during the same year was constrained to receive the same treatment; this constraint affected <15% of nests. Application of Amdro® at rates used in this experiment typically results in a 90% decrease in red imported fire ant abundance (Harlan et al. 1981). Initial measurements of effectiveness of the fire ant suppression treatment (described below) found many red imported fire ants in several treated nests. To more closely attain our goal of no ants in treated nests, we broadcast Amdro® directly over the nest for several seconds during the initial application, and we applied a second treatment of Amdro® to a 20- × 20-m area centered on the nest 1 week before the expected hatch date. These modifications affected treatment of 3 nests that hatched in 1997 and all treated nests in 1998.

We measured the effect of red imported fire ant reduction on hatching success and survival of chicks to 3 weeks of age; in addition, we obtained limited data on survival of chicks to 1 day of age. Hatching success was estimated on the day of hatch by examining eggs and eggshells in the nest. Successfully hatched eggs were easy to distinguish by the even line of pipping that separated the eggshell cap from the remainder of the eggshell.

Unsuccessfully hatched eggs were either unpipped or partially pipped with dead chicks inside. In addition, any dead chicks found in or around the nest were classified as an unsuccessfully hatched egg. On the day after hatch, we often approached hens and used the stereotypical behavior of hens with chicks to determine the number of broods that suffered 100% mortality within 1 day. This technique could not provide information beyond presence or absence of chicks; broods in which nearly all chicks died could not be distinguished from those that had 100% survival. Three weeks after the hatch, we flushed and counted the hen and chicks to measure chick survival. We repeated these counts with 1 or 2 observers and up to twice per day until we were confident of brood size.

We used a 30-mL plastic cup baited with a slice of hot dog (approx 1 g; Porter and Tschinkel 1987) to determine efficacy of the fire ant insecticide treatment and assess red imported fire ant foraging activity in the control nests. On the day of hatch, we removed all unhatched eggs and eggshells from the nest to eliminate residual yolk in the eggshells to which the ants might be attracted. On the morning after the eggs hatched, we placed a bait cup on its side in the nest. After 30 min, we capped the bait cup and placed it in a freezer (-20°C) to kill the ants. Bait-cup sampling was conducted within the thermal range of maximal foraging by red imported fire ants (22--36°C at depth of 2 cm; Porter and Tschinkel 1987). We counted the number of red imported fire ants in each cup and stored all ants in 70% ethanol for later verification of species identification.

We measured the density of active red imported fire ant mounds on the study area during August 1997. We counted the number of red imported fire ant mounds in 18 20- × 20-m areas centered on northern bobwhite nests that had received the placebo treatment. To assess presence of red imported fire ants in all potential mounds, we used a shovel to dig down to visible soil moisture where ants would be if present.

Data Analysis

We compared the proportions of eggs that hatched successfully and chicks that survived to 3 weeks of age between treatments and years using analysis of variance (ANOVA). The data were analyzed as a 2 × 2 factorial with years and treatments as main effects and year×treatment interaction. The year effect was analyzed first as a fixed effect (Model I ANOVA; Zar 1996:244) to draw inference to treatment effects during the 2 years of this study, and then analyzed as a random effect (Model III ANOVA or mixed model; Zar 1996:247) to draw inference to the probability of treatment effects during any year. Thus, the denominator for the *F*-test assessing differences

in treatment means when the year effect was fixed was the mean square error of the model, and the denominator for this test when the year effect was random was the mean of the sum of squares for the interaction term. We followed the recommendations of Cochran (1943) and determined that each nest or brood should receive equal weighting. The analysis was repeated using the ranks of the proportions due to heteroscedasticity and nonnormality (Zar 1996:269-270). Calculations were made with SPSS for Windows (Norušis 1993). We report means of the proportions of eggs that hatched successfully and chicks that survived to 3 weeks of age; these means were used for experimental comparisons. We also report mean chick survival to 3 weeks of age for each treatment as calculated via an equation for cluster sampling (Cochran 1963:65). We estimated the percentage of northern bobwhite mortality at 21 days attributed to red imported fire ants (m_a) at hatching by using the equation

$$m_a = \left(1 - \frac{m_o}{m_{a+o}}\right) \times 100,$$

where m_o = the percent mortality at 21 days for the fire ant suppression group, and m_{a+o} = the percent mortality at 21 days for the control group. Means are reported ± 1 standard error.

We used logistic regression to assess the effect of red imported fire ant foraging activity on chick survival (PROC LOGISTIC; SAS Institute 1989). Our model was (number of chicks from nest i alive at 3 weeks)/(number of chicks from nest i that hatched) = (number of red imported fire ants in the bait cup in nest i). We tested for a linear relation between the logit of chick survival and the number of red imported fire ants in the bait cup in the nest on the day after the hatch by using a full--reduced model likelihood ratio chi-square test (SAS Institute 1989).

RESULTS

Precipitation differed tremendously during the 2 years of the study. Rainfall from March through July 1997 was 165% of the long-term mean; during the same period in 1998, rainfall was only 23% of the long-term mean (National Oceanic and Atmospheric Administration 1997, 1998).

We radiomarked 77 northern bobwhite hens in 1997 and 71 in 1998. Of 79 nests found in 1997, 37 were depredated by vertebrates, 35 hatched, 6 were abandoned, and 1 was trampled by cattle. In 1998, we found 87 nests: 44 were depredated by vertebrates, 27 hatched, 13 were abandoned, 1 was lost when the hen died, and 2

were still being incubated when the study ended. Four of the 62 successful nests in 1997--98 were disturbed by predators before the number of eggs that hatched could be counted, and 1 nest could not be assigned to a treatment due to its proximity to both treated and untreated nests. These 5 nests were censored. Of the remaining 57 broods, 43 were successfully counted at 3-weeks of age to measure survival.

Effects of Red Imported Fire Ants

Hatching success did not differ by treatment ($F_{1,53} = 0.98, P = 0.327$) or year ($F_{1,53} = 1.89, P = 0.175$), and no treatment \times year interaction was detected ($F_{1,53} = 0.53, P = 0.472$). In the 35 control nests that were successful, 457 of 483 eggs (94.6%) hatched successfully. We found 10 of these unsuccessful eggs pipped or hatched, and observed dozens of red imported fire ants feeding on each dead chick. In the 22 nests treated with insecticide, 265 of 289 eggs (91.7%) hatched successfully. We found 4 of these unsuccessful eggs pipped and 1 cracked, but observed no red imported fire ants on these chicks.

Chick survival differed greatly between treatments. The proportion of chicks surviving to 21 days was higher for treated nests ($n = 18$) than control nests ($n = 25$; proportions of broods surviving: $53.5 \pm 8.6\%$ vs. $24.7 \pm 6.6\%$, year analyzed as a fixed effect, $F_{1,39} = 7.35, P = 0.010$; chick survival: $60.1 \pm 7.6\%$ vs. $22.0 \pm 6.2\%$). Death of all chicks occurred in 52% of broods from control nests and 22% of broods from treated nests. The percentage of mortality at 21 days attributed to red imported fire ants at hatching (m_a) was 48.8%. For this calculation, we did not include the 2% of chicks from control nests that were found dead and covered with red imported fire ants because these chicks may have died of other causes and inclusion of these mortalities had little effect on the resultant value. No year effect ($F_{1,39} = 0.26, P = 0.612$) or treatment \times year interaction ($F_{1,39} = 0.18, P = 0.670$) were detected. Results using ranked data were similar. When the year effect was analyzed as a random effect, results were less conclusive ($F_{1,1} = 39.91, P = 0.100$). The power of this test was low because the study was conducted for only 2 years, which resulted in only 1 degree of freedom for the denominator in the F -test. We monitored 12 broods on the day after hatch that were later found to have no chicks alive at 21 days. Six of 10 control broods and both treated broods in this category had lost all of their chicks within the first day.

The number of red imported fire ants in bait cups in control nests averaged 89 ± 59 more ants in 1997 ($n = 358 \pm 43$ ants in 1997 and 269 ± 39 ants in 1998). The logit of chick survival decreased as the number of red

imported fire ants in a bait cup increased ($\chi^2_1 = 95.0, P < 0.001$; Fig. 1). The probability of a chick surviving decreased by 0.5% with each additional red imported fire ant. Mean red imported fire ant mound density in August 1997 was 290 ± 60 mounds/ha (range = 50--1,000).

In 1998, 1 nest appeared to have been abandoned due to red imported fire ants. The nest had been constructed next to a clump of grass that contained an abandoned red imported fire ant mound. The ants reoccupied the mound after a 3.5-cm rainfall preceded by 2 months of drought, and they covered the eggs in the nearby northern bobwhite nest with soil litter.

DISCUSSION

Time of Mortality

The benefit of fire ant suppression in our study probably was experienced by northern bobwhite chicks only while hatching and for a few hours afterward. Chicks were led away from the nest by 1 or both parents, were always outside the treated area 24 hr following hatching, and were never relocated in a treated area. Thus, chicks from control and treated nests used the same environment after leaving the nest. Few chicks in our study were killed in the nest. After 3 weeks, however, survival of broods from control nests was less than one-half that of broods from treated nests. Much of the mortality may have occurred soon after the chicks hatched; this possibility is supported by our data showing that 60% of the control broods that suffered 100% mortality by 21 days had already experienced this mortality by the day after hatch. The following field observation also supports this possibility and illustrates that deaths may have resulted from indirect effects of red imported fire ant stings.

At a nest site with a red imported fire ant mound density of 1,000 mounds/ha, we found 3 dead chicks (2 still in their eggs) in the nest covered by red imported fire ants. We observed the hen flush 15 m from the nest and captured 1 chick that attempted to hide at our feet. This chick had 1 eye swollen shut from red imported fire ant stings, yellow marks on its feet that may have been sting sites, and 1 red imported fire ant still stinging its foot. Pedersen et al. (1996) reported that red imported fire ants often attacked and stung newly hatched and 1-day-old northern bobwhite chicks on the eyelids, legs, and toes; stings to the eyelids caused swelling that obscured vision, and stings to the legs and feet sometimes caused swelling that prevented normal movement. Undoubtedly, the probability of a chick surviving decreases if its vision and movement are hindered by red imported fire ant stings.

Chick Survival

Past studies that estimated northern bobwhite chick survival from repeated counts of chicks in broods associated with radiomarked adults found survival to 2 weeks of 13% ($n = 5$ broods; Cantu and Everett 1982) and 38% ($n = 22$ broods; DeVos and Mueller 1993) and survival to 3 weeks of 38% ($n = 59$ broods; DeMaso et al. 1997). The study area of DeVos and Mueller (1993) was within the range of the red imported fire ant. When we compared our results with these studies, survival of chicks that were protected from red imported fire ants (60%) was higher, and survival of chicks from control nests (22%) was within the range of previously reported estimates.

When we assumed survival rates of chicks in our 2 treatment groups were identical after 21 days, additive mortality of northern bobwhite due to stings from red imported fire ants at the time of hatching was $38.1 \pm 7.7\%$. Our estimate of additive mortality was the difference in chick survival for the 2 treatments. This difference would have been greater if indirect mortality from red imported fire ant stings did not occur within 21 days. Giuliano et al. (1996) observed that northern bobwhite chicks that survived the most severe exposures to red imported fire ants weighed less than controls after 9 days (the duration of their experiment), and they speculated that depressed growth could reduce subsequent survival because chick size may be related to survival. However, the difference in survival rates could have decreased subsequently due to density-dependent factors. Our data could not resolve if the difference in mortality between the 2 treatment groups changed after 21 days.

Northern bobwhite frequently attempt >1 nest/season (Rosene 1969:73, Roseberry and Klimstra 1984:83) and may occasionally raise >1 brood in a season (reviewed by Guthery and Kuvlesky 1998). These attributes allow the possibility that northern bobwhite could exhibit compensatory reproduction in response to losing broods rapidly due to red imported fire ant stings at hatching. However, when an entire brood is killed by red imported fire ants, it occurs after an investment of >37 days by the hen (average clutch size of 14 eggs [this study], lay rate of 1 egg/day, 23-day incubation period, and time to build the nest and lay the first egg that Rosene [1969] estimated at 6--12 days). Burger et al. (1995) found the interval between successful brood rearing and renesting was about 14 days longer than the interval between nest failure and renesting. Thus, if red imported fire ants kill only some chicks in a brood, even more time will pass before the hen will attempt to reneest. In either case, mortality by red imported fire ants at hatching is more severe than an equivalent proportion of mortality from nest destruction

because of the greater loss of time for reproduction.

We observed no renesting after the loss of an entire brood of chicks, but we only monitored hens through July. Guthery et al. (1988) found that nearly half the northern bobwhite hens in our region of Texas were in laying condition in August; thus, hens had ample time to produce additional clutches. Hens also may have produced clutches that were incubated by males (Burger et al. 1995, DeMaso et al. 1997). Further investigation is needed to determine how time constraints resulting from a limited reproductive season and a large investment of time in chicks that are killed by red imported fire ants affect potential compensatory reproduction.

The scope of inference from this study is limited to 2 years at 1 site. Here we consider the reasonableness of extrapolating these results to other times and areas. The statistical test of treatment effects in the context of all possible years (i.e., years as a random variable) was not conclusive ($P = 0.100$). Because precipitation affects northern bobwhite populations (Rice et al. 1993) and activity of red imported fire ants (Rhoades and Davis 1967), it was probably the most important variable in the "year" effect in this study. Fortuitously, precipitation during this study represented periods of flooding one year and drought the next. Treatment effects were consistent between these conditions. Thus, we expect effects of red imported fire ants on hatching northern bobwhites to vary little under the extremes of precipitation that occur on the Texas Coastal Prairie. However, many other variables could cause differences in the treatment effect, and our data were not adequate for supporting the effects we observed over the universality of time. Expanding the inference from this study to other regions is even more problematic. Because characteristics of red imported fire ants and northern bobwhite may vary among locations, replication of this study in other regions is needed to understand how well the functional relation described in Figure 1 applies to other regions.

Effectiveness of Treatment

The number of red imported fire ants in bait cups exceeded 200 ants at 3 of the first 9 treated nests in 1997 (Fig. 1); thus, we added a second treatment to attain better ant suppression. Changing our methodology probably did not confound results. First, chick survival for these 3 nests did not differ ($P = 0.6$) from that of other nests treated in 1997. Second, chick survival across years was not different ($P = 0.4$) between nests treated either once or twice. Thus, while the additional treatment was valuable for suppressing red imported fire ants at some nests, it did not cause a detectable change in chick survival. We recommend using 2 applications of fire ant insecticide to suppress red imported fire ants in research applications to ensure distinct differences in numbers of red imported fire ants between treatments.

Interpreting Evidence at Nests

We observed the outcome of eggs hatching in 3 nests abandoned by the hen (1 due to a western diamondback rattlesnake [*Crotalus atrox*] in the nest, 1 due to the initiation of brooding of the first chick that hatched days before the others, and 1 due to disturbance by us). The death of chicks in these nests would have been attributed to red imported fire ants if the circumstances had not been understood. These nests were tallied as abandoned nests in our study and not included in any subsequent analysis. Most of the chicks in 2 of these broods were found dead near their nests and appeared to have been killed by red imported fire ants. The third brood would probably have suffered the same fate, but that nest had been treated with fire ant insecticide and the chicks were seen alive around the nest without a parent for 2 days. These observations confirm that proving red imported fire ants are responsible for killing northern bobwhite chicks is difficult when researchers simply observe hatches (Johnson 1961).

MANAGEMENT IMPLICATIONS

Red imported fire ants have probably degraded suitability of habitat for northern bobwhites throughout the Texas Coastal Prairie due to the mortality they inflict on northern bobwhite chicks. Current methods of controlling red imported fire ants on large areas using insecticides are expensive ($\$20.00 \text{ ha}^{-1} \text{ treatment}^{-1}$; Drees et al. 1996) and may last <3 months (Apperson et al. 1984). Allen (1993) found that insecticidal control of red imported fire ants to increase northern bobwhite and white-tailed deer (*Odocoileus virginianus*) densities for lease hunting was

not warranted economically. However, insecticidal treatments may play a valuable role in management of threatened and endangered species (e.g., Lockley 1995). One endangered species that occurs near our study area that might benefit from insecticidal control of red imported fire ants is the endangered Attwater's prairie chicken (*Tympanuchus cupido attwateri*).

Because of the high cost of reducing red imported fire ants directly through insecticide, we think that the best current management for northern bobwhite populations is limited to mitigating red imported fire ant mortality. Thus, reduction of other mortality factors and increasing productivity via traditional means has become more critical with the invasion of northern bobwhite habitat by red imported fire ants. The most cost-effective solution presently is to manage land so that it provides usable space (Guthery 1997) for northern bobwhites in the form of an interspersed nesting, brood-rearing, feeding, loafing, and roosting cover demonstrated for decades to benefit northern bobwhites (Stoddard 1931, Rosene 1969).

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Fig. 1. Relation between the proportion of northern bobwhite broods surviving to 3 weeks and number of red imported fire ants in a bait cup after 30 min on the day after hatch ($n = 43$). Broods from nests treated to suppress red imported fire ants are displayed separately from broods from control nests. The logit of chick survival decreased with increasing numbers of red imported fire ants ($P < 0.001$). The equation depicted by the line is $y = \frac{e^{(0.55 - 0.0054x)}}{1 + e^{(0.55 - 0.0054x)}}$. Arrows and associated numbers denote the number of overlapping values at those points.

