

2007

Effect of red imported fire ant (*Solenopsis invicta* Buren) on the nesting success of Northern Bobwhite (*Colinus virginianus* L.)

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**EFFECT OF RED IMPORTED FIRE ANT
(*SOLENOPTIS INVICTA* BUREN) ON THE
NESTING SUCCESS OF
NORTHERN BOBWHITE (*COLINUS VIRGINIANUS* L.)**

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Entomology

by
Michael Andrew Seymour
B.S., Louisiana State University, 2001
May 2007

DEDICATION

I dedicate this work to my nieces and nephews: Anna, Claire, Andrew, Matthew, and Michael. My wish is that this work and others like it shed light on the importance of our natural heritage and the complexities of nature so that your generation and the ones that follow will have the same opportunities enjoying the natural world that I had.

ACKNOWLEDGMENTS

This collaborative effort benefited greatly from the assistance of intelligent, talented, and dedicated people. I thank my committee, Dr. Linda M. Hooper-Bùi, Dr. Dorothy P. Prowell, Dr. J. V. Remsen, Jr., and Dr. Dearl E. Sanders for their invaluable support, constructive criticism, and pep talks over the course of this project. The project certainly would not have succeeded without their help; in particular, Dr. Bùi's previous work with birds and fire ants significantly aided me in this work. Dr. James P. Geaghan assisted me in statistical analyses of the project.

I arrived in the LSU AgCenter's Red Imported Fire Ant Laboratory in 2002 after learning from Dr. Tom J. Riley that the lab would benefit from a GIS technician. I will be forever grateful to Tom for introducing me to this lab and its researchers. In fact, I stayed in the fire ant lab and became a graduate student, because I was so impressed with the ensemble that Dr. Bùi had assembled from the experienced field researchers like Keri Landry, Jessica Rosson, and Heather Story to the computer and graphics arts genius Stacy Clayton. I'm proud to have called them my friends and colleagues.

Other than conceptual and logistical issues resolved with assistance from my committee and colleagues, arguably, the most difficult portion of the work was likely the procurement and construction of the materials and infrastructure, namely 27 walk-in aviaries built on site inside forests. I thank the front office staff in the LSU Department of Entomology for helping me with purchase orders and LaCarte charges for what must have appeared to be bizarre purchases at times, including the large, outdoor dog kennels used for the aviaries. I will be forever indebted to Lee Womack for his help moving and building cages; in fact, it might be more correct to say that at times I helped him! Cary Hebert was a great asset who helped me build the majority of the aviaries in the study; his positive attitude and willingness to work in adverse weather

conditions was a major plus. I also thank Shawn Dash, E. Paul Mumma, Kimberly Seymour, and Dr. Beverly Wiltz for their assistance in cage construction. The folks at Marco Specialty Steel of Houston, Texas, were helpful in suggesting which wire might be best for such construction. I thank aviculture expert and exotic veterinarian Dr. Joseph Smith for his advice on aviary design and materials.

Several people helped to procure Northern Bobwhite for this project. I am grateful for my friendship with John Wenzel of the Mid America Hunting Association, who offered quail-trapping on his property in Kansas after I posted a request on an internet website, a request I assumed would go unanswered. The kindness of the people of Kansas will not be forgotten. Mike Olinde of LA Department of Wildlife and Fisheries supplied documents on quail trap designs. I also appreciate the quail growers and hunting clubs that supplied birds for the project. I thank Sherry Bond of S & D Game Bird Farm, Vernon Fusilier of Shallow Lake Hunting Preserve, Mike Guillory of Bayou Teche Hunting Preserve, Kathy and Wayne Robertson of The Quail's Nest, and Frank Cardwell of V & C Quail Farm.

I also thank Melvin "Brother" Bell of Tailwinds Air, Inc. and Allen Walker of North Star Helicopters for aerially treating plots with Amdro®. Kathie Kalmowitz of BASF supplied Amdro® for the project. I am grateful for the field assistance and 4-wheeler expertise of Lee Womack, who managed to park his 4-wheeler atop an active Yellow Jacket nest. His ability to drive 4-wheelers through dense brush of brambles and briars while avoiding stumps, logs, holes, and, usually, tree trunks is admirable. I also thank all the folks at Idlewild Experimental Research Station; I'm glad y'all have a tractor large enough to pull a Dodge Ram 1500 out mud up to the tailpipe. Thank you, Dr. Sanders and all those at Idlewild who offered their assistance

when I needed help. In some cases, I probably would have been spending the night in the truck if it were not for your help!

I greatly appreciate the field assistance of Rebecca Baillif, Caren Carney, Stacy Clayton, Andy Fulks, Kathryn O'Brien, Jessica Rosson, and Kimberly Seymour, which occasionally included caring for birds while I was away. Emily Marcum, Courtney Prejean, Rachel Strecker, Sowmya Subramanian, and Lee Womack saved me valuable time by gathering much of the literature used in this work or in the developmental stages of this project.

I used 1350 food traps in this study. Their contents were counted and cleaned by several student workers and myself. Student workers assisting in that phase of the project or providing general laboratory assistance include Douglass Alongia, Rebecca Baillif, Leon Cannizzaro, Heather DiMaggio, Jennifer Fleming, Thabit "Lola" Folami, Andy Fulks, Cary Hebert, Huei-Yang Ho, Joseph Lantz, Jesse Prejean, Kristin Prejean, Nathan Saucier, Bart Savant, Susan Scoby, Uduak Udoh, and C. Ryan Walker.

Lee Womack was integral in identifying all ant species that were not red imported fire ants trapped in this study. He spent several days behind a microscope with ant specimens and paging through dichotomous keys ensuring that my specimens would be identified correctly in time for the completion of my Master's work. He succeeded with time to spare, and, for that, I am truly grateful.

My greatest accomplishment during my time as a graduate student at LSU was likely the maintenance and culmination of a relationship with a young woman I met through colleague Kathryn O'Brien. Kimberly Kennedy and I dated during what may ultimately be the most stressful times in my life. Her love, support, and understanding helped get me through the rough

spots in the project and provided companionship and funny stories in the field I'll cherish till the day I die. Kimberly and I were married on 14 August 2005. Thank you, Kimmy.

I also thank my parents, Robert and Shirley Seymour, my siblings, Mark Seymour and Melissa McConnell, my mother- and father-in-law, Steve and Terri Kennedy, and my sister- and brothers-in-law, Shannon Seymour, David Kennedy, and John McConnell. I sincerely appreciate the love, support, and funding over the many years! Thank y'all very, very much!

I'd be remiss if I didn't thank several other folks in my life whose guidance and selflessness got me where I am today. I thank Dr. Marcella Hackney, who was my biology teacher at Scotlandville Magnet High School for Engineering Professions. Her passion for teaching life sciences certainly fueled my passion for knowledge of the subject. Several birders took me under their wings early in my birding career for which I am very appreciative: Elizabeth Brown, the late Margie Griffith, Grace Lutschg, and Joe Kleiman. My home-away-from-home for many years from high school through undergrad was the LSU Museum of Natural Science, where I benefited tremendously by the mentorships of Steve Cardiff, Donna Dittmann, and Dr. J. V. Remsen, Jr., who all provided valuable input on the project or specimen preparation expertise. I also thank Dr. Jeff Boundy, Dan Lane, Dr. Gary Lester, Steve Shively, Dr. Joseph Smith, Dr. Latimore Smith, M. Mark Swan, and Bill Vermillion who influenced my interest in birding and other pursuits of nature.

Projects such as mine are best pursued only with support garnered from authorities in the field. I was lucky enough to have all those required right here at LSU whether on campus or at a research station. Finally, I thank LSU and LSU AgCenter for funding for this project and for employing experts in the fields of entomology, ecology, ornithology, and animal husbandry.

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ABSTRACT

The Northern Bobwhite (*Colinus virginianus* L.) has experienced average yearly decline of 3% in the United States from 1966-2005. Factors implicated in decline include habitat destruction, alteration of farm practices, and red imported fire ant (RIFA) (*Solenopsis invicta* Buren). Effect of RIFA on bobwhites has been a hot topic leading to formation of polarized camps – those who believe RIFA have little effect and those who believe the effect will further threaten the species' survival. The main objective of this study was to determine if RIFA affect nesting of bobwhites and at what stage in nesting birds are most vulnerable. Effect of broadcast-spread fire ant bait on RIFA and non-target ant species also was studied.

In 2005, eight 4.45 ha plots, each with one aviary housing breeding bobwhites from Louisiana captive stock, were paired by habitat feature. Four plots were broadcast-treated aerially with Amdro® (0.73% hydramethylnon). Sticky traps indicated successful dispersal of bait, although percent composition of particle size on traps differed from that expected. Food traps were used to measure success of RIFA suppression and to determine effect of bait on non-RIFA ants. In 2006, number of plots was reduced to six (three pairs), but number of aviaries per plot increased to four. Two aviaries per plot contained captive-bred bobwhites from Arkansas, whereas the other two contained Louisiana captives.

In addition to RIFA, which was successfully suppressed on treated plots, *Aphaenogaster fulva-rudis-texana* was negatively affected by treatment. Data from other myrmecines suggested similar patterns of decrease by treatment. Only *Prenolepis imparis* showed a possible competitive release from RIFA.

In 2005, lack of nesting reduced number of replicates. One nest hatched successfully; another failed – overrun by RIFA. In 2006, mean number of nests, eggs, and chicks did not

differ significantly between treated and untreated plots nor between the two populations of bobwhites. A significantly greater proportion of AR and LA nests were attacked by RIFA on untreated plots. Three nests hatched successfully on treated plots versus one on untreated. RIFA appear to breach intact bobwhite eggshells. In areas of sympatry, RIFA may exacerbate bobwhite decline by attacking eggs prior to hatch.

CHAPTER 1

INTRODUCTION

THE ORGANISMS OF STUDY

The organisms featured in this work could not be more different; their body designs, breeding behaviors, and taxonomy are dramatically dissimilar. Their life histories unite them – particularly the range over which they occur in the USA and their habitat preferences, especially their utilization of edge and open areas.

THE RED IMPORTED FIRE ANT. A hymenopteran, the red imported fire ant (RIFA), *Solenopsis invicta* Buren, is in the family Formicidae, subfamily Myrmecinae. It is small (2-6 mm long) and polymorphic – different sizes of worker ants within the same colony. Workers are usually reddish brown to dark red with a darker, often black gaster (the last several segments of the abdomen). Female alates, or reproductives, look relatively similar to workers except for a more robust thorax that allows for wing musculature, and alates are significantly larger. Male alates are large, black, and have a disproportionately small head. Workers may be identified through a combination of characters – a ten-segmented antenna with a two-segmented club; a two-noded pedicel, the “waist” of the ant; a median clypeal tooth; four teeth on the mandible; an inverted “Y” on frons; lack of anteroventral tooth on petiole; and striations on the mesopleuron (Vinson and Sorensen 1986, Trager 1991, Vinson 1997). Voucher specimens of ants from this study were deposited in the Louisiana State Arthropod Museum at Louisiana State University.

THE NORTHERN BOBWHITE. A galliform, the Northern Bobwhite, *Colinus virginianus* L., in the family Odontophoridae, received its common name from its most recognized call. It is a small, stocky, short-tailed, ground-nesting, terrestrial bird of approximately 26 cm and 165 g. Both males and females have boldly patterned faces. The male has very dark brown, almost

black, eye stripes that start at the lores, continue back on the sides of the head, and meet with the sides of a dark band on the upper breast. In the male, the throat and supercilium, the stripe above the eye, are immaculate white; the female is buff on the throat and supercilium. Both sexes have varying degrees of cryptically patterned back and scapular feathers that allow the birds to remain hidden in ground cover (Stoddard 1931: 79-83, Terres 1991: 688-689). The Institutional Animal Care and Use Committee (IACUC) of Louisiana State University AgCenter accepted the protocol of this project under protocol number AE01-04. Voucher specimens from this study were deposited in the Louisiana State University Museum of Natural Science.

THE HISTORY OF ANTS AND QUAIL

THE INVASION OF THE RED IMPORTED FIRE ANT. The red imported fire ant, *Solenopsis invicta* Buren, is an invasive, exotic pest that occurs throughout much of the southern United States, as well as irrigated parts of the desert Southwest. A stowaway in a shipment or in ballast of trading ships from South America in the early 1930's, the ant was first introduced to the Port of Mobile, Alabama (Vinson 1997). Released from predators, competitors, and other ecological constraints it might have faced in its native lands in Argentina, Paraguay, and Brazil, the pest has been able to colonize new areas of the USA with little resistance from our native fauna or climate (Vinson 1997). RIFA has recently spread worldwide to Puerto Rico, several Caribbean Islands, several provinces in China, Taiwan, New Zealand, and Australia among other places (Callcott and Collins 1996, Davis *et al.* 2001, Harris 2001, Nattrass and Vanderwoude 2001, Pascoe 2001, Moloney and Vanderwoude 2002, Morrison *et al.* 2004, Chen *et al.* 2006, Tschinkel 2006: 16). Populations of native vertebrates and invertebrates, even whole community structures, have experienced drastic alterations and restructuring in the wake of the

invasion front (Glancey *et al.* 1976, Porter *et al.* 1988, Camilo and Philips 1990, Porter and Savignano 1990, Vinson 1994, Allen *et al.* 2004).

Prior to the arrival of RIFA, as the United States was at war overseas, a small, unobtrusive insect invaded the homeland (Blu Buhs 2004: 15-21). Like the more infamous species that would arrive some twenty years later, this ant would be allowed into the country with little notice until it was too late to stop its advance. The species we now know as the black imported fire ant (BIFA), *Solenopsis richteri* Forel, arrived in the Port of Mobile, Alabama, sometime in the late 1910's (Vinson 1997). BIFA soon spread to much of the Southeast, including much of Mississippi and Alabama (Vinson 1997). The black imported fire ant's more dominant congener, the red imported fire ant, quickly outcompeted the black as the red spread in the 1940's. Today, the black imported fire ant's range is restricted to northeast Mississippi and northwest Alabama, although its hybrid with the red imported fire ant occurs over a much broader range including parts of Georgia (Allen *et al.* 1994, Vinson 1997). Interbreeding with the red imported fire ant is another reason why the black imported fire ant became less common in the USA (Taber 2000: 113-114, Gibbons and Simberloff 2005).

A history lesson on the black imported fire ant may seem out of place in a work that discusses the impacts of the red imported fire ant on fauna, but until recently, the scientific community made no species level distinction between the two (Horton *et al.* 1975, Allen *et al.* 1994). Not until the 1970's was it clear that the USA had been invaded, and possibly on multiple occasions, by two different species of imported fire ant (Buren 1972). At the time of the invasions, both the red and black were referred to as *Solenopsis saevissima*. Several studies were performed that evaluated the effect of this "species" on native fauna, including Northern Bobwhite. Unfortunately, because little or no attempt was made to determine whether the ants

were the black form or red form, studies prior to the early 1970's may spark more confusion about the issue than they resolve (Horton *et al.* 1975, Camilo and Philips 1990, Allen *et al.* 1994). Over fifty years after the first imported fire ant invaded, Buren (1972) sorted out the taxonomy of the species; he assigned the black form to *S. richteri* Forel and the red form to *S. invicta* Buren. Unbeknownst to Buren, Santschi had already named the red species *S. wagneri* in 1916 based on South American specimens. This is a rare example of when Rules of Priority set forth by the International Commission on Zoological Nomenclature were overruled, and the popularized use of a name allowed a junior synonym to become the accepted name (Shattuck *et al.* 1999). That is, if a species is found to possess an older scientific name, then the new name must be retired, and the accepted scientific name should revert back to the older name – such was not the case with *S. invicta*.

No matter what name is given to the red imported fire ant, whether it's a tribute to a German entomologist or it's a reference to the fire ant's "invincible" nature, one thing is quite certain – red imported fire ant (hereafter RIFA) has the ability to rapidly invade new areas and the ability to assimilate into new ecosystems with ease.

Anthropogenic introduction of this invasive exotic continues today despite the USDA's 1958 Federal Imported Fire Ant Quarantine regulation regarding interstate transport of nursery root stock and soil-moving machinery (Vinson 1997). This regulation restricts movement of plants to those chemically treated to control RIFA. Vinson and Sorensen (1986) gave four methods of spread of RIFA colonies: (1) through the aforementioned transport of "colonies or mated queens in nursery root stock and sod or in soil used during construction; (2) during natural mating flights...; (3) mated queens may land in trucks, train beds or in other open containers that are moved from place to place; and (4) after being flooded from their mounds by heavy rains,

colonies form rafts that float to new locations.” As with other non-native organisms, RIFA has encountered few natural predators, which has allowed it to spread throughout much of the southern United States. In fact, RIFA are rarely vulnerable once a nest is established, although sizeable numbers may be taken during mating flights or in early stages of nest construction (Whitcomb *et al.* 1973, Nichols and Sites 1991). As of 2006, RIFA could be found in North and South Carolina, Georgia, Florida, Alabama, Tennessee, Mississippi, Louisiana, Arkansas, Texas, Oklahoma, New Mexico, California, and Puerto Rico, inhabiting a total of more than 128 million hectares (Williams *et al.* 2001) (Appendix). Several new quarantined counties are added each year (Anonymous 2006).

Minimum yearly temperatures and minimum amounts of precipitation may be main factors limiting the spread of RIFA. Colonies of *S. invicta* will likely not spread beyond a -15°C isoline in the southeastern USA (Korzukhin *et al.* 2001). The ant’s spread in the West has been restricted to “wetter lowlands and irrigated or watered land” (Vinson 1997). Areas with suitable temperatures may expect infestations if the area receives ≥ 10 inches (~25 cm) of rain per year. In areas of favorable conditions, the fire ant’s spread west is occurring at 20-30 miles (~32-48 km) per year (Vinson and Sorensen 1986).

Exotic species that have the ability to spread without strict boundaries will frequently do so and may become invasive pests. It is believed that RIFA experienced a “lag phase” in the early stages post-introduction. A lag phase may allow a species’ population to grow in one or many locales unnoticed until the population grows to a point at which rapid reproduction, and population growth, takes place (Mack *et al.* 2000). Disturbance, from which many invasives such as RIFA thrive, likely increased in the Mobile area during World War II and allowed rapid

expansion of this ant (Blu Buhs 2004: 21-38). Delayed disturbance could be the reason for the lag phase in the early invasion (Blu Buhs 2004: 21-38).

Mack *et al.* (2000) listed several factors affecting lag phase (or a perceived lag phase): limits on detection of population's growth, number and arrangement of infestations of immigrants, natural selection among rare or newly created genotypes adapted to new range, and vagaries of environmental forces. RIFA may have been able to remain undetected in Alabama, because few people at the time would have recognized the ant or that the ant was a new species for our continent. It is unknown if RIFA were introduced in one or multiple introductions, but some authorities believe that at least two may be required for a specialized breeding system called polygyny (discussed below) (Tsutsui and Saurez 2003). In the case of polygyne fire ants, Tsutsui and Saurez (2003) proposed that rather than a bottleneck, an increase in potential genotypes (from a presumed second introduction) is what caused a massive surge in fire ant numbers. In all introductions, the exotic species faces an ecological crapshoot; in RIFA's case, the environments of the southern United States seem well-suited for this alien.

Occasionally, a species changes so much fundamentally in its new home that new rules apply. Decades after first detection of RIFA in the USA, it was discovered that the ant had not one but two breeding systems (Glancey *et al.* 1973). The first type of breeding system, monogyne, or single-queen colonies, were well known in RIFA; it was that system that probably first arrived when the ant left the ballast soil piled up near the docks at Mobile. The monogyne breeding system in RIFA is quite simple. During times of reproduction, the colony will begin to produce alates, winged, reproductive male and female ants, to complement the all female worker ants. On warm days, usually following rain and with minimal winds present, the alates will emerge from the mound, climb to the tops of grass blades or other structures, and take to the air

to mate (Vinson and Sorensen 1986). Mating occurs hundreds of meters above the earth (Vinson and Sorensen 1986, Vinson 1997). Soon both males and females will fall to the ground. Males die immediately, their only purpose in life having been served. The females will drop their wings and dig new colonies as queens (Vinson 1997).

The second type of breeding system, polygyne, or multiple-queen colonies, were not known to occur in RIFA in the USA until discovered by Glancey *et al.* in 1971 in Mississippi (Glancey *et al.* 1973). Polygyne colonies of fire ants occur at much greater mound densities than monogyne colonies (average 700 mounds/ha vs. 300 mounds/ha), which results in packing hundreds of millions more worker ants per territory than monogyne (Porter *et al.* 1991). In fact, mound density of polygyne RIFA may reach 3000 mounds/ha in some areas (Porter *et al.* 1991). Colony spread in the polygyne form is by mating flights and by budding – a new queen will take a few workers to a new location to start a new colony (Vargo and Porter 1989). This new colony will share food resources and territory (Vinson 1997). Increased fire ant numbers of polygyne colonies obviously leads to an increase in overall effect on native organisms by RIFA (Porter *et al.* 1988, Porter *et al.* 1991, Kintz-Early *et al.* 2003).

Tsutsui and Saurez (2003) investigated polygyny in RIFA. The decrease in territoriality associated with polygyny is believed to occur due to two different genotypes in the general protein-9 allozyme locus. Monogyne fire ants are homozygous dominant at this locus, whereas polygyne fire ants are heterozygous. Homozygous queens are strongly selected against in polygyne territories; if a homozygous (monogyne) queen lands in a polygyne territory after touchdown from a mating flight, she will be killed if found by foraging workers. Heterozygous recessive queens are extremely rare; the condition is fatal in workers (Tsutsui and Saurez 2003).

Important distinctions must be made between several closely related and oftentimes confusing terms in invasion biology, namely “exotic,” “invasive,” “pest,” and “tramp.” Several of these terms are discussed in detail in Mack *et al.* (2000) and McGlynn (1999) but will be defined in simple terms here. Exotic species, also known as alien, introduced or nonnative species, are simply those transferred to a new geographic location previously unoccupied by that species. Such transfer might occur through natural means – high winds, flooding, etc. – or through anthropogenic means – movement of nursery stock, intentional stocking, etc. Exotics have a roughly 10% chance of success in their new range (Mack *et al.* 2000). Invasive species are those exotics that have escaped control measures and have significant impacts (usually negative) on native species or systems. Only one-percent of exotic species will reach invasive status (Mack *et al.* 2000). Pest and invasive have frequently been used interchangeably; this is not correct usage. To be sure, a species does not need to be exotic to be considered a pest of humans. Instead, pest should be reserved for instances when a species becomes an extreme nuisance to humans and causes economic damage. Invasiveness rarely arises from native species, but frequently from exotic organisms. Tramps are those species that rely on humans for long-distance transport and are usually closely associated with human activities (McGlynn 1999). In the ant world, these tramps frequently occupy unfilled niches (McGlynn 1999). Small, yellowish orange ants found inside offices at Louisiana State University are likely Pharaoh ants, *Monomorium pharaonis* (L.) – a species that lives in close association with humans; is moved about in potted plants; produces new colonies by budding, when a queen or workers with brood from the established colony leaves to start anew; and thrives in areas typically unoccupied by native ants (Hooper-Bùi, *pers. comm.*).

Many traits or combinations of traits make exotic ants more likely to become invasive: (1) polygyny, (2) unicoloniality, lacking intraspecific internest aggression, (3) small worker size, (4) quick recruitment to food items (and dominant species at resource), (5) omnivory, (6) catholic requirements for nesting environments, (7) thriving in disturbed areas, (8) extreme aggression towards other species, and (9) nest-raiding of other species (McGlynn 1999, Holway *et al.* 2002). A species need not possess all the aforementioned traits to become a successful invasive, nor does possession of some of the traits alone necessarily impart success. The success of RIFA as an invasive species should come as no surprise when one learns that all the traits above apply to this accidentally introduced species. McGlynn (1999) noted approximately 147 species of exotic ants worldwide from 49 genera. Twenty-six of those species are tramps, nine of those 147 are invasive, and five of those 147 are considered invasive tramps (McGlynn 1999).

A huge dichotomy exists between those flora and fauna intentionally introduced and those accidentally introduced. The vast majority of vertebrates, especially fish, mammals, and birds, have been intentionally introduced, usually for game or aesthetics, and, occasionally, at a great expense to our native organisms (Mack *et al.* 2000). However, with the exception of biological control agents, there have been very few intentional introductions of invertebrates. The Global Invasive Species Database includes RIFA in its “Top 100 of the World’s Worst Invasive Alien Species.” This list also includes four other ant species – a testament to the dominance of ants in ecosystems (Lowe *et al.* 2004). Indeed, ants play key roles as seed dispersers, ecosystem engineers (especially regarding soil nutrients), scavengers, predators, and even food items for other organisms (Hölldobler and Wilson 1990).

IMPACTS OF RED IMPORTED FIRE ANT ON NATIVE ARTHROPODS. The most obvious result of a successful ant invasion is that small arthropod species will likely be adversely affected either

directly or indirectly. Studies of indirect RIFA effects on arthropods are scarce and complicated. Eubanks (2001) presented indirect effects and the difficulty of quantifying the net impact of RIFA in an agricultural setting. Although fire ants attacked problematic pest species, the ants also attacked beneficial insects that also attack pest species. Compounding the problem is that beneficial insects may also attack other beneficials, namely those important in biological control efforts (Eubanks 2001). Sterling *et al.* (1979) concluded that RIFA do not affect entomophagous insects and spiders and that RIFA should be considered a valuable predator of cotton pests.

However, Eubanks *et al.* (2002) reported that densities of 12 of 13 and 8 of 8 natural enemies of cotton pests in 1999 and 2000, respectively, were negatively correlated with densities of foraging RIFA. RIFA also reduced the survival of lady beetles, *Coccinella septempunctata* L. and *Hippodamia convergens* Guérin-Méneville, by 50%, and green lacewing larvae, *Chrysoperla carnea* Stephens, by 38% in greenhouse experiments. Eubanks *et al.* (2002) considered RIFA to be “major intraguild predators of important beneficial arthropods in cotton.” Harris *et al.* (2003) also found significantly fewer lacewing larvae and pupae and lady beetle adults in pecan orchards on some sampling dates. Due to the lack of RIFA observations in foliage of the pecan trees and inconsistencies in results from different sampling dates, Harris *et al.* (2003) concluded that RIFA do not have major effects on distribution or abundance of canopy fauna.

Galarraga (2003) suggested similar results to Eubanks (2001); that is, abundance of certain groups of key predators in cotton, including minute pirate bugs, *Orius* spp., lacewings, *Chrysoperla* spp., and spiders was decreased by RIFA, whereas cotton aphid, *Aphis gossypii* Glover, abundance increased, although not reaching economic threshold (Galarraga 2003). Predation of sentinel bollworm, *Helicoverpa zea* (Boddie), and beet armyworm, *Spodoptera*

exigua Hubner, increased 20-30% in the presence of RIFA (Galarraga 2003). Galarraga (2003) suggested that RIFA “has a net positive impact on cotton pest management.”

Others also extol the value of RIFA as a pest of harmful insects in agroecosystems. The ant could even be used in lieu of potentially more dangerous options such as pesticides or at least with less quantity of pesticides in cotton fields (Breene 1991). Hensley *et al.* (1961) found that if sugarcane fields in Louisiana were treated with heptachlor to suppress imported fire ants, then “abnormally high [sugarcane] borer populations” would result for at least a year if the heptachlor treatment is not immediately followed up with a treatment to kill the borers, too. Also in Louisiana sugarcane fields, Reagan *et al.* (1972) found that after treatment with Mirex®, sugarcane borer, *Diatraea saccharalis* (F.), infestations increased 53% and crop damage 69%. Reagan *et al.* (1972) attributed these increases to suppression of arthropod predators, mainly RIFA, by Mirex®. It should be noted that RIFA was the only ant collected in this experiment (Reagan *et al.* 1972). Reagan *et al.* (1972) recommended that sugarcane farmers in Louisiana should refrain from treating their lands with Mirex® to avoid loss of RIFA. Sterling *et al.* (1979) and Sterling (1978) suggested that RIFA does not negatively affect predators of cotton herbivores and that the ant is more of a benefit than nuisance.

Cherry and Nuessly (1992) reported that since the 1970 discovery of RIFA in sugarcane fields in Florida, RIFA pressure coupled with insecticide use had potentially caused the extirpation of a once common, native fire ant, *Solenopsis geminata* (F.). In addition, RIFA was found to be the most dominant ant species present in the 28 sugarcane fields studied. At both bait cards and pitfall traps, RIFA was found to comprise the highest percentage of all ants caught, the greatest total number of ants caught, and the highest relative abundance of ants (Cherry and Nuessly 1992).

Trophic cascades and the influences of RIFA are frequently examined. Allen *et al.* (2001b) presented data that suggest RIFA negatively affect insect biomass, insect species richness, and insect diversity. Reductions in arthropods resulted in lower numbers of Loggerhead Shrikes (*Lanius ludovicianus*). Mechanisms for RIFA reductions of other arthropods were not addressed, but RIFA may feed upon other arthropods, compete with others for food resources, and scavenge from shrike larders (Allen *et al.* 2001b).

Porter and Savignano (1990) has become one of the most widely cited and best recognized work regarding the effects of RIFA on native arthropods. Polygyne fire ants were studied using paired plots – infested and uninfested – on a research station in Austin, Texas. Pitfall traps sampled for ants and other ground-dwelling arthropods, and bait vials and litter sampling were used for ants. Ant species richness dropped 70%, and total number of native ant individuals dropped 90%. A 10-30X increase in the number of ants was found at the sites; however, 99% of these ants were RIFA. With regard to other arthropods, a 30% and 70% drop in species richness and total individuals was observed, respectively. Some species of insects did increase, and these species were closely associated with RIFA. A brachypterous roach and ground cricket were believed to benefit from the increase in corpses provided by growing RIFA colonies; RIFA will pile corpses of their dead sisters outside the mound. A scarab, *Martineziana dutertrei* (Chalumeau), a fire ant symbiont, also increased.

Some years after Porter and Savignano's 1990 work, Morrison performed a study at the same research station using many of the same sampling techniques as the previous study. Fire ants were still the most abundant species of ant, but RIFA were much less common than in the previous study. Ant and other arthropod species richness did not differ significantly from their pre-invasion numbers. In addition, some arthropods actually increased over their pre-invasion

numbers, an increase not directly associated with RIFA unlike the previous study. It was suggested that the lower numbers of RIFA may have been caused by the fire ants themselves. Early in the invasion, fire ants were supplied with a great abundance of food resources that they quickly monopolized; if they were too successful, the ants may have overexploited their resources (Morrison 2002). It may be that after several generations, an equilibrium may be reached that allows for recolonization of native fauna (Glancey *et al.* 1976).

THE ONLY NATIVE QUAIL IN THE SOUTH. One of the United States' most beloved and widely recognized birds, the Northern Bobwhite, *Colinus virginianus*, occurs through much of the USA east of the Rocky Mountains (Appendix). The endangered subspecies Masked Bobwhite, *C. v. ridgwayi*, occurs in a small, disjunct population in extreme southern Arizona. As a testimony to the Northern Bobwhite's prized gamebird status, the species also occurs in pockets of introduction in some western states and various islands (Sauer *et al.* 2005, NatureServe 2006).

Bobwhites are not migratory (Stoddard 1931: 167-178, Rosene 1969: 80-81). Presumably, there has not been widespread exchange of genetic material among birds at the limits of the range – an important character to note in the experiments that follow. Bobwhite mating strategies appear quite diverse with various methods used to boost nest success. Bobwhites may exhibit monogamy, ambisexual polygamy, and rapid multiclutch polygamy (Stoddard 1931: 15-20, Curtis *et al.* 1993, Burger *et al.* 1995). Burger *et al.* (1995) described rapid multiclutch polygamy as “both sexes [attempting] to increase fitness by simultaneously incubating 2 clutches” (Emlen and Oring 1977). Ambisexual polygamy differs in that only a few females will lay multiple clutches and only a few males will incubate (Burger *et al.* 1995). Ambisexual polygamy is driven by sexual selection on the males due to an “increasingly male-

biased operational sex ratio” in the breeding season (Burger *et al.* 1995, Persson and Ohrstrom 1989). Burger *et al.* (1995) found that polygamy in the form of polyandry was, perhaps, common, with 40% of the females being associated with more than one male. Polyandry may result from a tendency of females to exploit the “skewed sex ratio by abandoning clutches” (Burger *et al.* 1995). Unlike rapid multiclutching, Burger *et al.* (1995) discovered that females would first incubate nest one, and “conditional on the fate of this nest, they sometimes laid a clutch that was incubated by a male.” They then may lay a third clutch, which the female will incubate (Burger *et al.* 1995). Burger *et al.* (1995) found that nesting females and males that survived the nesting period incubated an average of 1.8 and 1.0 nests, respectively. Of nesting birds that lived through 1 September, 74% of females and 26% of males hatched ≥ 1 nest, and males accounted for 29% of successfully hatched nests overall (Burger *et al.* 1995).

Bobwhites nest from May through August, although nesting in April or even through October is possible (Stoddard 1931: 20). Stoddard (1931: 21-26) suggested that nesting may occur in “any type of environment normally frequented by the birds at any season;” he found a “marked partiality for the vicinity of roads, paths, edges of fields, and similar open situations.” Almost three-quarters of all nests were located ≤ 50 feet (≤ 15.24 m) from these openings (Stoddard 1931: 21, Rosene 1969: 63). The vast majority of nests were located in “growth of the preceding season,” suggesting that early successional habitats are important nesting habitat (Stoddard 1931: 22). Because of the need for dead plant matter for nest construction and concealment, large-scale fires can be extremely detrimental (Stoddard 1931: 22). Construction is done primarily by males, although females are apparently capable of solitary nest-building (Stoddard 1931: 22-24). The bobwhite’s preference for early successional habitats and open areas places the bird in danger of predation by RIFA (Stoddard 1931: 21).

RIFA may be an exacerbating factor in a decline fueled mostly by habitat destruction and alteration and cleaner farming practices (Brennan 1991, Allen *et al.* 1995, Mueller *et al.* 1999, Allen *et al.* 2000, Dabbert *et al.* 2002). The Breeding Bird Survey estimates that the bobwhite population in the USA has decreased 3.0 % yearly from 1966-2005 (Sauer *et al.* 2005). Harvests of bobwhites have dropped > 90% since 1980 in Louisiana, Mississippi, and South Carolina, a combination of fewer birds and fewer hunters (Dimmick *et al.* 2002, Mike W. Olinde, *pers. comm.*). To be sure, the number of quail hunters in Louisiana has dropped from 30,000-50,000 to only 3000-5000 individuals in the past few decades (Mike W. Olinde, *pers. comm.*). Dimmick *et al.* (2002) suggested that the species could be extirpated in some areas by 2010 if countermeasures are not implemented immediately. Despite several studies that seem to link fire ants to the decline of bobwhite, two highly polarized camps have arisen – those who believe that fire ants are a large factor and those who think their effect is minimal (Brennan 1991, 1993, Allen *et al.* 1993). The issue is, in fact, clouded with many studies; studies of which are complicated by ant identification issues and varying quail environments.

IMPACTS OF RED IMPORTED FIRE ANT ON NORTHERN BOBWHITE. Early studies on bobwhites and fire ants may lead contemporary scientists to an erroneous conclusion on the fire ant-bobwhite debate. Because imported fire ants were originally lumped into one species *S. saevissima* Forel, it is difficult, and in many cases impossible, to determine which fire ant species is discussed in these early reports (Horton *et al.* 1975, Camilo and Philips 1990, Allen *et al.* 1994). Even native fire ants cannot be ruled out as culprits in some published studies. A landmark quail study by Johnson (1961) is frequently cited as strong evidence that RIFA do not affect nesting bobwhites, but based on the locale and year, given date of introduction of the ant in the study, and the scientific name used in the publication, "Solenopsis saevissima richteri

Forel,” the ant is most likely *S. richteri*, the black imported fire ant (BIFA). Entomologists have noted this less dominant species corralled by RIFA in the Southeast. It is likely that RIFA, the dominant congener, has greater potential for impact of native species than BIFA, especially given the range over which RIFA now occurs. Indeed, recent studies demonstrate that RIFA are causing measurable losses in Northern Bobwhite reproduction (Allen *et al.* 1995, Allen *et al.* 2000).

Although, no one could blame early researchers for not making the distinction between red and black imported fire ants, identification of the ant in question is only half the dilemma with early works. In 1973, only a few years after the splitting of *S. saevissima* Forel into two species (BIFA and RIFA), multiple-queen, or polygyne, colonies of RIFA were discovered in the USA (Glancey *et al.* 1973, Vinson and Sorensen 1986). Polygyne colonies may have twice the mound densities of monogyne RIFA (over 700 mounds/ha). In fact, it was polygyne colonies in Texas that were found to significantly affect bobwhites in one study (Allen *et al.* 1995, Porter *et al.* 1991). Polygyne colonies have been found in Texas, Louisiana, Mississippi, Florida, South Carolina, and Georgia (Vinson and Sorensen 1986, Porter *et al.* 1991, Porter *et al.* 1992, Glancey *et al.* 1973, Kintz-Early *et al.* 2003). Louisiana is a mosaic of monogyne and polygyne colonies (Michael A. Seymour and Linda M. Hooper-Bùi, *unpublished data*). Allen *et al.* (2000) also found that post-fire ant introduction, bobwhite abundance showed a significant downward trend. This occurred despite the fact that the vast majority of RIFA colonies in the southeastern United States are monogyne (Allen *et al.* 2000). In light of these recent developments, bobwhite-fire ant projects should be of utmost importance to wildlife managers.

Adverse effects on bobwhites by RIFA occur through direct means such as depredation of hatchlings and indirect means such as competition for food resources or behavior changes.

Allen *et al.* (1995) suggested a third category that includes negative effects from exposure to fire ant stings. Indirect factors with reference to bird foraging are largely unstudied. Travis (1938a) noted that seed stores created by ants may limit the availability of this resource for seed-eating birds such as bobwhites. Allen *et al.* (2001b) studied the effect of fire ants on wintering Loggerhead Shrikes, *Lanius ludovicianus*, and found that shrikes avoided areas with lower arthropod density, which was attributed to fire ant predation. Because invertebrates are an important food for both young bobwhite chicks and reproductive females, fire ant-induced invertebrate prey shortages could negatively affect quail populations (Giuliano *et al.* 1996).

Several field and laboratory studies attempted to elucidate the effect of RIFA on bobwhites. Pedersen *et al.* (1996) noted that bobwhite chicks spent less time sleeping and more time actively avoiding fire ants in outdoor arenas with RIFA present than those chicks in fire ant-free arenas. Perhaps, most important in this work is that Pedersen *et al.* (1996) found seasonality in responses, with greater reactions to RIFA occurring later in the season when temperature was greater than earlier in the season. Giuliano *et al.* (1996) also studied the effect of RIFA exposure on bobwhite chicks and found that “exposure to as few as 50 RIFA for 60 seconds and 200 RIFA for 15 seconds negatively affected survival of quail chicks.” Giuliano *et al.* (1996) conceded that such exposure of so many ants seems unlikely in the field, but field experiments are lacking. Deaths in the study were attributed to fire ant stings, although no toxicological tests were performed. Mueller *et al.* (1999) examined the hatching success and the survival of bobwhite chicks in Texas and discovered that hatching success of nests in treated and untreated areas did not differ significantly. However, only half as many chicks survived in the untreated areas compared to those on fire ant-suppressed areas after three weeks (Mueller *et al.* 1999).

While identifying predators at Northern Bobwhite nests in southern Georgia, Staller *et al.* (2005), found fire ants, probably RIFA based on locale, to be responsible for 13 complete depredation events – seven prior to hatch and six during hatching. Fire ants ranked third in the list of known bobwhite nest depredations, with 12% of the depredated nests. In attacks prior to hatching, the ants “created a mound over the eggs,” and these eggs were still intact 2 weeks later (Staller *et al.* 2005).

Studies done in one state may not be comparable to those in others simply due to differences in habitat quality, hunting pressures, how long RIFA have infested the areas, and whether the RIFA colonies are monogyne or polygyne, and some differences may simply be caused by adaptation by the birds to RIFA presence. In the decades Northern Bobwhites have been exposed to RIFA, it may be possible that the species has evolved mechanisms to avoid direct or indirect predation by RIFA. If this is the case, then bobwhites found in the southern USA, where RIFA have occurred the longest, should have more success raising broods than bobwhites in the northern extent of its range, where RIFA have more recently invaded.

THE COMMON GARDEN

As there is likely little mixing of genetic material between bobwhite populations of the North and South, especially given the recent scarcity of the species, a common garden experiment may be the most appropriate method for determining the effect of RIFA on nesting bobwhites. That is, if experienced birds from the South are compared to naïve birds of the North, bringing them into a common garden will allow control of most variables and should reveal if RIFA affect bobwhites at different stages of RIFA invasion. If RIFA affect northern birds more dramatically, then it may be that these naïve birds are not adapted to RIFA presence, because they have never been exposed to this aggressive ant species.

The first common garden experiment was described by Clausen *et al.* (1940), who observed that the same species of plant, *Potentilla glandulosa*, was showing distinct growth forms upon an elevation gradient. Clausen *et al.* (1940) determined that by transplanting the plants into a common garden, the phenotypes of plants remained distinct. His simple, yet brilliant, experiment showed that the climate induced by the elevation gradient was less responsible for the morphological differences than genetics; that is, genetics, not environmental conditions, are responsible for producing these particular morphological differences. A similar experiment with widely separated (northern and southern USA) populations of bobwhites might elucidate whether southern Northern Bobwhite populations have indeed evolved mechanisms to avoid RIFA predation during nesting and if bobwhites experience RIFA-related mortality during nesting.

CHAPTER 2

EFFICACY OF BROADCAST-SPREAD AMDRO® AGAINST RED IMPORTED FIRE ANT IN FOREST, FIELD, AND PASTURE SYSTEMS

INTRODUCTION

Many attempts have been made to rid the USA of the red imported fire ant (RIFA), *Solenopsis invicta* Buren with invariable results, namely failure. Ultimately, it is unlikely that the USA will ever be RIFA-free. Historical and present-day chemical, biological, and cultural control measures for imported fire ants (IFA) are discussed in detail by Collins (1992). His work, being the most comprehensive to date, is summarized here.

As successful biological control and cultural measures have evaded discovery or are problematic in implementation, those methods will only be discussed in passing. Collins (1992) included descriptions of viruses, bacteria, fungi, protozoa, nematodes, ectoparasitic arthropods, endoparasitic arthropods, social parasites, predators, and use of sterile males. Cultural measures including altered tillage techniques, vegetation management, controlled burning and flooding, and physical removal of nest mounds, only allowed a maximum decrease of IFA nests by approximately 50% (Collins 1992).

THE EARLY DAYS (1937-1960). The earliest, organized, control program began in 1937 in Baldwin County, Alabama, ten years after the first detection of IFA in the USA (Loding 1929, Collins 1992). Individual mound treatments of 1-3 oz (~ 28 – 85 g) of “A” grade dust (Cyanogas®, 48% calcium cyanide) per nest were applied; nests were dug, dusted, and recovered with soil. Such treatment was applied to “approximately 2,000 acres [> 800 ha] of vegetable cropland” (Eden and Arant 1949, Collins 1992).

Soon, other states followed suit creating control programs. Mississippi’s control and research program began in 1947, and in their second year, the Mississippi State Plant Board

received \$15,000 funding for the battle (Wilson and Eads 1949). Five-percent chlordane dust was used in 1947 and 1948 Mississippi with good results (Collins 1992). Mississippi joined Alabama, Florida, and the US Department of Agriculture (USDA) in 1949 to study the “biology, control, distribution, and economic importance of IFA” in Alabama (Collins 1992). North Carolina implemented a successful bid to prevent establishment of IFA in their state in 1952. Also in 1952, the “Louisiana Legislature appropriated funds... to purchase chlordane to be furnished at cost to farmers” (Collins 1992). Twelve-thousand acres (4856 ha) in Union County, Arkansas, were subjected to an aerially applied treatment of granular heptachlor at rate of 2 lbs/acre (2.24 kg/ha) in 1957 with “excellent results” (Collins 1992, Anonymous 1958).

Also by 1957, US Congress provided funding and authorization to USDA to begin a cooperative Federal-State control and eradication program after it was discovered that the range of IFA was expanding (Collins 1992). For twelve months, starting July 1957, Congress appropriated \$2.4 million, funds to be matched by “State agencies, local sources, and/or individual farmers,” for the treatment of 2.5 million acres (> 1 million ha) with granular dieldrin or heptachlor (Brown 1961, Collins 1992). Rate of application, first at 2 lbs/acre (2.24 kg/ha) in 1957, dropped to 1.25 lbs/acre (1.4 kg/ha) in 1959, and later to 0.25 lbs/acre (0.28 kg/ha) after concerns over wildlife and residue problems. The lowest rate, 0.28 kg/ha, was applied twice at three to six month intervals (Collins 1992). The combined efforts of Senator J.J. Sparkman and Congressman F.W. Boykin of Alabama sought to suspend the use of these chemicals “until benefits and dangers could be properly evaluated” (Collins 1992). These gentlemen were soon vindicated; the US Food and Drug Administration learned that residues of heptachlor epoxide had been found in meat and milk and lowered the tolerance of these residues on harvested crops to zero (Collins 1992). Before the end of the heptachlor era, hundreds of animals – mammals,

birds, reptiles, amphibians, and fish – were documented dead, all purportedly attributed to the failed eradication attempt rather from the ants themselves (Blu Buhs 2004: 94-106 and references therein).

THE ERA OF MIREX® (1960-1980). It was well known even before aerial treatments began in the 1950's that contact insecticides with chlorinated hydrocarbons were potentially hazardous to the environment due to the amount of active ingredient used and chemistry of the compounds. Baits, on the other hand, are usually considered safer, because they contain “relatively small amounts of pesticide” (Collins 1992). Stringer *et al.* (1964) noted three important characteristics of successful baits: the “bait toxicant must (1) exhibit delayed kill over at least a 10-fold dosage range and preferably above a 100-fold dosage range, (2) be readily transferred from one ant to another via tropholaxis and kill the recipient, and (3) not be repellant to foraging ants” (Collins 1992).

Mirex® met these requirements and appeared promising, but success of the product was short-lived. Early studies with the chemical produced extremely high levels of control on the order of 99- to 100-percent (Lofgren *et al.* 1962). Reagan *et al.* (1972) demonstrated up to 90% control of RIFA in sugarcane fields in Louisiana treated aerially with 6 lbs/acre (6.72 kg/ha) Mirex®. By the time the first restriction by the US Environmental Protection Agency was placed on this pesticide in 1971, millions of acres had already been aerially treated at rates varying from 1.25 lbs/acre (1.4 kg/ha) to 10 lbs/acre (11.2 kg/ha). Considering the base bait particle was composed of light weight, ground corn cob grit, 11.2 kg/ha was a substantial amount of chemical (Collins 1992). Restrictions were placed on the use of Mirex® after evidence surfaced that the pesticide was persistent in the environment and that the compound

bioaccumulated in tissues of non-targets (Collins 1992). Applications of Mirex® were allowed through 1978 if applications were not performed aerially.

Mirex® did not disappear from the environment as quickly as it did from the market. The compound's half-life is "about 12 years, longer even than that of DDT" (Tschinkel 2006: 55). Mirex® not only bioaccumulated, but it also biomagnified up the food chain (Tschinkel 2006: 55-56). The disastrous effects of Mirex® provided Rachel Carlson with the essentials she needed to write *Silent Spring* (1962) (Vinson 1997).

THE PRESENT-DAY (1980-PRESENT). August 1980 saw the successful registration of another fire ant bait. American Cyanamid's Amdro® (Ambrands®, Atlanta, GA) is similar to Mirex® in that its carrier, ground corn cob, and its attractant, soybean oil, are the same. Amdro® is also highly effective against fire ants. The active ingredient is hydramethylnon, which may be formulated at 0.036% or 0.73%, depending on whether the product is marketed for broadcast yard treatments or for individual mound or large-scale broadcast treatment.

Amdro® is highly photolabile or readily degraded by UV light, and the active ingredient is undetectable in soil samples two days after application (Apperson *et al.* 1984, Vander Meer *et al.* 1982). Hydramethylnon is less likely to bioaccumulate and has negligible impacts on non-target ant species (Apperson *et al.* 1984). It is not toxic to birds and has an LD₅₀ of 1828 mg/kg for bobwhites (EXTOXNET 2003). It is expected that RIFA numbers will be reduced significantly using Amdro®; suppressions using this bait have decreased RIFA populations > 99% (Mueller *et al.* 1999). Collins *et al.* (1992) found that summer and spring applications of Amdro® at 1.7 kg/ha both resulted in peak suppression two to four months after application and that reinfestation occurred 11 months or six months after treatment for summer and spring applications, respectively. Lofgren and Williams (1985) showed that aerial treatments with

Amdro® (9.88 g (AI)/ha) could last at least 44 weeks. Reinfestation occurred after 44 weeks with colony numbers increasing 327%; the increase in colony numbers, however, was due to small, incipient colonies. Incipient colonies, because of their small size, caused only slight increases in total of RIFA workers. RIFA worker population did not reach that of the untreated controls until 100 weeks after treatment based on quadratic regression analysis (Lofgren and Williams 1985).

Womack (2006) found RIFA suppression could be sustained for three to seven months post-treatment with Amdro® administered at label rate (1.68 kg/ha) with hand-spreaders. RIFA were suppressed 99-42.3% and 97-48% at two research sites in Louisiana, respectively – Alexander State Forest and Sandy Hollow Wildlife Management Area. Evening treatments and amount of mid- and understory vegetation affected success of treatments, probably because timing and cover lessened photodegradation of the bait (Womack 2006).

With the exception of Womack (2006), the majority of early studies on Amdro® and RIFA were confined to highly disturbed habitats such as roadsides or pastures. In this study, Amdro® was broadcast via aircrafts and all-terrain-vehicles over forests and fields in addition to pastures to determine the chemical's efficacy against RIFA in these more complex and less disturbed environments. Sticky traps were used to capture bait particles to determine pattern of dispersal of particles and what particle size(s) most likely reached the target. Food traps were used to gauge RIFA numbers before and after treatments.

MATERIALS AND METHODS

STUDY AREA. Louisiana State University AgCenter's Idlewild Experimental Research Station is located at 30°48'33"N/-090°57'39"W in East Feliciana Parish, Louisiana. The nearest town is Clinton, Louisiana. Idlewild Experimental Research Station consists of "1,800 acres

[~730 ha], including 300 acres [~120 ha] in improved pasture, 150 acres [~60 ha] in open grass, 70 acres [~28 ha] in lakes and ponds, 1,100 acres [~445 ha] in woodlands, 50 acres [~20 ha] in tree fruit research and 50 acres [~20 ha] in deer impoundments” (Louisiana State University AgCenter 2002). The main foci of this research station are “beef, forage and tree fruit research as well as wildlife research in quail, turkey and wildlife habitat management” (Louisiana State University AgCenter 2002). These factors make Idlewild Experimental Research Station an ideal environment in which to conduct field research of this type.

Idlewild is a mosaic of habitats managed for cultivation of crops, raising of cattle, and logging of timber. Different portions of the station are prescribed burned yearly for teaching purposes. Pastures, fields, and orchards are nested within a matrix of mixed pine-hardwood forest. The forested areas of Idlewild can be categorized in three major groups – mesic hardwood, pine-hardwood mix, and pine plantation. The canopy of mesic hardwood areas is predominantly composed of oaks, *Quercus* spp., Sweetgum, *Liquidambar styraciflua*, Southern Magnolia, *Magnolia grandiflora*, Sugarberry, *Celtis laevigata*, American Sycamore, *Plantanus occidentalis*, Red Maple, *Acer rubrum*, and hickory, *Carya* spp. with a dense mid-story of Yaupon, *Ilex vomitoria* and Chinese Privet, *Ligustrum sinense*. Poison Ivy, *Toxicodendron radicans*, and *Vitus* spp. are common groundcover species. Light gaps are filled by *Vitus* spp. and *Rubus* spp. Pine-dominated habitats include a small area of young planted pines and large expanses of mixed pine-hardwood forest. Young planted pines are all Loblolly Pine, *Pinus taeda*, with no mid- or understory due to tight spacing of the trees. The pine-hardwood forests consist mostly of Loblolly Pine with a dense mid- and understory. The mid-story consists of hardwoods such as oaks, hickories, Wax Myrtle, *Myrica cerifera*, and sweetgum. The understory is mostly

Rubus spp., *Rhus* spp., *Toxicodendron radicans*, French Mulberry, *Callicarpa americana*, and various grass species.

DESIGN 2001. Data from a study performed in 2001 were kindly provided by L. M. Hooper-Bùi. The primary field technician for this study was J. L. Rosson.

AMDRO® SIEVING. Aliquots of Amdro® were sieved through USA Standard Testing sieves 10, 18, 20, 30, and 40 (Fisher Scientific, Pittsburgh, PA) with mesh sizes of 425 µm through 2 mm. Sieves were placed on a Ro-Tap™ shaker (WS Tyler, Mentor, OH) to expedite the separation process. These data were to be used to determine the distribution of particle sizes in a given formulation of Amdro®. In addition, 500 particles of well-mixed Amdro® were weighed. Extrapolations of number of particles expected to hit a sticky trap of known size at a known application rate were calculated and compared with field data.

AMDRO® STICKY TRAPS. Sticky traps were created from 1 m² double-sided adhesive squares similar in weight to clear contact paper used to line shelves. One side of the paper was adhered to 1 m² cardboard squares for rigidity. Each trap was staked to the ground with long nails in case of wind. Immediately prior to crop-dusting, the protective sheet was removed from the adhesive squares to expose an adhesive surface towards the sky. These traps were used to capture Amdro® bait particles as they fell from a crop-dusting airplane. Spent sticky traps were covered with clear plastic wrap and returned to the lab for processing. In the lab, all traps were transferred to black construction paper to make Amdro® particles more visible. All particles trapped on the adhesive squares were counted and their longest sides measured with Absolute Digimatic calipers (Mitutoyo Corp., Japan).

CROP-DUSTING OF PLOTS. Three plots were established at Idlewild in May 2001. Each plot contained two distinct habitats – forest with canopy cover > 50% and pasture with no

canopy. One sticky trap was placed on the ground per habitat in each plot for a total of six traps. A crop-dusting airplane modified to drop solid particles was used to treat two plots with Amdro® at label rate of 1.5 lbs/acre (1.68 kg/ha).

In July 2001, three new plots were established at Idlewild. Each plot was separated in four parts – two forested areas and two open areas. One sticky trap was placed on the ground per part for a total of 12 traps. The same crop-dusting airplane was used to treat all three plots with Amdro® at label rate of 1.5 lbs/acre (1.68 kg/ha).

DESIGN 2005. For the study in 2005, a helicopter was used. Such an aircraft was expected to be more adept at applying such a treatment, because of flight dynamics of the helicopter.

AMDRO® SIEVING. Four aliquots of Amdro®, one 1500 g, two 1000 g, one 500 g, were sieved through USA Standard Testing sieves 10, 18, 20, 30, and 40 with mesh sizes of 425 µm through 2 mm. Sieves were placed on a Ro-tap™ shaker for twenty minutes to expedite the separation process. These data were to be used to determine the distribution of particle sizes in this formulation of Amdro®. In addition, three replicates of 500 particles of well-mixed Amdro® were weighed. Extrapolations of the number of particles expected to hit a sticky trap of known size at a known application rate were calculated and compared with field data.

AMDRO® STICKY TRAPS. Sticky traps were created from aluminum oven liner pans (0.1840 m²) made by Hefty (Pactiv Corporation, Lake Forest, IL). Each of 17 pans was spray painted matte black (ColorPlace, Wal-Mart Stores, Inc., Bentonville, AR) and allowed to air dry. The spray paint was simply used to make Amdro® particles, which are pale yellow, more visible on the surface. On the day of treatment, ReMount™ removable mounting adhesive (3M™, St. Paul, MN) similar to Post-It® note adhesive was lightly sprayed onto each pan, and each pan

was staked to the ground in case of wind. These traps were used to capture Amdro® bait particles as they fell from a crop-dusting helicopter. Spent sticky traps were carefully wrapped in Glad® ClingWrap (The Glad Products Co., Oakland, CA) and returned to the lab for processing. All particles trapped by the adhesive were counted and their longest sides measured with Absolute Digimatic calipers (Mitutoyo Corp., Japan).

CROP-DUSTING OF PLOTS. Nine 11-acre (4.45 ha) plots corresponding to the quail project, described in a later chapter of this work, were established at Idlewild Experimental Research Station in Clinton, Louisiana in June 2005. Not all plots were treated, as controls were required for the quail experiments. Nevertheless, five plots were treated aerially by crop-dusting helicopter (North Star, Jasper, TX) with Amdro® (0.73% hydramethylnon) at label rate of 1.5lbs/acre (1.68 kg/ha) on 21 June 2005. The helicopter used was equipped with hoppers and an auger to push bait into the path of a large blower used to apply solid particles and was believed to be one of only two such equipped helicopters in existence at the time. In each of the treated plots, three sticky traps were placed, more or less, equidistant along a diagonal transect across the plots. If any treated plot was close to a plot that was to be left untreated, then individual sticky traps were placed at the edge of those nearby untreated plots to check for overspray. Two untreated plots met this proximity criterion.

ANT MONITORING. To determine the efficacy of Amdro® treatments, food traps were used to capture foraging worker ants. Although not selective for RIFA, food traps, particularly those baited with protein, are highly efficient at attracting such ants. Traditionally, mound ratings have been used to assess the efficacy of treatments (Pranschke and Hooper-Bùi 2003). Harlan *et al.* (1981) presented a system for rating fire ant activity using worker number and brood number estimates within mounds that was subsequently used by RIFA researchers across

the southeastern USA. Although novel in its approach and still used in many studies, the system has major flaws, not the least of which is that ant behavior is affected by temperature and humidity. To obtain accurate ratings, the researcher must determine if brood is present in the nest mound. Pranschke and Hooper-Bùi (2003) found that presence of brood near the top of the mound, the portion exposed for mound rating, was strongly correlated with temperature. Mounds temperatures between 25 - 30°C were more likely to contain brood in the above ground portion of the nest, whereas temperatures above 32°C resulted in workers moving brood below ground, where detection by field researchers is highly unlikely (Pranschke and Hooper-Bùi 2003). Increased temperatures also mean increased ant activity. If the same mound rated in the morning is disturbed again in the afternoon, then the new afternoon rating may double due to increased afternoon temperatures. Individual skill and diligence of field technicians may also greatly affect mound rating outcomes. Although not subjective by nature, mound ratings potentially could be determined to be different by separate observers viewing the same nest at the same time. Obstacles on the ground such as tall grass or other underbrush make mound detection difficult and make detection of incipient colonies practically impossible at times. Use of mound ratings in forest systems is especially unreliable, because RIFA frequently nest in stumps or logs, which makes brood detection particularly difficult.

Because of these problems, the traditional mound rating system was abandoned for this project. In lieu of that system, food traps were used to determine numbers of foraging ants, which are apt to forage throughout the day provided temperatures do not become too high. Use of food traps was a necessity in forest systems where nests may occur in debris, and in fields, where grass may obscure nests. Each food trap was composed of a glass, 20-mL scintillation vial (Kimble Glass, Inc., Vineland, NJ) labeled with date, plot number, and trap number and

covered in aluminum foil (Diamond®, Reynolds Consumer Products, Richmond, VA). Traps were baited with approximately 4 g Vienna sausage (Armour, The Dial Corporation, Scottsdale, AZ). Ten food traps were placed equidistant along a diagonal transect across each treated and untreated plot (nine plots total) at each sampling date. Sampling occurred monthly from June (a pretreatment count) through December 2005. Ten food traps placed across each plot were allowed to stay open for 1 hr. At the end of that period, vials were carefully and quickly capped and returned to the lab and frozen to kill any ants collected. All sampling occurred prior to 1100 hrs to ensure that the temperature was not too high to affect foraging of ants. All ants were initially determined to be either RIFA or non-RIFA and were stored in 95% EtOH. Further identification of non-RIFA was performed later by Lee A. Womack (LSU Entomology). Those data are described and analyzed in Chapter Three of this work.

DESIGN 2006. The study in 2006 appeared ill-fated initially as our aerial applicator contact reneged. Instead, plots were treated by two all-terrain-vehicles (4-wheelers) with seeders (spreaders) attached to the back. ATV treatments occurred in the last week of May 2006. Reapplication was required when ants were observed on a treated plot; hand-spreaders were used in the last week of July 2006 to suppress these fire ants.

GROUND TREATMENT OF PLOTS. The same five plots treated in 2005 were treated again in 2006, but this time with ATVs. Treatment occurred in the last week of May 2006. Herd spreaders (Herd Seeder Co., Inc., Logansport, IN) were calibrated to release Amdro® (0.73% hydramethylnon) at label rate 1.5 lbs/acre (1.68 kg/ha) provided the rider on the 4-wheeler maintained a speed of approximately 6 mph (9.7 km/hr) and was only accomplished through trial-and-error due to terrain and understory. The plots had been adequately treated, and no extra Amdro®, which had been measured for each plot, remained in the spreaders after each treatment.

In late July 2006, a reapplication was required. This was performed with a Handy Green II hand-spreader (Scotts, Marysville, OH) and with Amdro® at label rate 1.5 lbs/acre (1.68 kg/ha). Only the central area of each 11-acre (4.45 ha) plot, that is, where aviaries were erected, was re-treated in this manner as treating the entire plots was not feasible.

ANT MONITORING. To determine the efficacy of Amdro® treatments, food traps were used to capture foraging worker ants. Design of the food traps and their placement across the plots are the same as aforementioned in the design for 2005. Sampling occurred monthly from January 2006 through August 2006. All ants were initially determined to be either RIFA or non-RIFA and were stored in 95% EtOH. Further identification of non-RIFA was performed later by Lee A. Womack (LSU Entomology). Those data are described and analyzed in Chapter Three of this work.

STATISTICAL ANALYSES. SAS 9.1 software (SAS Institute Inc. 2002) and Microsoft® Excel 2002 (Microsoft® Corp. 1985-2001) were used to analyze all data collected in these experiments. T-tests were used to detect significant ($\alpha = 0.05$) differences in Amdro® particle size classes between formulated Amdro® and broadcast Amdro® caught on traps, as well as number of particles trapped versus expected. Proc Mixed, using Satterthwaite or Kenward-Rogers adjustments on degrees of freedom, was used to detect significant differences between mean number (square root transformation) of RIFA collected on treated versus untreated plots during Period A (pretreatment, June 2005), Period B (July 2005 – August 2005), Period C (September 2005 – April 2006), and Period D (May 2006 – August 2006). Because data were sliced into four periods in a Tukey mean separation test, Bonferroni adjustments indicated significance at $\alpha = 0.05/4$ or 0.0125. Four plot pairs, treated versus untreated were analyzed; a

fifth treated plot, which was not paired, was not used in data analyses or presented in Results below.

RESULTS

Formulated Amdro® sieved in 2001 was grouped into three size classes < 1.0 mm, 1.0 mm – 1.99 mm, and ≥ 2.0 mm. Percentages of Amdro® in each of these size classes sieved in 2001 were approximately 19.5%, 64%, and 16%, respectively. Percentages of these size classes in particles caught on sticky traps in wooded areas in 2001 were $11.9 \pm 7.3\%$ (mean \pm SEM), $26.5 \pm 9.5\%$, and $51.7 \pm 14.9\%$ for classes < 1.0 mm, 1.0 mm – 1.99 mm, and ≥ 2.0 mm, respectively. In pasture areas in 2001, these same size classes were composed of $7.6 \pm 3.1\%$ (mean \pm SEM), $31.4 \pm 8.7\%$, and $61.1 \pm 10.2\%$ of the particles trapped. All size class percent compositions from woods were significantly different ($t = -2.5$, $df = 14$, $P = 0.026$; $t = -6.0$, $df = 14$, $P < 0.0001$; $t = 3.2$, $df = 14$, $P = 0.007$, respectively) from size class percent composition found in sieved Amdro®. In addition, all size class percent compositions from pastures differed significantly ($t = -3.9$, $df = 14$, $P = 0.002$; $t = -4.7$, $df = 14$, $P = 0.0003$; $t = 5.0$, $df = 14$, $P = 0.0002$, respectively) from size class percent composition found in sieved Amdro®. No significant difference was found between size class percent compositions between woods and pastures (Figure 2.1).

In 2005, the percent composition of formulated Amdro® particle size was different from that in 2001. Amdro® sieved in 2005 contained $0.6 \pm 0.1\%$, $86.0 \pm 1.2\%$, and $11.6 \pm 1.2\%$ in size classes < 1.0 mm, 1.0 mm – 1.99 mm, and ≥ 2.0 mm, respectively. Percentage of Amdro® of these size classes caught on sticky traps in the 2005 study, which included both wooded and non-wooded areas, were $4.5 \pm 0.6\%$ (mean \pm SEM), $39.7 \pm 3.4\%$, and $55.8 \pm 3.1\%$, respectively. All size class percent compositions were significantly different ($t = 3.2$, $df = 6$, $P = 0.019$; $t = -$

12.9, df = 6, $P < 0.0001$; $t = 13.3$, df = 6, $P < 0.0001$, respectively) from size class percent composition found in sieved Amdro® (Figure 2.2).

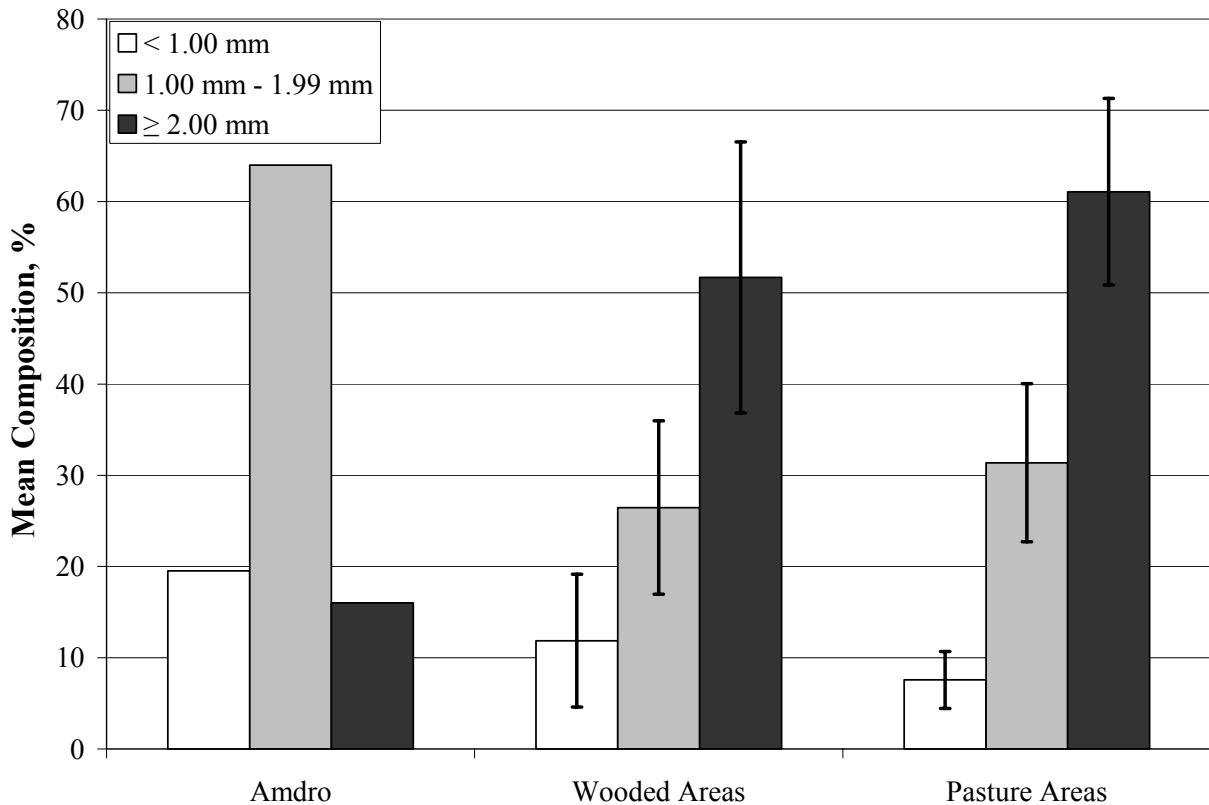


Figure 2.1. Mean percent composition of formulated Amdro® and Amdro® caught on sticky traps in woods and pastures in the 2001 field study. Bars represent SEM.

Number of particles of Amdro® expected to fall on each sticky trap was calculated using trap size and an extrapolated value of number of Amdro® particles found in the label rate 1.68 kg/ha. Expected number to fall on each 1 m² sticky trap used in 2001 was approximately 148 ± 8.2 particles (mean ± SEM). Sticky traps from the field study in 2001 contained 10.3 ± 4.6 particles (mean ± SEM) and 105.5 ± 60.5 particles on wooded areas and pasture areas, respectively. Number of particles caught on sticky traps on wooded plots was significantly less than expected ($t = -13.8$, df = 9, $P < 0.0001$). Number of particles caught on sticky traps on

pasture plots was not significantly different from expected ($t = -0.8$, $df = 9$, $P = 0.47$), nor were the number of particles caught in woods versus pastures ($t = -1.6$, $df = 8$, $P = 0.16$) (Figure 2.3).

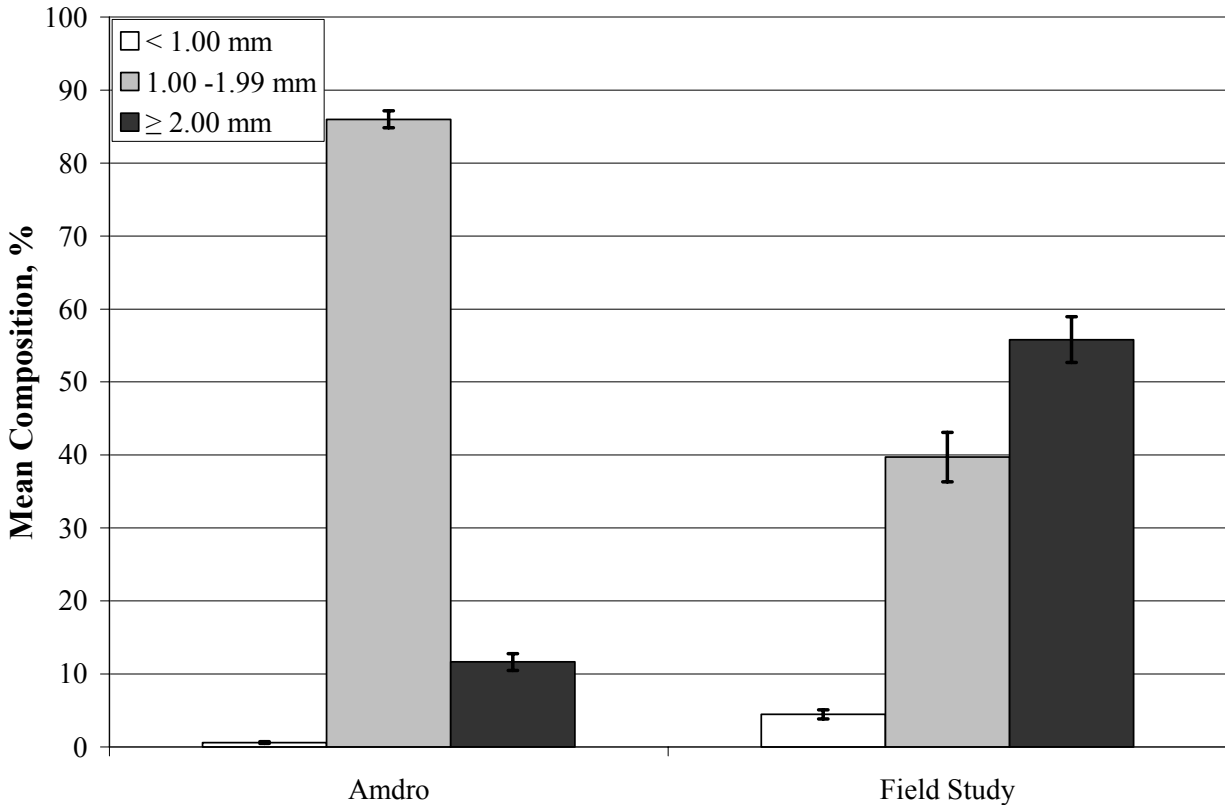


Figure 2.2. Mean percent composition of formulated Amdro® and Amdro® caught on sticky traps in the 2005 field study. Bars represent SEM.

In 2005, the expected number of particles to fall on each 0.1840 m² sticky trap was approximately 23 particles if Amdro® was applied at 1.68 kg/ha. Sticky traps from the field study in 2005 contained 24 ± 6.2 particles; no distinction was made between wooded versus non-wooded areas of the plots because of previous results. Number of particles caught on sticky traps did not differ significantly ($P > 0.5$) from the expected value of 23 ± 1.2 particles per sticky trap (Figure 2.4). Amdro® dispersion was not measured in 2006, because it was applied from the ground in a conventional manner.

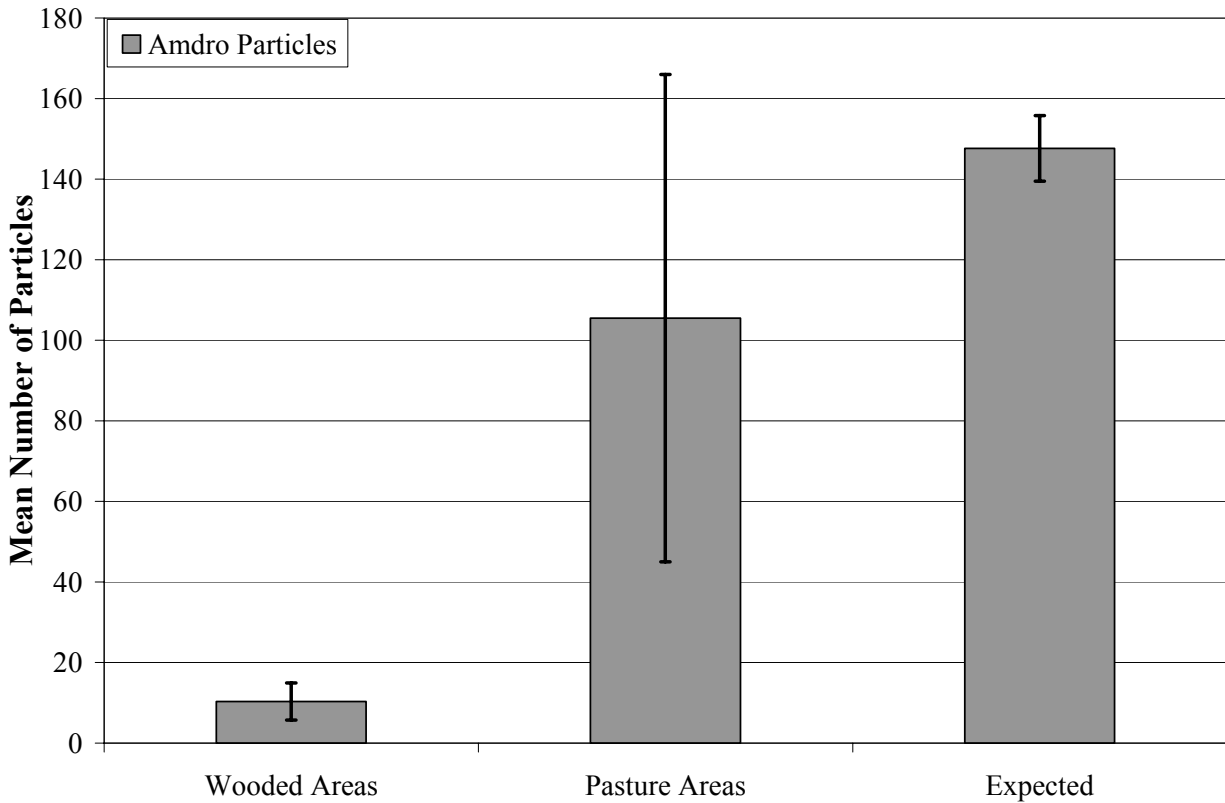


Figure 2.3. Mean number of Amdro® particles caught on sticky traps in woods and pastures in 2001 field study compared with expected number of caught particles. Bars represent SEM.

Only ant monitoring data from 2005 and 2006 will be presented here. Non-RIFA ant species are discussed in Chapter Three. To facilitate proper testing of the hypotheses, particularly those investigated in Chapter Four, dates were separated in SAS based on pretreatment and absence or presence of bobwhite eggs. The splitting of dates occurred as follows: (1) Period A was pretreatment (June 2005); (2) Period B was post-treatment from July 2005 to August 2005; (3) Period C was post-treatment from September 2005 to April 2006; and (4) Period D was post-treatment from May 2006 to August 2006.

Treatment of plots with Amdro® was successful at decreasing RIFA abundance. RIFA numbers were not significantly different ($F = 0.32$, $df = 1, 3$, $P = 0.61$) on treated (1103 ± 426 individuals) and untreated plots (911 ± 497 individuals) in June 2005 prior to treatment (Period

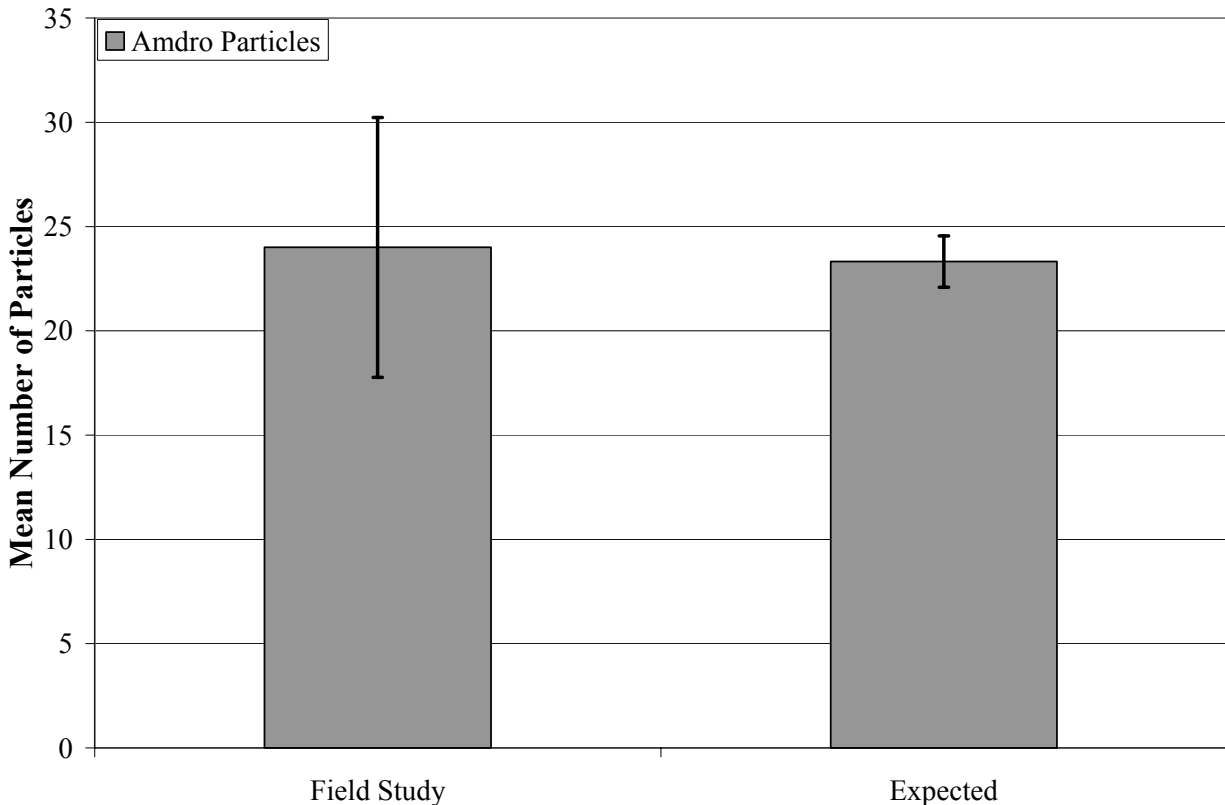


Figure 2.4. Mean number of Amdro® particles caught on sticky traps in 2005 field study compared with expected number of caught particles. Bars represent SEM.

A). Twenty-percent of vials on both treated and untreated plots contained only RIFA (at least one individual ant) at that time. After treatment (Periods B through D), treated plots contained significantly ($F = 13.9$, $df = 1, 11$, $P = 0.0003$) fewer RIFA than untreated plots. In 2005, vials from treated plots contained only RIFA in approximately 4.6% of vials, whereas untreated plots contained only RIFA in 9.6% of vials. In 2006, vials from treated plots contained only RIFA in approximately 6.7% of vials, whereas vials from untreated plots contained only RIFA in 14.2% of vials. Thirteen other vials from 2005 and 2006 also contained RIFA and at least one other ant species. Because competition for the food item was in progress and because it was not possible to predict the victor for certain, these vials were not included in the above numbers. Two-hundred-thirty-five vials (49.0% of all vials) were left empty in 2005; 274 (42.8%) were empty

in 2006. One-hundred-seventy-two (35.8%) and 229 vials (35.8%) contained only non-RIFA ants in 2005 and 2006, respectively (Table 2.1). See Chapter Three for a discussion of those species.

Table 2.1. Percent of vials containing only red imported fire ants and total number of red imported fire ant, *Solenopsis invicta*, individuals collected for each sampling period on treated and untreated plots at Idlewild Experimental Research Station.

	Pretreatment 2005 Treated	Pretreatment 2005 UTC	2005 Treated	2005 UTC	2006 Treated	2006 UTC
Percent of vials containing only RIFA ^a	20%	20%	4.6%	9.6%	6.7%	14.2%
Total RIFA collected ^b	4411	3642	3767	15035	6592	24237
Corrected total RIFA ^c	4411	3642	628	2506	824	3030

^aRIFA was also found with other ant species in vials an average of approximately twice per column above (13 vials total not included in table)

^bTotal RIFA collected in all treated or all untreated areas in that year

^cTotal RIFA as above, but total divided by number of sampling dates in that year

Analyzing the interactions between periods and treatment showed that Periods A and C were not significant ($F = 0.17$, $df = 1$, 112, $P > 0.1$ and $F = 3.7$, $df = 1$, 112, $P > 0.05$, respectively) with respect to RIFA numbers on treated and untreated plots. Periods B and D, which occurred during predominantly warm months, however, were significant ($F = 11.9$, $df = 1$, 112, $P < 0.001$ and $F = 9.4$, $df = 1$, 112, $P < 0.005$, respectively) (Table 2.2). In addition, post-treatment, Periods B, C, D, always showed fewer RIFA on treated plots compared with untreated plots. RIFA numbers were 79.4% and 79.9% lower on treated plots than on untreated plots in Period B, July 2005 and August 2005, respectively. RIFA numbers were 55.6%, 88.0%, 10.1%, and 100% lower on treated plots than on untreated plots in September, October, November, and December 2005, respectively. In January, February, March, and April 2006, RIFA numbers were 96.1%, 88.5%, 83.3% and 57.8% lower on treated plots than untreated plots, respectively.

In Period D, May, June, July, and August 2006, RIFA numbers were 57.6%, 92.2%, 61.5%, and 75.8% lower on treated plots than untreated plots, respectively.

Beginning in October 2005 and ending in February 2006, when temperatures were low, RIFA catches were correspondingly low. In fact, the lowest numbers of RIFA captured on both treated and untreated plots were collected during those months: 19 ± 19 (mean \pm SEM) and 160 ± 124 , 25 ± 17 and 27 ± 20 , 0 ± 0 and 97 ± 97 , 2 ± 2 and 52 ± 27 , and 3 ± 2 and 22 ± 13 for October 2005, November 2005, December 2005, January 2006, and February 2006 for treated and untreated plots, respectively. Note that the individuals collected in cold months were often from only one plot as shown by the standard errors (Table 2.2, Figure 2.5).

Warmer months, particularly months in spring and summer, meant higher numbers of RIFA in food traps. June through September 2005 and March through August 2006 provided the highest amount of trapped RIFA throughout the study. June, July, August, and September 2005 resulted in trap numbers of 1103 ± 426 (mean \pm SEM) and 911 ± 497 , 239 ± 238 and 1156 ± 340 , 306 ± 210 and 1522 ± 548 , and 354 ± 232 and 797 ± 290 on treated and untreated plots, respectively. March, April, May, June, July, August 2006 resulted in trap catches of 187 ± 108 and 1117 ± 652 , 102 ± 79 and 240 ± 92 , 519 ± 267 and 1224 ± 542 , 71 ± 50 and 907 ± 351 , 437 ± 195 and 1136 ± 455 , and 329 ± 119 and 1362 ± 457 on treated and untreated plots, respectively (Table 2.2, Figure 2.5).

DISCUSSION

Composition of formulated Amdro® differed between 2001 and 2005 with larger particles occurring in greater proportion in the 2005 aliquots. Makers of Amdro® may have increased particle size due to publications suggesting that larger particle size, particularly particles ≥ 2.0 mm, would be more readily accepted by fire ant foragers, thereby increasing kill

(Hooper-Bùi *et al.* 2002). Hooper-Bùi *et al.* (2002) found that formulated Amdro® was composed of 16% < 1.0 mm particles, 64% 1.00 – 1.99 mm particles, and 20% ≥ 2.00 mm particles. Rank of particle size composition for Amdro® measured in 2001 from smallest proportion to greatest proportion of formulation went “greater than or equal to 2.00 mm” < “less than 1.00 mm,” <<< “1.00 mm – 1.99 mm.” In 2005, the trend was “less than 1.00 mm” << “greater than or equal to 2.00 mm” <<< “1.00 mm – 1.99 mm.” The smallest particles were recovered in the lowest numbers of the particles trapped. The low number of small-sized particles (< 1.00 mm) found on sticky traps in 2001 in wooded areas may be the result of those lightweight particles becoming caught in canopy vegetation. The low number of small-sized particles (< 1.00 mm) found on sticky traps in 2001 in pasture areas may be the result of those lightweight particles becoming blown outside the plot by winds or simply the action or speed of the crop-dusting airplane. The most interesting result of aerial application by airplane is that the largest percentage of particles trapped by the sticky traps ($51.7 \pm 14.9\%$ and $61.1 \pm 10.2\%$ in woods and pastures, respectively) were of the particle size (≥ 2.00 mm) that occurred as the smallest proportion in laboratory measured, formulated Amdro®. Higher numbers of larger particles suggest that larger particles more readily reach target areas than smaller particles. Particles ≥ 2.00 mm are preferred by RIFA in field trials (Hooper-Bùi *et al.* 2002).

Surprisingly, smaller particles were actually trapped in a higher percentage on sticky traps in 2005 than expected based on laboratory measurements. Perhaps, the auger system and blower required to spread Amdro® from the helicopter chopped the bait into smaller pieces, which may have shifted the size composition of the bait in the field. Again, larger particles, ≥ 2.00 mm, were best represented on sticky traps in 2005, although in 2005, that size class was not of the smallest proportion in the laboratory-measured, formulated Amdro® as in 2001. This

study suggests that in aerial applications of fire ant bait, larger particles of a weight that can still be easily carried by foragers, would be most beneficial as those particles were more often trapped by sticky traps in both years. That is, larger, heavier particles appear less likely affected by the drop from an aircraft. Ambrands® should consider creating an aerial formulation with Amdro® of larger particle size.

Each application, whether performed by air or land, had its strengths and weaknesses. When crop-dusting by airplane, the Amdro® simply drops out the bottom of the aircraft (Hooper-Bùi, *pers. comm.*). Because the Amdro® does not travel through augers or mixing systems, the bait particles remain intact as they fall to the ground. Preserving large-sized bait particles is beneficial if targeting RIFA. Unfortunately, most crop-dusting airplanes are equipped with sprayer systems, which are not functional for dispersal of dry products like baits. Most crop-dusting pilots are hesitant to perform the conversion from sprayer systems to dry dispersal, because of the limited market for dispersal of dry products. Also, the speed of a fixed-wing aircraft may not be optimal for dispersal of lightweight bait. Crop-dusting by helicopter, on the other hand, may benefit from the slower airspeed allowed by rotary-wing aircraft. Like airplanes, few helicopters are equipped with dry dispersal systems. Unlike airplanes, helicopters equipped with such systems cannot switch back and forth between sprayer and dry systems. The problem with dry dispersal in helicopters is that an auger is used to push bait into the path of a large blower fan that forces the bait out. Some bait will likely be inherently ground to smaller particle size – perhaps, small enough so that bait particle size may no longer be preferred by the target ant species.

Many of the drawbacks of aerial treatment are quickly outweighed, however, by the difficulties of treating wooded plots with dense mid- and understory as at Idlewild. Driving all-

terrain-vehicles (4-wheelers) with broadcast spreaders through dense mid- and understory is difficult. Dangers abound, including tree stumps, holes left by rotten stumps, sharp broken branches, hidden logs or ditches, brush piles, briars, and ground-nesting wasps. The benefit is that bait is applied from approximately one meter above ground, which leaves little chance for bait lost in canopy or wind.

Table 2.2. Mean number of red imported fire ants, *Solenopsis invicta*, collected on treated and untreated plots for each sampling date at Idlewild Experimental Research Station. Note that significance values refer to the Period not sampling Date. Significance occurs at $\alpha = 0.0125$, because data were sliced by period.

Date	Period	Num DF	Den DF	Treated (Mean \pm SEM)	Untreated Control (Mean \pm SEM)	F value	P value
Jun-05	A	1	112	1103 \pm 426	911 \pm 497	0.17	0.684
Jul-05	B			239 \pm 238	1156 \pm 340		
Aug-05	B	1	112	306 \pm 210	1522 \pm 548	11.9	0.001
Sep-05	C			354 \pm 232	797 \pm 290		
Oct-05	C			19 \pm 19	160 \pm 124		
Nov-05	C			25 \pm 17	27 \pm 20		
Dec-05	C			0 \pm 0	97 \pm 97		
Jan-06	C			2 \pm 2	52 \pm 27		
Feb-06	C			3 \pm 2	22 \pm 13		
Mar-06	C			187 \pm 108	1117 \pm 652		
Apr-06	C	1	112	102 \pm 79	240 \pm 92	3.7	0.057
May-06	D			519 \pm 267	1224 \pm 542		
Jun-06	D			71 \pm 50	907 \pm 351		
Jul-06	D			437 \pm 195	1136 \pm 455		
Aug-06	D	1	112	329 \pm 119	1362 \pm 457	9.4	0.003

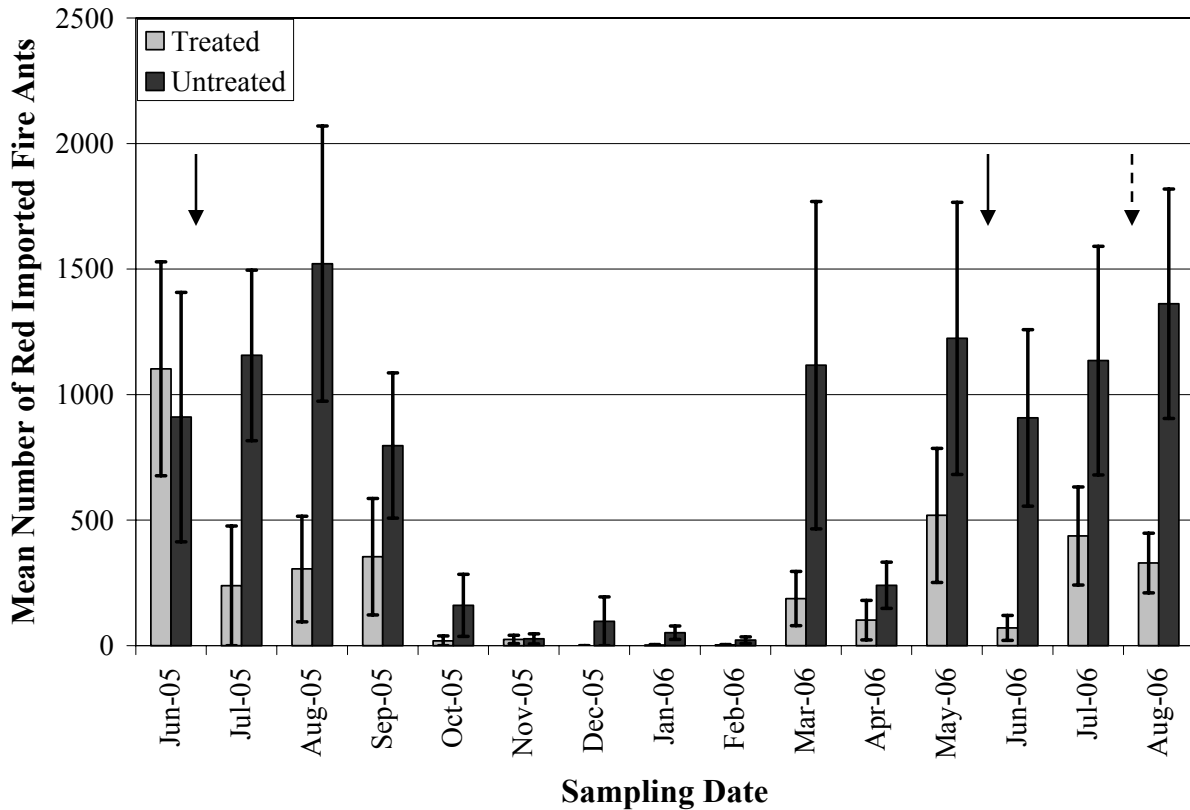


Figure 2.5. Mean number of red imported fire ants, *Solenopsis invicta*, collected in food traps for each sampling date at Idlewild Experimental Research Station. Solid arrows indicate large-scale, broadcast treatments of plots with Amdro®. The dashed arrow represents broadcast treatments of Amdro® confined to plot centers. Bars represent SEM.

As expected, the aerial application study of 2001 showed that presence of canopy greatly affects dispersion of baits. The ground level of wooded areas received significantly ($P < 0.0001$) less Amdro® than expected. Sticky traps in pasture areas, which, hypothetically, have few or no obstructions from above, contained fewer particles than expected (105.5 ± 60.5 versus 148 ± 8.2 particles), but the difference was not significant. The lower number of particles may simply be due to wind blowing smaller particles off the plot.

In 2005, plots were not divided into wooded versus open areas, because, in fact, most plots were a matrix of the two. Traps in the field contained more Amdro® than calculated (24 ± 6.2 versus 23 ± 1.2 particles), but the difference was not significant. If the results from woods

and pastures in 2001 are lumped and compared with expected values of that year, the relationship is not significant. That is, as in the 2005 study, 2001's dispersion might be considered a success, but the insignificance may result from extremely high variability in the pasture trap counts. Ant trap counts from 2001 suggest that similar levels of suppression can be obtained with less Amdro®, potentially; despite less Amdro® reaching the ground in forests, RIFA in forests were suppressed in similar amounts to that of RIFA in pastures. Dispersion of the bait may have suffered in 2005 from canopy catching and wind as in 2001, but lack of significance suggests a well dispersed product.

Canopy catch of aerially dispersed bait products limits the number of particles reaching the ground and may, therefore, limit the number of particles reaching target species. Canopy catch may also result in an increase in non-target ant poisonings as less dominant, mostly arboreal ant species such as *Camponotus* spp. and *Crematogaster* spp. are more likely to recruit to a bait in the canopy than are fire ants. Baits capitalize on aggressive foraging of targets such as RIFA, which may find some baits in less than ten minutes. Therefore, baits not found by RIFA such as those particles caught in the canopy could be foraged by less aggressive and non-target ant species (MacKay *et al.* 1994). This could result in decreased non-target ant populations, which, ultimately, may result in increased RIFA abundance (Summerlin *et al.* 1977). Such possibility is discussed in detail in Chapter Three.

Canopy may also act as a sieve that traps larger bait particles allowing smaller particles to reach the ground if not blown off target. Larger particles, ≥ 2.0 mm, preferred by RIFA may not reach optimal foraging areas for RIFA. Instead, smaller particles potentially foraged by non-target ants will reach the ground. Particle size should not be overlooked when targeting different species of ants (Hooper-Bùi *et al.* 2002).

RIFA were adequately suppressed after treatment with Amdro®. Analysis of Period A, June 2005, showed that the number of RIFA on both untreated and soon-to-be treated plots was not significant ($P = 0.684$). Insignificance in this period suggests that plots were paired well for the remainder of the study. Number of RIFA on untreated plots in Period B, July and August 2005, was significantly greater ($P = 0.0008$) than RIFA on treated plots. Published reports of efficacy of Amdro® (0.73% hydramethylnon) suggest that RIFA suppression may last three to 11 months with peak suppression in the first one to four months post-treatment (Apperson *et al.* 1984, Collins *et al.* 1992). Treatment occurred in late June 2005; treated plots contained fewer foragers in July and August 2005 (79.4% and 79.9% suppression) and, marginally, fewer in September 2005 (55.6% suppression). Once cold weather arrived in October, difficulty arises when assigning importance of treatment versus weather on suppression. Months when bobwhite eggs were not present, September through December 2005 and January through March 2006, composed Period C. RIFA numbers at food traps on treated and untreated plots did not differ significantly ($P = 0.057$) during Period C. Insignificance may have resulted from low trap counts during winter combined with extremely high variability, which plagued most sampling dates. Untreated plots in Period D, May through August 2006, contained significantly greater ($P = 0.0027$) numbers of foraging RIFA than treated plots. These data suggest that treatments in late May and July 2006 were effective in decreasing RIFA numbers.

The most dramatic reductions of RIFA on treated versus untreated plots occurred on sampling dates either immediately after RIFA became active after cold winter-like temperatures (e.g., March 2006) or immediately after treatments (e.g., July, August 2005; June 2006; and August 2006). RIFA trap numbers remained extremely low on treated plots in colder months particularly December 2005 and January and February 2006, but colder temperatures also meant

fewer captures on untreated plots during those months. Therefore, the > 95% reductions of RIFA on treated plots during December 2005 and January 2006, for instance, may be misleading, because trap counts were extremely low. In fact, RIFA were captured from only one plot, an untreated plot, in December 2005 and only one treated plot in January 2006. Reductions of RIFA presumably not influenced by cold temperature-related, low trap counts averaged approximately $71.5 \pm 4.5\%$ (mean \pm SEM).

Effective treatment did not persist throughout the sampling period. RIFA were not successfully suppressed for several consecutive months, particularly September 2005 through April 2006. Treatment did not suppress foragers for longer than three months. Due to timings of treatments, however, one should not use this particular field study to cite length of effectiveness of Amdro®. That is, treatment within a few months of cold weather and termination of study before RIFA numbers returned to pretreatment levels preclude determination of length of effective treatment.

Although foraging activity of RIFA depends strongly on temperature, food traps are, nevertheless, more reliable than mound ratings (Porter and Tschinkel 1987). Mound counts and mound ratings are affected by habitat, temperature, humidity, and skill of observer. Times of drought or excessive temperature may force ants below the above ground portion of the nest mound, which may make ratings inaccurate (Pranschke and Hooper-Bùi 2003). Food traps were set out and collected prior to 1100 hrs to ensure temperature would not adversely affect foraging, which begins to decrease above 36°C and terminates at 43°C (Porter and Tschinkel 1987). Except during cold weather, temperature was likely not a factor in RIFA food trap counts; RIFA will not forage at temperatures below 15°C and prefer temperatures above 22°C (Porter and Tschinkel 1987). Prior to 1100 hrs, temperatures never exceeded 36°C. Rain may decrease

foraging approximately 40%, but rainfall never occurred during ant sampling (Porter and Tschinkel 1987).

Amdro® was an effective means of suppressing RIFA numbers at Idlewild Experimental Research Station, but several applications of Amdro® were required to provide satisfactory suppression. More importantly, however, was the fact that even though RIFA was not suppressed for all sampling dates or periods, RIFA was suppressed during times of bobwhite egg presence, which is the most critical part of this work.

CHAPTER 3

EFFECT OF AMDRO® ON ANT FAUNA AT IDLEWILD EXPERIMENTAL RESEARCH STATION

INTRODUCTION

The red imported fire ant (RIFA), *Solenopsis invicta* Buren, an invasive, exotic, pest ant species, has posed serious problems in areas where introduced. The species is known to affect both vertebrate and invertebrate faunae, causes economic losses on the scale of hundreds of millions of dollars annually in the USA, and thwarts most control efforts (Porter and Savignano 1990, Collins 1992, Allen *et al.* 1994, Vinson 1997, Pimentel *et al.* 2000, Allen *et al.* 2004). The war waged against these ants, although fought with good reason, a lot of emotion, and, what passed for sound science at the time, ironically, may have actually assisted RIFA in its spread throughout the USA (Blu Buhs 2004: 175-176, Tschinkel 2006: 73-74).

Early treatments to control fire ants were problematic (Collins 1992). Heptachlor and chlordane, both contact insecticides, had the potential to kill all insects indiscriminately. Mirex®, a bait, which is presumably safer for the environment and non-target species, proved to be extremely damaging (Vinson 1997, Tschinkel 2006: 55-56). These pesticides' effects on vertebrate fauna are staggering with the direct loss of adult animals – mammals, birds, reptiles, amphibians, and fish – and the possibility of passing the chemicals or chemical products to offspring (Blu Buhs 2004: 94-106 and references therein, Tschinkel 2006: 55-56).

In the 1970's, entomologists first addressed the alarmingly real possibility that treating large areas with pesticides, particularly Mirex®, the RIFA insecticide of choice in that era, might affect non-target ant species and may backfire. Markin *et al.* (1974) noted reductions in the numbers of southern fire ant, *Solenopsis xyloni* McCook, Florida harvester ant, *Pogonomyrmex badius* (Latreille), and little black ant, *Monomorium minimum* (Buckley) in an area treated with

Mirex® in Louisiana. Summerlin *et al.* (1977) found that within two weeks post-treatment with Mirex®, ant species that were generally omnivorous or highly predacious were eliminated from those treated areas. Species included RIFA; tropical fire ant, *S. geminata* (F.); little black ant; a big-headed ant species, *Pheidole dentata* Mayr; and a fungus-growing ant, *Trachymyrmex septentrionalis* (McCook). After seven months post-treatment, RIFA, the pyramid ant, *Dorymyrmex insana* (Buckley), and the elongate isopod-eating ant, *Leptogenys elongata* Mayr, were reestablishing populations on the plot. After 14 months, RIFA and the pyramid ant were the most prolific reinvaders and were building population numbers far greater than pretreatment populations. After one year post-treatment, little black ant, *Forelius pruinosus* (Roger), and *P. dentata* populations were reduced even beyond pretreatment population numbers (Summerlin *et al.* 1977).

Summerlin *et al.* (1977) used gas liquid chromatography (GLC) to determine the amount of Mirex® residue left in ants. The highest levels of these residues were found in red harvester ant, *Pogonomyrmex barbatus* (F. Smith), and pyramid ant, both non-target species. Red harvester ant and black carpenter ant, *Camponotus pennsylvanicus* (DeGeer), were the only two species left on the plot after eight weeks, and specimens of both species contained Mirex® residues.

Stimac and Alves (1994), also interested in potential indiscriminate mortality of non-target ant species, treated one plot in a Florida pasture with Amdro® (A.I.: hydramethylnon) and left another plot in the same pasture untreated as a control. At pretreatment and 30 months post-treatment, nests of ant species on the control plot, including RIFA, showed no change in the proportion. At pretreatment the plot that would be treated with Amdro® was completely RIFA-free. The majority of nests were tropical fire ant, *S. geminata*, (> 50% nests) and *Dorymyrmex*

spp. (~ 33% nests). Thirty months after treatment, RIFA (> 50% nests) had almost exactly replaced *S. geminata*, which was now almost absent from the treated plot. *Dorymyrmex* spp. were almost eliminated (Stimac and Alves 1994). The study, which does not appear to be replicated, warns of the possible deleterious effects some pesticides may inflict on non-targets, especially when applied in areas with low population levels of RIFA.

Apperson *et al.* (1984) used Logic® (A.I.: fenoxycarb) and Amdro® to determine the effects of insecticides on RIFA and non-target ants. Apperson *et al.* (1984) showed that Amdro® was effective against RIFA, but warned that rapid recolonization by incipient colonies would require multiple reapplications. Apperson *et al.* (1984) reported that Amdro® had negligible impacts on non-target ant species. In a similar study, Zakharov and Thompson (1998) reported that in RIFA-infested plots treated with Logic® (1% fenoxycarb), non-target ants appeared to fair well against the bait; in fact, native ant species actually increased in these plots, which suggests competitive release from RIFA and lower susceptibility or acceptability of fenoxycarb baits for those ant species (Zakharov and Thompson 1998). Effects of fenoxycarb and hydramethylnon on Formicinae and Dolichoderinae were comparatively low, although the effect of hydramethylnon on Formicinae was apparent for some species. Effects of Amdro® (0.73% hydramethylnon) were even more apparent on Myrmecinae (Zakharov and Thompson 1998). Practically all species of Myrmecinae including those in genera *Aphaenogaster*, *Crematogaster*, *Monomorium*, *Pheidole*, and *Solenopsis*, virtually disappeared in the hydramethylnon-treated plots. Only one species of Myrmecinae, RIFA – the target of the treatment – was not eliminated, although its numbers were suppressed (Zakharov and Thompson 1998).

Results of the aforementioned studies suggest, explicitly or implicitly, that treatments designed to eliminate RIFA, ultimately, may have the opposite effect. Although RIFA may be suppressed for several months after treatment, recolonization occurs more rapidly by this species, and occupation by RIFA alone may suppress native ants. Several studies attempt to resolve whether RIFA cause suppression of native species of ants. For the most part, studies in the current literature fail to provide these answers, because it is extremely difficult to disentangle the effects of RIFA from those of disturbance. In other words, because RIFA is a species that thrives with disturbance, it is difficult to determine whether native ants are suffering from RIFA introduction or the disturbance to the environment that allowed RIFA introduction (Tschinkel 2006: 568-594). Nevertheless, many researchers have attempted to answer the question of whether or not RIFA affect native ants.

RIFA was found to be the most dominant ant species present in 28 sugarcane fields in Florida (Cherry and Nuessly 1992). At both bait cards and pitfall traps, RIFA was found to comprise the highest percentage of all ants caught, the greatest total number of ants caught, and the highest relative abundance of ants (Cherry and Nuessly 1992). In fact, presence of RIFA in combination with earlier periods of pesticide use likely caused the extirpation of a native fire ant, *Solenopsis geminata*, which had once been common (Cherry and Nuessly 1992).

Camilo and Philips (1990) studied the diversity of ants along a transect through RIFA-infested and uninfested areas in central Texas. They produced a dendrogram of four distinct ant assemblages using cluster analysis. The first split in the tree separates undisturbed from disturbed habitats. Then the disturbed branch is split by sites that contained RIFA and those that did not. The final major division occurs in the branch of sites that contained RIFA; this split resulted in sites with low density (< 2 foragers/day/trap) and high density (> 200

foragers/day/trap) of RIFA (Camilo and Philips 1990). Assemblage one, which occurred in the undisturbed areas without RIFA, contained the most ecologically specialized species and was the most species rich with 22 species. Assemblage two, which occurred in the disturbed areas without RIFA, was also species rich with 13 species and contained a few specialized species. Camilo and Philips (1990) suggested that the main factor causing the difference in the number of species present was likely the amount of disturbance. In assemblage three, low numbers of RIFA may have been caused by the presence of a subterranean species of thief ant, *Solenopsis (Diplorhoptrum)* sp., which may attack founding queens; ten species of ants were assigned to this assemblage. Assemblage four contained low species richness – only six species – and high RIFA density. In one area of extremely high RIFA density (> 1000 foragers/day/trap) only one other ant species, the little black ant, was collected.

Eight species were associated with RIFA, and four of these associations were significant. Little black ant and pyramid ant were both positively associated with higher RIFA densities, whereas two species of granivorous *Pheidole* were negatively associated with RIFA and were replaced more than the other ants in the assemblages containing RIFA (Camilo and Philips 1990). Tschinkel (2006: 574-575) warns that caution must be exercised when attempting to assign cause and effect to RIFA and/or disturbance.

Jusino-Atresino and Phillips (1994) found that pitfalls placed in RIFA-infested versus uninfested plots captured 18 and 20 species, respectively. Three species were only collected in the uninfested plots, albeit in very low numbers – *Pheidole lamia* Wheeler (9 individuals collected), *Crematogaster minutissima missouriensis* Emery (7 individuals), and the thief ant, *Solenopsis (Diplorhoptrum) molesta* (Say) (2 individuals). *Formica gnava* Buckley (5 individuals), on the other hand, was the only species collected on infested areas but not

uninfested (Jusino-Atresino and Phillips 1994). It is believed that the arboreal nature of *F. gnava* keeps it out of contact with RIFA much of the time. Seventeen species were common to both areas; most of these species were adversely affected by RIFA (Jusino-Atresino and Phillips 1994). Numbers of individuals of these species between infested and uninfested areas displayed a RIFA-related intraspecific reduction $> 20\%$ (Jusino-Atresino and Phillips 1994). The most affected of these species was little black ant, which experienced a reduction of 76% (Jusino-Atresino and Phillips 1994). Like Camilo and Philips (1990), Jusino-Atresino and Phillips (1994) found that pyramid ant, *Dorymyrmex insana*, was coexisting with RIFA in higher numbers than in uninfested areas.

Over the course of 21 years beginning in 1972, Wojcik (1994) collected almost one million ants from 13,600 bait samples resulting in 55 species of ants from five subfamilies and 22 genera in Florida! RIFA, the tropical fire ant, and *Pheidole dentata* were the three most frequently collected species composing 58.3% of total occurrences and 82.5% of specimens (Wojcik 1994). In September 1992, RIFA was the dominant ant at 43.3% occurrence and 63.1% specimens, which was likely assisted by urbanization (Wojcik 1994). In stark contrast, tropical fire ant and *Pheidole dentata* both declined significantly over those 21 years. Where the tropical fire ant once occurred at 30%, 35%, and 55% of occurrences, sites, and specimens, respectively, it had declined to only 15%, 15%, and 25% of occurrences, sites, and specimens by 1992 (Wojcik 1994, Tschinkel 2006: 577). Nine additional species that occurred in sufficient numbers for analysis also showed negative correlations with increasing RIFA populations and habitat disturbance. Four species, three of which are exotic, showed increases. The one native ant that showed a slight increase in number was a trap-jawed ant, *Odontomachus brunneus* (Patton); specialized defenses may have allowed it to succeed where other natives had not (Wojcik 1994).

Increased urbanization along the transect is a confounding factor when trying to determine effects actually attributable to RIFA (Tschinkel 2006: 578).

Adding to the growing evidence that RIFA may adversely affect the little black ant and *Pheidole* spp., among others, Cook (2003) studied the effects of RIFA on native ants in treated and untreated areas in Texas. In the treated area, Cook (2003) used mound treatments of Amdro® or Extinguish® (A.I.: methoprene), which did cause a decrease in RIFA colonies. Untreated plots were at an early stage of invasion by RIFA. At the start of the study, both treated and untreated plots, more or less, contained the same species richness (12 vs. 11 species). At the end of the study, all species previously found in the treated plot were still extant in that plot along with a new species, an undescribed species of *Cyphomyrmex* (Cook 2003). Like Jusino-Atresino and Phillips (1994), Cook (2003) found that little black ant was adversely affected by RIFA; in fact, little black ant was eliminated on the untreated plot. During the study, Cook (2003) also found that two species of *Pheidole* (not identified to species) were eliminated on the untreated plot much like Camilo and Philips (1990) had discovered before. A fourth species, red harvester ant, *Pogonomyrmex barbatus*, was also eliminated on the plots with RIFA. Interestingly, *P. comanche* had also been eliminated from the untreated plots only to reestablish itself, however tenuously, with one colony (Cook 2003).

Arguably the most recognized and most often cited work regarding the effects of RIFA on native invertebrates was performed by Porter, Morrison, and their colleagues at the Brackenridge Field Laboratory (BFL) in Texas, a 32-ha tract of woods and fields with vegetation “characteristic of the Edwards Plateau and the Blackland Prairie of central Texas” (Porter *et al.* 1988, Porter and Savignano 1990, Morrison 2002). Porter *et al.* (1988) reported the events and results of an invasion of polygyne RIFA at BFL. Polygyne RIFA occur at greater mound

densities and are likely to produce more dramatic effects on native fauna than their monogyne form (Kintz-Early *et al.* 2003). Porter *et al.* (1988) discovered that RIFA eliminated the native tropical fire ant colonies and prevented founding queens from creating new colonies inside the invasion front. RIFA did not cause exact replacement, but instead, RIFA replaced tropical fire ant colonies at a ratio of 6:1. In other words, the invasion of polygyne RIFA indicates “a radical restructuring of the arthropod community rather than a simple one-for-one substitution of an equivalent species” (Porter *et al.* 1988).

In fact, a radical restructuring of the arthropod community is precisely what they had discovered. Porter and Savignano (1990) used pitfalls and baits to measure ant abundance and diversity at BFL. In the RIFA-infested areas, ant species richness was 70% less than that of uninfested areas. Total number of native ant individuals dropped 90% in infested areas. Twenty-three of 35 ant species collected at BFL were either significantly less common or absent from RIFA-infested areas (Porter and Savignano 1990). There was a 10-30X increase in total number of ants at the infested sites; RIFA, the only ant more common at infested sites, was responsible for > 99% of this increase (Porter and Savignano 1990).

Twelve years after the Porter and Savignano (1990) study was published, Morrison (2002) presented evidence that the ant community at BFL had recovered in part. Morrison (2002) used similar sampling techniques as the previous study and found that native ant abundance and species richness had returned to their preinvasion numbers; that is, the native ants had found a way to effectively coexist with RIFA. Morrison (2002) also discovered that RIFA, although still the most abundant ant species, actually declined in number. Whereas RIFA had accounted for 99.6% of individuals in pitfall traps in Porter’s and Savignano’s (1990) study, RIFA accounted for only approximately 33% of the catch in Morrison’s pitfalls (Morrison 2002). Morrison (2002)

warns that although it appears that ant communities may return to preinvasion levels, one should not interpret the study “as an indication that detrimental effects of invasive ants will simply disappear with time;” in fact, two species, tropical fire ant and red harvester ant were still rare in the infested areas (Morrison 2002). Mechanisms of this flip-flop in ant species richness and the reduction of RIFA abundance are not known (Morrison 2002). Morrison and Porter (2003) found that in areas of mostly monogyne RIFA, 37 species of ants were able to coexist with RIFA. Morrison and Porter (2003) suggest that invaded communities may be more resilient to RIFA than previously believed.

Womack (2006) studied the effects of RIFA and broadcast Amdro® (0.73% hydramethylnon) treatments on non-RIFA in pine-dominated forests in Louisiana for four years. Studies were conducted at Alexander State Forest, Rapides Parish, and Sandy Hollow Wildlife Management Area (WMA), Tangipahoa Parish. At Alexander State Forest, the pine-mixed hardwood site, odorous house ant, *Tapinoma sessile* (Say) and a species of rover ant, *Brachymyrmex* sp., displayed a positive response to treatment; their numbers increased in trap catches after treatment of plots with Amdro®. Little black ant and *Paratrechina faisonensis* (Forel) did not respond to treatment and appear to coexist with RIFA in this ecosystem. *Aphaenogaster rudis-texana* (Umphrey), *Crematogaster lineolata* (Say), and *Pheidole dentata* showed random population fluctuations that may not be attributed to RIFA. In his plots at Sandy Hollow WMA, the longleaf pine dominated site, Womack (2006) found that *Paratrechina faisonensis*, *Pheidole dentata*, *P. metallescens* Emery, and a rover ant, *Brachymyrmex* sp. did not respond to treatment and exhibit random population fluctuations that may not be attributed to RIFA. *Dorymyrmex bureni* Buckley did not respond to treatment or the presence of RIFA, which suggests it may coexist with RIFA in this ecosystem. Little black ant and *Prenolepis*

imparis (Say) exhibited negative response to treatments; their numbers decreased in traps after treatment (Womack 2006).

From the above studies, it appears that despite the difficulty of assigning responsibility to RIFA or to disturbance in the reduction of our native ants, RIFA do cause significant losses in some ant species. RIFA has almost certainly caused the local extirpations of tropical fire ant and severe reductions in some *Pheidole* spp. and *Pogonomyrmex* spp., and may cause reductions in little black ant populations in some environments. Still other ant species such as thief ant, *Solenopsis (Diplorhoptrum) molesta* (Say), may prevent RIFA from easily spreading to previously uninvaded locales (Camilo and Philips 1990, Vinson and Rao 2004). RIFA may fill niches left vacant by native ants that have been suppressed or eliminated by insecticide use, expanding their range and foothold while the other species are most vulnerable.

In this study, Amdro® was broadcast via helicopter and all-terrain-vehicles over forests and fields to determine the product's efficacy against RIFA and potential non-target effects in these complex ecosystems. Food traps were used to gauge ant numbers before and after treatments. Effects of both Amdro® and RIFA on non-target ant species are discussed.

MATERIALS AND METHODS

STUDY AREA. Please see Chapter Two of this work for description of Idlewild Experimental Research Station, its purpose and its habitats.

DESIGN 2005. A specially modified helicopter was used to treat plots with Amdro® in 2005.

CROP-DUSTING OF PLOTS. Nine 11-acre (4.45 ha) plots corresponding to the quail project, described in a later chapter of this work, were established at Idlewild in June 2005. Not all plots were treated, because controls were required for the quail experiments. Nevertheless, five plots

were treated aerially by crop-dusting helicopter (North Star, Jasper, TX) with Amdro® (0.73% hydramethylnon) at label rate of 1.5lbs/acre (1.68 kg/ha) on 21 June 2005. The helicopter used was equipped with hoppers and an auger to push bait into the path of a large blower used to apply solid particles and was believed to be one of only two such equipped helicopters in existence at the time.

ANT MONITORING. To determine the efficacy of Amdro® treatments, food traps were used to capture foraging worker ants. Although not selective for RIFA, food traps, particularly those baited with protein, are highly efficient at attracting such ants. Justification for the use of food traps versus more traditional mound counts is provided in Chapter Two. Also, nest detection of non-RIFA would be overly time-consuming in such complex habitats. Each food trap was composed of a glass, 20-mL scintillation vial (Kimble Glass, Inc., Vineland, NJ) labeled with date, plot and trap numbers and covered in aluminum foil (Diamond®, Reynolds Consumer Products, Richmond, VA). Traps were baited with approximately 4 g Vienna sausage (Armour, The Dial Corporation, Scottsdale, AZ). Ten food traps were placed equidistant along a diagonal transect across each treated and untreated plot (nine plots total) at each sampling date. Sampling occurred monthly from June 2005 (a pretreatment count) through December 2005. Food traps were allowed to stay open for 1 hr. At the end of that period, vials were carefully and quickly capped and returned to the lab and frozen to kill any ants collected. All sampling occurred prior to 1100 hrs to ensure that the temperature was not too high to affect foraging of ants. All ants were initially determined to be either RIFA or non-RIFA and were stored in 95% EtOH.

DESIGN 2006. In 2006, two all-terrain-vehicles (4-wheelers) with Herd seeders (spreaders) were used to treat plots with Amdro®. Reapplication was required when ants were observed on

a treated plot; hand-spreaders were used in the last week of July 2006 to suppress these fire ants in the center of treated plots

GROUND TREATMENT OF PLOTS. The same five plots treated in 2005 were treated again in 2006, but this time with ATVs. Treatment occurred in the last week of May 2006. Herd spreaders (Herd Seeder Co., Inc., Logansport, IN) were calibrated to release Amdro® (0.73% hydramethylnon) at label rate 1.5 lbs/acre (1.68 kg/ha) provided the rider on the 4-wheeler maintained a speed of approximately 6 mph (9.7 km/hr) and was only accomplished through trial-and-error due to terrain and under-story. The plots had been adequately treated and no extra Amdro®, which had been measured for each plot, remained in the spreaders after each treatment. In late July 2006, a reapplication was required. This was performed with a Handy Green II hand-spreader (Scotts, Marysville, OH) and with Amdro® at label rate 1.5 lbs/acre (1.68 kg/ha). Only the central area of each 11-acre (4.45 ha) plot, that is, where aviaries were erected, was re-treated in this manner as treating the entire plots was not feasible.

ANT MONITORING. To determine the efficacy of Amdro® treatments, food traps were used to capture foraging worker ants. Design of the food traps and their placement across the plots are the same as aforementioned in the design for 2005. Sampling occurred monthly from January 2006 through August 2006. All ants were initially determined to be either RIFA or non-RIFA and were stored in 95% EtOH.

ANT IDENTIFICATION. Representatives of all ants from 2005 and 2006 that were determined as non-RIFA were mounted on points. All non-RIFA species determination was performed by Lee A. Womack (LSU Entomology). Voucher specimens were deposited into the Louisiana State Arthropod Museum (LSAM).

STATISTICAL ANALYSES. SAS 9.1 software was used to analyze all data collected in these experiments (SAS Institute Inc. 2002). Chi-square analyses were performed to test for significant differences between mean number of non-RIFA ant species on treated (observed) versus untreated (expected) plots during pretreatment 2005, post-treatment 2005, and post-treatment 2006. One of the five treated plots was removed from analyses, because it was not paired with an untreated plot. Because many species were collected in very low abundance, several species could not be analyzed statistically. Alpha was set at 0.05.

RESULTS

The effects of Amdro® on RIFA are discussed in Chapter Two; the results on fire ants will only be discussed as they relate to non-RIFA here. Sampling dates were split into two periods – Period A for pretreatment (June 2005) and Period B for post-treatment (July 2005 through August 2006). Results will be expressed as Period A, Period B (2005 dates), and Period B (2006 dates).

During the pretreatment period, six species of ants from five genera of two subfamilies (Myrmecinae and Formicinae), including RIFA, were collected from both treated and untreated plots – *Aphaenogaster fulva-rudis-texana* (Umphrey), *Crematogaster lineolata*, *Pheidole dentata*, *Solenopsis invicta*, *Solenopsis molesta*, and *Paratrechina faisonensis*. *Aphaenogaster fulva-rudis-texana* and *P. faisonensis* were significantly more numerous ($\chi^2 = 22.9$, $P < 0.0001$ and $\chi^2 = 14.6$, $P < 0.0001$, respectively) in food traps on the soon-to-be-treated plots at pretreatment. *P. dentata* and *S. (Diplorhoptrum) molesta* were significantly ($\chi^2 = 7.9$, $P = 0.005$ and $\chi^2 = 22.7$, $P < 0.0001$, respectively) more numerous on untreated plots at pretreatment.

After treatment, a total of 22 ant species was collected on treated and untreated plots. Note that there was only one pretreatment sample versus 14 post-treatment. Also note that when

these species are plotted on a species accumulation curve in Microsoft® Excel 2002 (Microsoft® Corp. 1985-2001) using Mao Tau values calculated by EstimateS 7.5 (Colwell 2005), the curve does not plateau, which suggests more species may be present (Figure 3.1). Fourteen species of Myrmecinae, including RIFA, were trapped in addition to seven species of Formicinae and one species of Ponerinae. Species of Myrmecinae included *Aphaenogaster fulva-rudis-texana*, *Crematogaster ashmeadi* Mayr, *Crematogaster lineolata*, *Crematogaster pilosa* Emery, *Monomorium minimum*, *Myrmecina americana* Emery, *Myrmica punctiventris* Roger, *Pheidole dentata*, *Pheidole dentigula* Smith, *Pheidole moerens* Wheeler, *Solenopsis invicta*, *Solenopsis (Diplorhoptrum) molesta*, *Temnothorax curvispinosus* (Mayr), and *Temnothorax pergandei* (Emery) (Table 3.1). Species of Formicinae included *Brachymyrmex patagonicus* (Mayr), *Camponotus castaneus* (Latreille), *Camponotus pennsylvanicus* (De Geer), *Paratrechina arenivaga* (Wheeler), *Paratrechina faisonensis*, *Paratrechina vividula* (Nylander), and *Prenolepis imparis*. *Hypoponera opaciceps* (Mayr) was the only species of Ponerinae that was collected (Table 3.2).

Myrmecine ants, with the exception of *Solenopsis* spp. and *Pheidole* spp., were almost wiped out by treatment especially in 2005. *Aphaenogaster fulva-rudis-texana* was the most notable example. Prior to treatment in 2005, the species was significantly more numerous on to-be-treated plots versus plots that would remain untreated, but was adversely affected by treatment. This species was found in significantly fewer ($\chi^2 = 13.2$, $P < 0.0003$ and $\chi^2 = 24.7$, $P < 0.0001$) numbers on treated versus untreated plots in both 2005 and 2006, respectively. This translated to 100% and 98.4% reductions of *A. fulva-rudis-texana* on treated versus untreated plots in 2005 and 2006, respectively (Table 3.3). *Crematogaster ashmeadi* was collected in

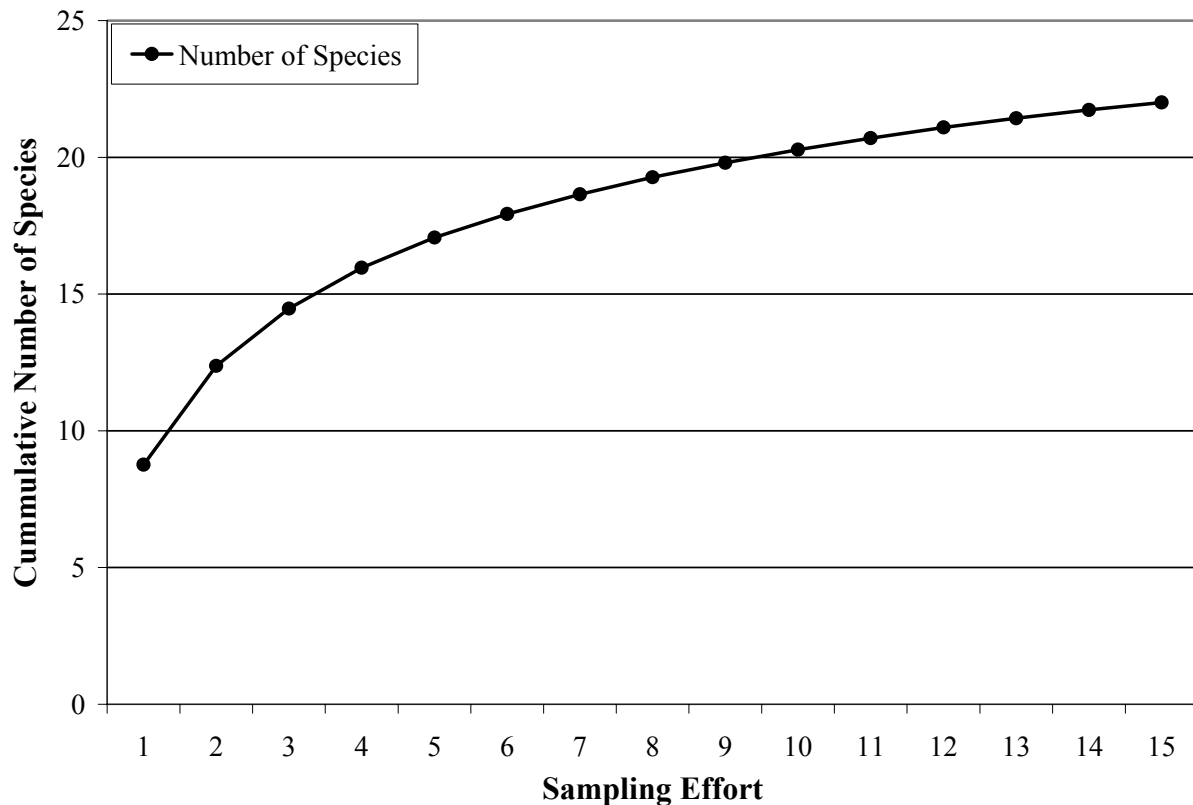


Figure 3.1. Species Accumulation Curve of all species trapped in food traps at Idlewild Experimental Research Station. Note that the curve has not reached a plateau, which suggests there are more species potentially present.

small numbers for most sampling dates; sufficient numbers in 2006 showed no significant ($\chi^2 = 0.08$, $P = 0.78$) difference between treated and untreated areas (Table 3.4). Post-treatment, *Crematogaster lineolata* was never collected in sufficient quantities for analysis. *Crematogaster pilosa* was significantly ($\chi^2 = 23.0$, $P < 0.0001$ and $\chi^2 = 31.2$, $P < 0.0001$) more common on untreated plots post-treatment than treated plots in 2005 and 2006, respectively. In fact, *C. pilosa* was never collected on treated plots (Table 3.5). *Monomorium minimum* was also significantly ($\chi^2 = 4.3$, $P = 0.04$ and $\chi^2 = 8.8$, $P = 0.003$) more common on untreated plots than treated plots in 2005 and 2006 after treatment. This species, too, was never collected on treated

plots (Table 3.6). *Myrmecina americana* and *Myrmica punctiventris* were both collected in insufficient numbers for analyses.

Pheidole dentata, the best represented non-RIFA, provided interesting results. After treatment in 2005, *P. dentata* was significantly ($\chi^2 = 188.5$, $P < 0.0001$) more common on untreated than treated plots. In 2006, the occurrence of this species on treated plots did not differ significantly from untreated plots ($\chi^2 = 0.0$, $P = 1.00$) (Table 3.7). *Pheidole dentigula* and *P. moerens* were both collected in extremely low numbers. Only *P. moerens* could be analyzed and only for 2006 when its occurrence was found not significant ($\chi^2 = 0.08$, $P = 0.78$) (Table 3.8). *Solenopsis molesta* was significantly ($\chi^2 = 12.2$, $P = 0.0005$) more common on untreated plots than treated plots after treatment in 2005, but was not in 2006 ($\chi^2 = 2.6$, $P = 0.11$) (Table 3.9). *Temnothorax curvispinosus* and *T. pergandei* were not collected in sufficient numbers for analyses.

Prenolepis imparis was the only formicine ant collected in sufficient numbers for analysis. Post-treatment in 2005, *P. imparis* was significantly ($\chi^2 = 6.9$, $P = 0.009$) more commonly collected on treated plots than untreated plots. In 2006, however, there was no significant difference ($\chi^2 = 0.08$, $P = 0.78$) between treatments for this species (Table 3.10). The ponerine ant *Hypoponera opaciceps* was not collected in sufficient numbers for analysis.

Overall, only three non-RIFA ant species were collected in sufficient numbers during pretreatment, and post-treatment 2005 and 2006 for analyses. These species were all myrmecines – *A. fulva-rudis-texana*, *P. dentata*, and *S. molesta*. *C. pilosa* and *M. minimum* of Myrmecinae and *P. imparis* of Formicinae were not collected in sufficient numbers pretreatment but were post-treatment. *C. ashmeadi* and *P. moerens* were only collected in sufficient numbers in 2006.

Table 3.1. Number of individual ants of subfamily Myrmecinae collected at Idlewild Experimental Research Station on treated and untreated control plots for each sampling period.

Subfamily	Species	Pretreatment 2005 Treated	Pretreatment 2005 UTC	Post- treatment 2005 Treated	Post- treatment 2005 UTC	Post- treatment 2006 Treated	Post- treatment 2006 UTC
Myrmecinae	<i>Aphaenogaster fulva-rudis-texana</i>	438	197	0	384	14	883
	<i>Crematogaster ashmeadi</i>	0	0	36	0	192	225
	<i>Crematogaster lineolata</i>	30	23	0	315	30	228
	<i>Crematogaster pilosa</i>	0	0	0	619	0	1092
	<i>Monomorium minimum</i>	0	0	0	162	0	367
	<i>Myrmecina americana</i>	0	0	0	3	6	0
	<i>Myrmica punctiventris</i>	0	0	5	9	1	20
	<i>Pheidole dentata</i>	399	574	882	6762	4934	4931
	<i>Pheidole dentigula</i>	0	0	0	0	0	35
	<i>Pheidole moerens</i>	0	0	34	127	744	685
	<i>Solenopsis invicta</i>	4411	3642	3767	15035	6592	24237
	<i>Solenopsis molesta</i>	4	102	0	358	135	332
	<i>Temnothorax curvispinosus</i>	0	0	0	0	1	0
	<i>Temnothorax pergandei</i>	0	0	0	4	0	4
	All Myrmecinae	5282	4538	4724	23778	12649	33039
	All Myrmecinae minus <i>S. invicta</i>	871	896	957	8743	6057	8802
	All Myrmecinae minus <i>S. invicta</i> and <i>P. dentata</i>	472	322	75	1981	1123	3871
	Percent of Myrmecinae catch explained by <i>S. invicta</i>	83.5%	80.3%	79.7%	63.2%	52.1%	73.4%

Table 3.2. Number of individual ants of subfamilies Formicinae and Ponerinae collected at Idlewild Experimental Research Station on treated and untreated control plots for each sampling period.

Subfamily	Species	Pretreatment 2005 Treated	Pretreatment 2005 UTC	Post- treatment 2005 Treated	Post- treatment 2005 UTC	Post- treatment 2006 Treated	Post- treatment 2006 UTC
Formicinae	<i>Brachymyrmex patagonicus</i>	0	0	41	0	74	2
	<i>Camponotus castaneus</i>	0	0	0	0	1	1
	<i>Camponotus pennsylvanicus</i>	0	0	10	2	33	23
	<i>Paratrechina arenivaga</i>	0	0	0	0	0	3
	<i>Paratrechina faisonensis</i>	64	2	2	152	19	4
	<i>Paratrechina vividula</i>	0	0	0	2	0	0
	<i>Prenolepis imparis</i>	0	0	459	143	192	162
	All Formicinae	64	2	512	299	319	195
Ponerinae	<i>Hypoponera opaciceps</i>	0	0	0	6	0	1

Table 3.3. Mean number of *Aphaenogaster fulva-rudis-texana* from food traps at Idlewild Experimental Research Station for each sampling period.

Sampling Period	Treated (Mean \pm SEM)	Untreated Control (Mean \pm SEM)	χ^2	P value
Pretreatment 2005	110 \pm 69	49 \pm 21	22.9	< 0.0001
Post-treatment 2005	0 \pm 0	16 \pm 5	13.2	0.0003
Post-treatment 2006	0.4 \pm 0.4	28 \pm 7	24.7	< 0.0001

Table 3.4. Mean number of *Crematogaster ashmeadi* from food traps at Idlewild Experimental Research Station for each sampling period.

Sampling Period	Treated (Mean \pm SEM)	Untreated Control (Mean \pm SEM)	χ^2	P value
Pretreatment 2005	0 \pm 0	0 \pm 0	0.0	1.00
Post-treatment 2005	2 \pm 1	0 \pm 0	0.10	0.75
Post-treatment 2006	6 \pm 5	7 \pm 4	0.08	0.78

Table 3.5. Mean number of *Crematogaster pilosa* from food traps at Idlewild Experimental Research Station for each sampling period.

Sampling Period	Treated (Mean \pm SEM)	Untreated Control (Mean \pm SEM)	χ^2	P value
Pretreatment 2005	0 \pm 0	0 \pm 0	0.0	1.00
Post-treatment 2005	0 \pm 0	26 \pm 15	23.0	< 0.0001
Post-treatment 2006	0 \pm 0	34 \pm 13	31.2	< 0.0001

Table 3.6. Mean number of *Monomorium minimum* from food traps at Idlewild Experimental Research Station for each sampling period.

Sampling Period	Treated (Mean \pm SEM)	Untreated Control (Mean \pm SEM)	χ^2	P value
Pretreatment 2005	0 \pm 0	0 \pm 0	0.0	1.00
Post-treatment 2005	0 \pm 0	7 \pm 6	4.3	0.04
Post-treatment 2006	0 \pm 0	11 \pm 11	8.8	0.003

Table 3.7. Mean number of *Pheidole dentata* from food traps at Idlewild Experimental Research Station for each sampling period.

Sampling Period	Treated (Mean \pm SEM)	Untreated Control (Mean \pm SEM)	χ^2	P value
Pretreatment 2005	100 \pm 57	144 \pm 63	7.9	0.005
Post-treatment 2005	37 \pm 9	282 \pm 70	188.5	< 0.0001
Post-treatment 2006	154 \pm 45	154 \pm 53	0.0	1.00

Table 3.8. Mean number of *Pheidole moerens* from food traps at Idlewild Experimental Research Station for each sampling period.

Sampling Period	Treated (Mean \pm SEM)	Untreated Control (Mean \pm SEM)	χ^2	P value
Pretreatment 2005	0 \pm 0	0 \pm 0	0.0	1.00
Post-treatment 2005	1 \pm 1	5 \pm 2	2.2	0.14
Post-treatment 2006	23 \pm 16	21 \pm 15	0.08	0.78

Table 3.9. Mean number of *Solenopsis (Diplorhoptrum) molesta* from food traps at Idlewild Experimental Research Station for each sampling period.

Sampling Period	Treated (Mean \pm SEM)	Untreated Control (Mean \pm SEM)	χ^2	P value
Pretreatment 2005	1 \pm 1	26 \pm 26	22.7	< 0.0001
Post-treatment 2005	0 \pm 0	15 \pm 15	12.2	0.0005
Post-treatment 2006	4 \pm 3	10 \pm 8	2.6	0.11

Table 3.10. Mean number of *Prenolepis imparis* from food traps at Idlewild Experimental Research Station for each sampling period.

Sampling Period	Treated (Mean \pm SEM)	Untreated Control (Mean \pm SEM)	χ^2	P value
Pretreatment 2005	0 \pm 0	0 \pm 0	0.0	1.00
Post-treatment 2005	19 \pm 14	6 \pm 3	6.9	0.009
Post-treatment 2006	6 \pm 5	5 \pm 4	0.08	0.78

Low capture rates for many species made proper statistical analyses of most data impossible. Less than half of the species collected were represented by > 300 individuals. In addition, comparisons between pretreatment and post-treatment numbers are confounded by low captures pretreatment for most species, which may be remedied by additional pretreatment samples.

DISCUSSION

Several species could be analyzed in sufficient numbers. *Aphaenogaster fulva-rudis-texana* was clearly affected by Amdro® treatment. *Crematogaster pilosa* and *Monomorium minimum* were only found on untreated plots, but the complete lack of captures pretreatment on either types of plots makes assigning responsibility to Amdro® treatments risky. None of the aforementioned genera is listed as a target ant species on Amdro®'s label (Ambrands®, http://www.amdro.com/Amdro/downloads/amdro_label_041503.pdf). *Solenopsis molesta* was found during pretreatment and post-treatment on both treated and untreated plots, although always in lower numbers on treated plots. *Pheidole dentata* was well represented on both treated and untreated plots, but was significantly more common on untreated plots in 2005. Big-headed ants, which would presumably include *P. dentata*, are listed as target species on the Amdro® label.

Of all ants collected, *Aphaenogaster fulva-rudis-texana* was most negatively affected by Amdro® treatments. In fact, *A. fulva-rudis-texana* was significantly more abundant on the to-be-treated plots at pretreatment than the untreated plots. Even RIFA, the target ant species, did not show such high percentage of control. *A. fulva-rudis-texana* occurs most frequently in wooded portions of the plots, which it shares with only low densities of RIFA. The majority of the *A. fulva-rudis-texana* were collected in hardwood forest in particular, areas not usually colonized by RIFA, which benefit from disturbed habitats (Taber 2000: 26-28). Had RIFA occurred more regularly in habitats preferred by *A. fulva-rudis-texana*, then, perhaps, RIFA would have collected the majority of the bait. Dominance of RIFA at the baits might have prevented suppression of *A. fulva-rudis-texana* by Amdro®. Therefore, treatment of wooded areas, especially those with little RIFA presence, should not be broadcast treated with Amdro® as such

treatment may eliminate non-target ant species. Elimination of large predatory ants such as *A. fulva-rudis-texana* may be doubly deleterious, because ants are major predators of founding RIFA queens and may help suppress RIFA populations or expansions (Nichols and Sites 1991). Reduction of a species of this genus in this study concurs with Zakharov and Thompson (1998), who found that myrmecine ants including *Aphaenogaster* were most negatively affected by treatment of hydramethylnon baits.

Crematogaster pilosa, a primarily arboreal ant, is also an ant of the wooded areas of plots. Before treatment, this species was not trapped on either untreated or soon-to-be-treated plots. Although the species was not trapped before treatment, it is difficult to believe that treatment did not have some negative effect on *C. pilosa*. Paired plots in wooded areas were nearly identical with similar canopy, mid- and understory composition and age. There is no other obvious reason but treatment effect for no captures on treated plots. Zakharov and Thompson (1998) included *Crematogaster* as one of the myrmecine genera eliminated by hydramethylnon baits.

Zakharov and Thompson (1998) also list *Monomorium* as a genus negatively affected by hydramethylnon baits, although the species is not explicitly listed on Amdro®'s label. This study concurs with that conclusion with a caveat that *M. minimum* was not collected prior to treatment; circumstantial evidence supports this conclusion. In addition to hydramethylnon baits, *M. minimum* has been reduced by Mirex®, and treatments may be responsible for keeping little black ant populations low while allowing recolonization by invasives like RIFA (Markin *et al.* 1974, Summerlin *et al.* 1977). Although little black ant has been found to co-exist with even high densities of RIFA (Camilo and Philips 1990), other studies report that little black ant has been suppressed or eliminated by RIFA presence (Jusino-Atresino and Phillips 1994, Cook

2003). It appears that little black ant can co-exist in small numbers at least in the presence of RIFA in the pine-hardwood forests used in this study and in others in Louisiana (Womack 2006).

Solenopsis (Diplorhoptrum) molesta was collected in greater numbers on untreated plots throughout the study, significantly so at pretreatment and post-treatment 2005. It is not possible to determine if treatment had an effect on this species, because it was collected in low numbers on the to-be-treated plots at pretreatment. Thief ant has been reported as a main driving force preventing RIFA from establishing incipient colonies in previously uninfested areas (Camilo and Philips 1990, Vinson and Rao 2004). If hydramethylnon bait is responsible for fewer trap captures in 2005 and 2006 on treated versus untreated plots, then Amdro® may serve to functionally eliminate RIFA predators.

Pheidole dentata did not show a clear response to treatment. Post-treatment, the species was significantly ($\chi^2 = 188.5$, $P < 0.0001$) more common on untreated plots in 2005, but was not significant ($\chi^2 = 0.0$, $P = 1.00$) in 2006. In fact, the difference between numbers of individuals collected on treated plots versus untreated plots was only three individuals in 2006 totals. Significance post-treatment in 2005 may have simply been a holdover from significance in pretreatment or may represent actual treatment effect. If the decreased number in 2005 was due to treatment, then the reduction could occur by two means: particles trapped in trees were foraged by this species and increased numbers of smaller particles made particle size more attractive to the species. Overall, this species may be unaffected by both treatment and RIFA suppression, which suggests that the species can co-exist with RIFA and in undiminished numbers. That is, even with significant RIFA suppression, *P. dentata* did not show a significant increase in population. *Pheidole* spp. have been shown to be susceptible to poison baits and the presence of RIFA. Summerlin *et al.* (1977) found that *P. dentata* could be eliminated by

Mirex® in roadside communities. Amdro® eliminated *Pheidole* spp. from a number of habitats in southeastern Arkansas (Zakharov and Thompson 1998). Camilo and Philips (1990) and Cook (2003) found that *Pheidole* spp. could be eliminated by RIFA, and Wojcik (1994) found that RIFA, probably coupled with urbanization, was responsible for significant reductions in *P. dentata* on roadsides in Florida.

Occurrences of other ant species could be statistically analyzed with limited success. *Crematogaster ashmeadi* was unaffected by treatment or presence of RIFA; it was found in not significantly different numbers on treated and untreated plots throughout sampling. *Pheidole moerens* gave similar results; there was no significant difference in any sampling period. *Prenolepis imparis* was significantly ($\chi^2 = 6.9$, $P = 0.009$) more common on treated plots in 2005 and not significant at other times. Interestingly, Zakharov and Thompson (1998) found that this species was potentially negatively affected by Amdro®; the first year of their study showed fewer *P. imparis* on treated plots. The second year of their study suffered from low trap counts of the species on treated and untreated areas.

Prenolepis imparis may be a special case as it is frequently called the “winter ant” due to increased activity in colder months and cooler weather, a trait not shared with most other ant species in Louisiana. Whether this increase in activity is due to competitive release from species not as cold-tolerant as *P. imparis* or from a marked preference for cooler temperatures could not be determined by these experiments. It seems highly unlikely that RIFA had any actual impact on *P. imparis*, because RIFA were not very active during colder months when *P. imparis* was present in traps (October through December). In those months, RIFA was not common on treated or untreated plots; the average mean number of RIFA recorded during these cold months

was 55 ± 25 (mean \pm SEM.), compared with the lowest mean recorded during warmer months in 2005, which was 729 ± 214 individuals.

The most surprising result from this study is that with the possible exception of *P. imparis*, no ant species showed increased trap counts with the suppression of RIFA. *P. imparis* seemed to show random or seasonal population fluctuations, a pattern not readily attributable to RIFA or Amdro®. Eight of thirteen non-RIFA were myrmecines, a subfamily with members known to decrease after Amdro® treatments (Zakharov and Thompson 1998). Therefore, the use of Amdro® alone may have precluded detection of RIFA impacts. Of the seven species of formicines collected, only the problematic *P. imparis* was trapped in sufficient numbers for statistical analysis. Because formicines are less affected by Amdro®, if more formicine individuals were collected to allow analyses, impacts of RIFA might be discovered (Zakharov and Thompson 1998). Effects of Amdro® on ponerine ants such as *Hypoponera opaciceps* is not well understood; this species was poorly represented in food traps.

Aerial broadcast treatments to suppress RIFA may be best suited for pasture areas or other areas with little or no canopy and should only be used in areas where RIFA are causing significant harm to native faunae or agro-business interests. The use of chemicals to suppress pest species should be performed only after all opportunity costs are fully understood. That is, broadcast treatment of an area with little RIFA presence may result in suppression or elimination of native ants. The open niche left by eliminated ants will be recolonized by RIFA and frequently in nest densities far greater than that of the native ants. Treatment of forested landscapes may be unwarranted much of the time as RIFA are far less common inside forests. At forest edges where RIFA may occur, hand-spreaders or mound treatments may be utilized to suppress RIFA. Mound treatments would likely cause less disturbance to native ant fauna as

foraging would be further restricted to active RIFA territories. Unfortunately, mound treatments would be extremely difficult to execute in forested areas, where RIFA frequently nest in decaying woody debris such as logs or stumps and may not exhibit obvious nest mounds.

Research on effects of RIFA on non-targets may be confounded by effects from Amdro®. Amdro® is effective against many species in addition to the target group of fire ants. Many myrmecines collected in sufficient numbers for analysis were negatively affected by Amdro®. Several of these species occurred in areas not usually associated with strong RIFA presence, especially forest interiors. These non-RIFA ants may have recruited to baits that would have otherwise been immediately collected by RIFA. In addition, bait particles trapped in trees would be immediately gathered by more arboreal ant species rather than RIFA. Treatment of densely wooded areas or other areas where RIFA may not be the dominant ant species should be performed with great care to avoid eliminating non-targets, some of which may be effective predators on founding RIFA queens.

CHAPTER 4

IMPACTS OF RED IMPORTED FIRE ANT ON CAPTIVE NORTHERN BOBWHITE NESTING

INTRODUCTION

Since its introduction in the 1930's, the red imported fire ant (RIFA), *Solenopsis invicta* Buren, has proven to be a difficult species to control, and little has been done to curb its effects in natural ecosystems due to the procedures and costs involved. Such factors have allowed RIFA's unchecked spread into suitable environments, which RIFA only briefly shared with its native congeners. With the threat of this newly introduced and highly efficient predator, the native wildlife of the USA face a species with which they have had no previous experience. The Northern Bobwhite, *Colinus virginianus*, is one such native species that now occurs sympatrically with RIFA in much of its range (Appendix).

One of the most economically important and widely recognized birds within its range, the Northern Bobwhite occurs throughout much of the eastern USA from Wisconsin in the north, the Gulf of Mexico in the south, the eastern seaboard in the east, and to the Rocky Mountains in the west (Appendix). A small, disjunct population of the endangered subspecies Masked Bobwhite (*C. v. ridgwayi*) occurs in extreme southern Arizona. As a testimony to the Northern Bobwhite's prized gamebird status, the species also occurs in pockets of introduction in some western states and various islands (Sauer *et al.* 2005, NatureServe 2006).

Unfortunately, bobwhite numbers have decreased rapidly throughout much of its range. According to US Breeding Bird Survey estimates, the species has experienced an average yearly decline of 3.0% from 1966-2005 (Sauer *et al.* 2005). In Louisiana, Mississippi, and South Carolina, harvests of bobwhites have dropped > 90% since 1980, a combination of fewer birds and fewer hunters (Dimmick *et al.* 2002, Mike W. Olinde, *pers. comm.*). Dimmick *et al.* (2002)

suggested that the species could be extirpated in some areas by 2010 if countermeasures are not implemented immediately. Paralleling the precipitous decline of the bird, the number of quail hunters in Louisiana has dropped from 30,000-50,000 to only 3000-5000 individuals in the past few decades (Mike W. Olinde, *pers. comm.*). Although habitat destruction and alteration and cleaner farming practices are the most likely reasons for the quail's drastic decline, RIFA has often been cited as an exacerbating factor (Brennan 1991, Allen *et al.* 1995, Mueller *et al.* 1999, Allen *et al.* 2000, Dabbert *et al.* 2002). That being said, the effect of fire ants on bobwhites remains elusive; the two camps – those who believe that fire ants are a large factor and those who think their effect is minimal – are highly polarized (Brennan 1991, 1993, Allen *et al.* 1993). The issue is, in fact, clouded with many studies; studies of which are complicated by ant identification issues and varying quail environments.

The red imported fire ant is believed to have been a stowaway in a shipment or, perhaps, in the ballasts of ships that docked in the Port of Mobile, Alabama in the 1930's. It is a native of the Pantanal of northern Argentina, Paraguay, and southern Brazil (Vinson and Sorensen 1986). It has very few predators in the USA, though sizeable numbers may be preyed upon during mating flights and colony-founding (Whitcomb *et al.* 1973, Nichols and Sites 1991). Approximately 20 years earlier, the black imported fire ant (BIFA), *Solenopsis richteri* Forel, was introduced into, perhaps, the same port and rapidly spread across Alabama and Mississippi (Vinson and Sorensen 1986, Vinson 1997). As RIFA became sympatric with BIFA, BIFA was outcompeted and now is restricted to northeastern Mississippi and northwestern Alabama (Vinson 1997, Vinson and Sorensen 1986). As BIFA and RIFA were once lumped into one species, *S. saevissima* Forel, early research on imported fire ants and subsequent publications made little attempt to specify whether the ants were of the red form or the black form. In fact, a

landmark quail study by Johnson (1961) almost certainly dealt with the black form. It wasn't until the 1970's that the two forms were elevated to species status by Buren (Buren 1972, Vinson and Sorensen 1986). Therefore, it becomes rather problematic to determine which species is being studied and reported upon in works prior to the early 1970's (Horton *et al.* 1975, Camilo and Philips 1990, Allen *et al.* 1994). This translates to much confusion when one wants to distinguish between early reports of impacts by BIFA and RIFA on wildlife.

Early studies on bobwhites and fire ants tend to show that fire ants were not actually having significant effects on the quail (Johnson 1961). But more recent studies demonstrate the opposite; that is, that RIFA are, indeed, causing measurable losses in Northern Bobwhite reproduction (Allen *et al.* 1995, Allen *et al.* 2000). Either something has changed in our experimental procedures or something has changed in the biology of the fire ant or Northern Bobwhite (Brennan 1999). Interestingly, in 1973, only a few years after the splitting of *S. saevissima* Forel into two species (BIFA and RIFA), multiple-queen, or polygyne, colonies of RIFA were discovered (Glancey *et al.* 1973, Vinson and Sorensen 1986). Single-queen, or monogyne, colonies of RIFA contain one queen per colony, and mound density of monogyne colonies may average 300 mounds/ha (Porter *et al.* 1991). Polygyne colonies may contain 20 or more queens with average mound densities of approximately 700 mounds/ha (Porter *et al.* 1991). Mound densities of polygyne RIFA may be as high as 3000 mounds/ha in some areas (Porter *et al.* 1991). It was polygyne colonies in Texas, where polygyne colonies occur regularly, that were found to significantly affect bobwhites (Allen *et al.* 1995, Porter *et al.* 1991). Polygyne colonies have been found in Texas, Louisiana, Mississippi, Florida, South Carolina, and Georgia (Glancey *et al.* 1973, Vinson and Sorensen 1986, Porter *et al.* 1991, Porter *et al.* 1992, Kintz-*Early et al.* 2003). Louisiana is a mosaic of monogyne and polygyne colonies (Michael A.

Seymour and Linda M. Hooper-Bùi, *unpublished data*). Allen *et al.* (2000) also found that post-fire ant introduction, bobwhite abundance showed a significant downward trend. This occurred despite the fact that the vast majority of RIFA colonies in the southeastern United States are monogyne (Allen *et al.* 2000). In light of these recent developments, bobwhite-fire ant projects should be of utmost importance to wildlife managers.

Certainly, RIFA has a well-deserved reputation for wreaking havoc among our native faunae (Allen *et al.* 1994) – vertebrate taxa believed affected are a diverse group including small mammals (Masser and Grant 1986, Flickinger 1989, Killion *et al.* 1990, Smith *et al.* 1990, Lechner and Ribble 1996, Holtcamp *et al.* 1997, Pedersen *et al.* 2003, Womack 2006), birds (Ridleyhuber 1982, Wilson and Silvy 1988, Drees 1994, Lockley 1995, Giuliano *et al.* 1996, Pedersen *et al.* 1996, Sikes and Arnold 1986, Mueller *et al.* 1999, Allen *et al.* 2000, Allen *et al.* 2001b, Dabbert *et al.* 2002, Stake and Cimprich 2003, Smith *et al.* 2004), reptiles and amphibians (Landers *et al.* 1980, Mount 1981, Mount *et al.* 1981, Montgomery 1996, Reagan *et al.* 2000, Allen *et al.* 2001a, Parris *et al.* 2002, Epperson and Heise 2003, Wetterer and Moore 2005, Womack 2006), and even fish (Contreras and Labay 1999). Readers interested in RIFA's effects on native ground-dwelling mammals and herpetofauna (and arthropods) in Louisiana are strongly encouraged to view Womack (2006) and Landry (2004).

RIFA have been known to attack many bird species and usually during nesting, the most vulnerable time in a bird's life. In fact, all published reports to date on birds and RIFA concern attacks by RIFA at bird nests. RIFA may be an important predator at bird nests due to their ability to bite and sting, and published reports of RIFA-related reduction of nesting success relative to RIFA-suppressed areas ranges “between 26.7-92%” (Saurez *et al.* 2005). Many of

these studies on birds and RIFA concern colonial nesting birds, more specifically swallows, terns, and wading birds (Sikes and Arnold 1986, Drees 1994, Lockley 1995, Kosciuch *et al.* 2001).

Drees (1994) studied nesting colonial waterbirds, in particular, Great Egret, *Ardea alba*; Great Blue Heron, *A. herodias*; Neotropic Cormorant, *Phalacrocorax brasilianus*; Snowy Egret, *Egretta thula*; Tricolored Heron, *E. tricolor*; Roseate Spoonbill, *Platalea ajaja*; Laughing Gull, *Larus atricilla*; Gull-billed Tern, *Gelochelidon nilotica*; and Forster's Tern, *Sterna forsteri*.

Drees (1994) used fenoxycarb fire ant bait, an insect growth regulator, to suppress RIFA numbers on man-made spoil islands in Texas. The birds experienced little RIFA-related mortality until June, probably because fire ants become more active at that season due to increased demands for protein for their brood (Drees 1994, Pedersen *et al.* 1996). RIFA-suppressed parts of one island produced 72 successful offspring compared to only 6 successful offspring in the presence of RIFA, resulting in a 92% reduction of waterbird production due to RIFA. Many nests in RIFA-suppressed areas were reused up to three times and often by different bird species (Drees 1994). Lockley (1995) found that mortality of Least Tern, *Sternula antillarum*, on sites treated to suppress RIFA (fenoxycarb bait at 1.63 kg/ha) was an average of 26.7% lower than fire ant-infested sites.

Hooper-Bùi *et al.* (2004) studied the effects of southern fire ant (SFA), *Solenopsis xyloni* (McCook) on the endangered California Least Tern, *Sternula antillarum browni*, nesting in colonies on tarmacs in California. Least Terns chicks are semiprecocial, which might expose young to additional predation by fire ants, because although mobile, the chicks remain in the nest during development after hatching (Gill 2000: 434). SFA was observed breaching intact eggshells of tern eggs as well as Coturnix Quail, *Coturnix coturnix*, eggs placed near active SFA colonies in the field. In the laboratory, quail eggs were breached by SFA in 30 min with initial

attacks occurring within the first 30 sec. Interestingly, when the same laboratory experiment used RIFA, no eggs were breached, which agrees with the current dogma concerning breaches of bird eggs by RIFA: RIFA do not breach bird eggshells (Hooper-Bùi *et al.* 2004).

Many swallow species are also colonial nesters. Cliff Swallows, *Petrochelidon pyrrhonota*, frequently nest in culverts and other man-made structures increasing the risk of potential encounters with RIFA, which thrives in disturbed habitats (Sikes and Arnold 1986, Kosciuch *et al.* 2001). Risk of fire ant attack likely increases dramatically for altricial birds like swallows, the young of which remain in the nest for 2-3 weeks after hatching and much of that time as helpless, naked chicks (Gill 2000: 434). Sikes and Arnold (1986) observed extremely high mortality of Cliff Swallows in the presence of RIFA in east-central Texas. In 1982, they observed 13 of 21 nests in one culvert destroyed by RIFA (Sikes and Arnold 1986). Fire ants were responsible for a 34.4% decrease in nesting success of these swallows; nesting success was compared between culverts treated with Amdro® to suppress fire ants and those left untreated (Sikes and Arnold 1986). Sikes and Arnold (1986) also found that RIFA were most damaging from the time the eggs pipped, at which time RIFA would enter the eggs and kill the chicks, to the time the chicks developed their feather tracts seven to ten days post-hatch.

Kopachena *et al.* (2000) found that RIFA depredated 24.7% of culvert nesting Barn Swallows, *Hirundo rustica*, at one site in Texas, whereas a site with three times the RIFA mound density suffered no depredation by the ants. It was suggested that microclimate and habitat features – particularly humidity and temperature – may affect the attractiveness of nests at the two sites. The unaffected bird nests at site two were covered with a potentially desiccating chalk-like dust, were exposed to indirect sunlight, and were in hotter temperatures, which may explain the protection from three times the number of ant colonies, whose foragers would have

faced more exposed and dangerous conditions at site two (Kopachena *et al.* 2000). Three nests attacked by ants at the egg stage, were abandoned. In two cases, eggs were left intact; in the third nest, eggs were emptied by the ants. The authors do not mention how the ants gained access (e.g., from hairline cracks, etc.) to these eggs' contents. RIFA also attacked swallow chicks as also found by Sikes and Arnold (1986). Eighteen nests with nestlings were destroyed. Two other nests fledged early (Kopachena *et al.* 2000). Implications of early fledging are not discussed, but such an event may lead to decreased fitness or expose a fledgling to a whole suite of predators too early in its life. Using Breeding Bird Survey data to investigate trends in Barn Swallows in RIFA-infested areas, Kopachena *et al.* (2000) concluded that RIFA are not significantly affecting populations of Barn Swallows in the southeastern USA.

Other authors also report attacks on pipping or newly hatched chicks (Ridlehuber 1982, Dickinson 1995, Legare and Eddleman 2001). Ridlehuber (1982) discovered three instances of RIFA attack on Wood Duck, *Aix sponsa*, ducklings and pipped eggs from 20 successful clutch hatchings in Wood Duck nest boxes placed on poles above land in Texas. Ridlehuber (1982) also noted that 70% of natural cavities (n = 20) were used by foraging RIFA and that such occupation likely decreases available nesting cavities for Wood Ducks. Dickinson (1995) noted two instances of RIFA predation on hatchling Crested Caracaras, *Caracara cheriway*, in prairie land in Texas. It should be noted, however, that caracara nests earlier in the season showed no RIFA attacks, which, as in other studies, suggests that seasonality plays a key role in RIFA bird nest attacks (Dickinson 1995). Legare and Eddleman (2001) observed one nest of Black Rail, *Laterallus jamaicensis*, in Florida destroyed by RIFA, which entered pipped eggs and killed a hatchling.

Endangered species, including Golden-cheeked Warbler, *Dendroica chrysoparia*, and Black-capped Vireo, *Vireo atricapilla*, are also vulnerable to RIFA attacks at the nest (Stake and Cimprich 2003, Smith *et al.* 2004, Stake *et al.* 2004). Stake *et al.* (2004) reported an attack on a Golden-cheeked Warbler nest by RIFA. Golden-cheeked Warbler nests are usually built an average of 4.8 m above the ground (U.S. Fish and Wildlife Service 1992: 13). Prior to the nest attack documented by Stake *et al.* (2004), a chick from the same nest had fallen to the ground and been attacked by RIFA. Ten hours later, the same nest was depredated by RIFA climbing into the nest (Stake *et al.* 2004). Stake *et al.* (2004), however, did not consider RIFA to be a major threat to these warblers.

The same is not true for Black-capped Vireo, a species that nests closer to the ground, usually between 40 and 120 cm above ground (U.S. Fish and Wildlife Service 1991: 21). Stake and Cimprich (2003) found RIFA to be second only to snakes in the number of nests depredated. In this study, 48 of 142 observed nests failed due to predators. In 31% of the depredated nests, RIFA was the predator (Stake and Cimprich 2003). Most RIFA predation (92%) took place at night when RIFA flushed brooding adults off the nest and killed nestlings (Stake and Cimprich 2003). Despite RIFA visits also taking place in the egg stage, no RIFA attacks on eggs were observed, and it is believed that the ants could not damage the vireo's eggshells (Stake and Cimprich 2003).

Smith *et al.* (2004) reported observations of Black-capped Vireo nests attacked by RIFA. At one nest, both adults, hours apart, attempted to remove the ants from the nest via aerial defense displays and actively pecking the ants, but the nest was ultimately abandoned due to RIFA. Examination of eggs revealed a small hole in one egg. This breach was attributed to an eastern woodrat, *Neotoma floridana*, that had visited the nest during the ant attack, but

subsequently left, possibly deterred by RIFA presence. Smith *et al.* (2004) reiterated that RIFA likely do not breach vireo eggs. In a second nest, RIFA swarmed over three nestlings, which were soon eaten by a Texas Rat Snake (*Elaphe obsoleta lindheimeri*) despite the nestlings being covered with attacking ants. The authors suggested that the snake may have been attracted to the nest due to commotion caused by stinging and biting ants and that increased nest defense by adults may also, ultimately, lure predators to the nests (Smith *et al.* 2004).

Adverse effects on bobwhites by RIFA occur on many scales through both direct means such as predation upon hatchlings and indirect means such as competition for food resources. Allen *et al.* (1995) suggested a third category that includes negative effects from exposure to fire ant stings. Indirect factors with reference to bird foraging are largely unstudied. Travis (1938a) noted that seed stores by ants may limit the availability of this resource for seed-eating birds such as bobwhite. Allen *et al.* (2001b) studied the effect of fire ants on wintering Loggerhead Shrikes, *Lanius ludovicianus*. Shrikes avoided areas with lower arthropod density, which was attributed to fire ant predation. As invertebrates are important for fitness of both young bobwhite chicks and reproductive females, fire ant-induced invertebrate prey shortages could negatively impact quail populations (Giuliano *et al.* 1996).

Pedersen *et al.* (1996) noted changes in time allotted for sleeping, moving, and foraging in bobwhite chicks. Chicks were placed in paired outdoor, 30 m² areas and observed in 15 min intervals (with a different chick after each interval) during their first six days after hatching. The impacts of RIFA on behaviors were highly seasonal with the most significant results occurring later in the season. During the early period (March through April), chicks spent less time sleeping in the untreated area than the treated area, and spent more time responding to RIFA in the untreated area than the treated area. The latter response, however, was confined to those

chicks standing atop RIFA nest mounds. Otherwise, chicks reacted similarly on the treated and untreated areas in the early period. In the later period (May through June) on the untreated area, chicks spent more time responding to RIFA and moving about and spent less time sleeping than on the treated area. Seasonality of response may have important implications on bobwhite nesting. Increased soil temperature and seasonal food preferences of RIFA (increased protein is needed during brood production) may cause particularly increased predation of bobwhite hatchlings later in the season. As far as RIFA predation of quail is concerned, early nesting may be the best mechanism to avoid increased loss by RIFA (Pedersen *et al.* 1996).

Giulliano *et al.* (1996) investigated the effect of RIFA exposure on bobwhite chicks. To accomplish this, 4-day-old chicks were exposed to either 0, 10, 25, 50, 100, or 200 RIFA for 60 sec or 50, 100, or 200 RIFA for 15 sec in 4 L glass beakers. After the allotted interval had passed, chicks were placed back into a brooder and weighed at days 1, 3, 5, 7 and 9 post-treatment. Unexposed birds served as controls. Giulliano *et al.* (1996) found that “exposure to as few as 50 RIFA for 60 seconds and 200 RIFA for 15 seconds negatively affected survival of quail chicks.” Such exposures corresponded to “as few as 6.5 and 22.5 ants on a chick,” respectively (Giulliano *et al.* 1996). Exposure to 200 ants for 60 sec negatively affected chick body mass, which may result in decreased fitness and survival. Giulliano *et al.* (1996) conceded that such exposure of so many ants seems unlikely in the field, but field experiments are lacking. Deaths in the study were attributed to fire ant stings, although no toxicological tests were performed.

Mueller *et al.* (1999) examined the hatching success and survival of bobwhite chicks in Texas. Using radio-tagged females, Mueller and colleagues were able to locate quail nests. A square area 60 m x 60 m, centered on each quail nest, was treated with Amdro® at 1.7 kg/ha.

Two applications resulted in > 99% suppression of RIFA. Interestingly, hatching success of nests in treated and untreated areas did not differ significantly, suggesting that despite this most vulnerable time in a bobwhite's life, hatching is unaffected by RIFA. It should be noted that bobwhites have precocial chicks, which means the young birds can follow adults out the nest within hours of hatching, lessening the risk of RIFA encounters in the nest. Surprisingly, treatment effect on chick survival at 3 weeks post-hatching was notable; death of all chicks occurred in 52% of broods from untreated nests and 22% of broods from treated nests. Only half as many chicks survived in the untreated areas compared to those on fire ant-suppressed areas after three weeks (Mueller *et al.* 1999). Implications on renesting are discussed, namely that even more time will be lost for reproduction if chicks die after hatching than if a nest has been destroyed prior to hatch (Mueller *et al.* 1999). It should be noted that only nests incubated by females were included in this study. Males may make up 26.5% of the incubators in some populations (Stoddard 1931: 29-32). It would be interesting to see if altricial chicks would show the same pattern of delayed treatment effect as those chicks would be confined to the nest for longer periods post-hatch.

A landmark quail study by Johnson (1961) made a valiant attempt to address the bobwhite-fire ant debate. Unfortunately, the study occurred early in the invasion of the USA by RIFA, and the species is given as "imported fire ant (*Solenopsis saevissima richteri* Forel)" in Johnson's abstract, the name that usually refers to what became known as the black imported fire ant, *S. richteri* Forel, over a decade later (Mueller *et al.* 1999). Also, Johnson (1961) gave the introduction date as "about 1920," a date we regard as closer to BIFA's introduction date, not RIFA's. We now know that RIFA pose an especially difficult problem with the newly discovered polygyne form as nest densities of this form produce extremely high numbers of

foragers. Regardless of the *Solenopsis* species in question, Johnson's study is still valuable. The study found that 6% of chick mortality could be attributed to fire ants, but that number could be as high as 8.2% if all fire ant-covered chicks were actually killed by fire ants (not simply scavenged). If including all fire ant-covered chicks, 34.5% of all nests contained chicks that were potentially killed by fire ants. Johnson (1961) suggested that fire ants reserve most of their damage to chicks having difficulty hatching. Difficulties included nest desertion by parents, drought conditions, and irregularity in the time of hatching (Johnson 1961). Johnson (1961) concluded that fire ants "are of little importance as predators on quail" and that "the invasion by the imported fire ant has had little if any effect on total quail populations." Allen *et al.* (1994) believed that Johnson's conclusions regarding the observed 6% mortality by fire ants to be "de-emphasized."

While identifying predators at Northern Bobwhite nests in southern Georgia, Staller *et al.* (2005) found fire ants, probably RIFA based on locale, to be responsible for 13 complete depredation events – seven prior to hatch and six during hatching. Fire ants ranked third in the list of known bobwhite nest depredations, with 12% of the depredated nests. In attacks prior to hatching, the ants "created a mound over the eggs," and these eggs were still intact 2 weeks later (Staller *et al.* 2005).

Other *Solenopsis* species have been historically linked to bobwhite nest attacks. Stoddard (1931: 193-194) reported that thief ants, *S. molesta* (Say), were observed entering eggs while chicks were attempting to hatch. He reported that 12.5% of bobwhite nests at Forshala Plantation, Florida, were "taken over by ants," possibly tropical fire ant, *S. geminata*, according to Stoddard (1932). The thief ants were even observed attempting to breach the eggshells by using clusters of workers concentrated on one small area of shell. Stoddard (1931: 194) did not

observe any successful breaches, but suggested that “it seems probable that they sometimes do [breach the shells], for such ant clusters often make noticeable progress in their work on the shell.” Travis (1938b) reported number of bobwhite nests destroyed all or in part by fire ants at Forshala Plantation for several years (1924-1937). Fire ant predation on nests ranged from one to nine percent (avg. = 6.5%) (Travis 1938b, Allen *et al.* 1994). Ants from the Travis (1938a, 1938b) works are most likely *S. geminata*.

Studies done in one state may not be comparable to those in others simply due to differences in habitat quality, hunting pressures, how long RIFA have infested the areas, and whether RIFA colonies are monogyne or polygyne, and some differences may simply be caused by adaptation by the birds to RIFA presence. In the decades Northern Bobwhites have been exposed to RIFA, it may be possible that the species has evolved mechanisms to avoid direct or indirect predation by RIFA. If this is the case, then bobwhites found in the southern USA, where RIFA have occurred the longest, should have more success raising broods than bobwhites in the northern extent of its range, where RIFA have more recently invaded.

I tested multiple null hypotheses, (1) RIFA have no effect on Northern Bobwhite nesting success rate, (2) bobwhite populations exposed to RIFA for 40+ years will have a nesting success rate that does not differ significantly from Northern Bobwhite populations that have never been exposed to RIFA when both of these populations are experimentally exposed to RIFA in a controlled nesting situation, and (3) naïve, wild bobwhites should not differ significantly in their nesting success rate from captive-raised bobwhites when exposed to fire ants in these tests. Because differences between populations might have resulted from natural selection driven by RIFA pressure, the most appropriate form of testing would likely be a common garden experiment, as first described by Clausen *et al.* (1940).

MATERIALS AND METHODS

STUDY AREA. Please see Chapter Two of this work for description of Idlewild Experimental Research Station, its purpose and its habitats.

DESIGN 2005. Nine 11-acre (4.45 ha) plots were established at Idlewild Experimental Research Station. Plots occurred in edge habitats, hardwood forest, and pine-hardwood forest and were paired by these features. That is, if one plot occurred in pine-hardwood forest, a second plot, paired with the first, would also occur in a portion of pine-hardwood. Four pairs were created in this way. One of the nine plots was established as an extra plot to house surplus birds to be used in cases of restocking aviaries. One plot of each pair and the extra plot were treated aerially with Amdro® (A.I. 0.73% hydramethylnon). Application of Amdro® bait used to suppress RIFA and those sampling techniques used to measure success of treatment are discussed in previous chapters.

Three aviaries were erected per 11-acre (4.45 ha) plot, one for each population of Northern Bobwhite to be used in the study. Wild-caught birds from Kansas and Louisiana and captive stock from Louisiana were to be housed in the pens. Kansas birds are naïve to red imported fire ant presence, whereas Louisiana birds have been exposed to the ants for decades. Birds from captive stock were used as what the PI hypothesized was potentially interesting contrast to wild birds. Scientific collecting permits that allowed live-trapping bobwhites were obtained from both states.

Designs for traps used to attempt to catch wild birds were supplied by Mike Olinde at Louisiana Department of Wildlife and Fisheries. The trap used was similar to the Kniffen modified funnel trap used for Mourning Doves, *Zenaida macroura* (Reeves *et al.* 1968). Modification to the design included the use of ¼ inch (0.635 cm) welded wire and UV Black zip

ties (Thomas & Betts, Memphis, Tennessee) for connecting sides and attaching funnels. The entire trap folds flat for ease of transport. White millet is used to bait birds into funnels and into the center of the trap. Birds usually are not able to navigate out the mouth of the funnel once in the trap.

Aviaries were built from chainlink dog kennels lined inside with 1 inch (2.54 cm) chickenwire; the roof was made with the same chickenwire with some slack so that birds were less likely to injure themselves if flushed. A ¼ inch (0.64 cm) welded wire skirt ran two feet (61 cm) up the sides of the outside of the aviary and two feet (61 cm) out from the sides of the aviary. The skirt was staked down by tent stakes (Bucket O' Pegs, Reliance, Winnipeg, Canada). The skirt was used to foil digging predators that would quickly consume caged birds. The final dimensions of the aviaries were 10 feet x 10 feet x 6 feet (~3 m x ~3 m x ~1.8 m). One-third of the roof and all of the back side (side opposite the door) of the aviary was covered with shade cloth that allowed birds relief from midday sun. Each aviary was furnished with a small, A-frame style shelter built from 2 feet x 2 feet (61 cm x 61 cm) pieces of ¾ inch (1.9 cm) thick plywood. This shelter was for the birds, but also served as a rainguard for quart-sized galvanized feeders. A one-gallon (3.8 L) quail waterer was also placed in each aviary. Quail were provided food and water *ad libitum*. Food was a gamebird grower feed from LoneStar Feeds. Natural ground cover was allowed to grow inside pens to provide additional forage and shelter.

Despite observing large coveys of > 20 birds, the PI was unsuccessful at capturing wild bobwhites in Kansas, which precluded their use in the study. It was determined from that attempt that trapping in an area with fewer quail like Louisiana would be almost impossible. Only captive birds from Louisiana stock were included in this year's experiment. As such, one aviary per plot was stocked with one male and two female Northern Bobwhites to ensure

breeding. The other two aviaries per plot were left empty. Note that the use of only captive birds prevented testing of null hypotheses two and three listed above.

Birds inside the enclosures were allowed to build nests, lay eggs, incubate eggs, and raise young with minimal impact from the PI. As birds were placed in aviaries prior to treatment with Amdro®, some eggs had already been laid by the start of the experiment. So that nesting occurred synchronously in all cages, eggs were pulled from all aviaries on the day of treatment. Birds and eggs were counted and monitored semiweekly. Deaths of adult birds were investigated to attempt to solve problems and to prevent further deaths, and aviaries were restocked to maintain similar numbers of adults throughout the experiment.

DESIGN 2006. Seven 11-acre (4.45 ha) plots were established at Idlewild Experimental Research Station in a similar manner to 2005. Three plot pairs were created. One of the seven plots was established as an extra plot to house surplus birds to be used in cases of restocking aviaries. One plot of each pair and the extra plot were treated by all-terrain-vehicles (4-wheelers) with Amdro® (A.I. 0.73% hydramethylnon), a poisonous bait for fire ants, in an attempt to provide those plots with as little fire ant pressure as possible. Plots were sampled monthly to determine extent of fire ant presence on all plots. Those sampling techniques are discussed in previous chapters.

In 2006, four aviaries (as described above) were erected per plot, two aviaries for each population of Northern Bobwhites to be used in the study. After failing to capture birds in the previous year, the PI decided to use only captive-raised birds in the second year. Captive birds were obtained from bobwhite breeders in Arkansas and Louisiana. Note that use of these two “populations” of bobwhites allowed testing of null hypotheses one and, marginally, two. Null

hypothesis three, ultimately, was not tested in this project due to the difficulty in obtaining wild birds. Food was a premium gamebird feed from Nutrena Feeds.

Birds inside the enclosures were allowed to build nests, lay eggs, incubate eggs, and raise young with minimal impact from the PI. As birds were placed in aviaries prior to treatment with Amdro®, some eggs had already been laid by the start of the experiment. Unlike 2005's study, eggs were not pulled from aviaries on the day of treatment as such manipulation may have adversely affected the first year study. Birds and eggs were counted and monitored at least semiweekly, and adults were restocked if needed.

STATISTICAL ANALYSES. SAS 9.1 software (SAS Institute Inc. 2002) was used to analyze all data collected in these experiments. Chi-square analyses were performed to test for significant differences between mean number of nests, eggs, and chicks produced by bobwhite of Louisiana and Arkansas origin on treated (observed) versus untreated (expected) plots. In addition, percentage of nests attacked by RIFA were also compared in this manner between treated and untreated plots. Comparisons between treated and untreated plots, as well as between the two populations are discussed. Alpha was set at 0.05.

RESULTS

RIFA were significantly suppressed post-treatment on treated plots in 2005 and 2006 on treated plots overall. See Chapter Two for those data.

In 2005, due to the dearth of available captive birds and the lack of trapping success in Kansas, only one aviary per plot was populated with birds. All birds from 2005 were captive birds of Louisiana origin. Birds from all six aviaries produced at least one nest structure, but only four of those aviaries produced eggs in those nests. Birds in the other aviaries produced eggs, but those eggs were dumped throughout the aviaries rather than within the nests. One of

the four nests was never incubated; the other three nests were incubated. One incubated nest of eggs was eaten by a vertebrate predator, most likely a snake. Rat snakes, *Elaphe obsoleta*, are common predators at Idlewild and were discovered inside aviaries on two occasions in 2005. Two remaining nests, both in aviaries on untreated plots, were incubated by tight-sitting males and were expected to hatch during the second or third week of August 2005. Due to the reduced number of replicates, no statistical analyses could be performed on the nesting data from 2005.

The untreated plots in which nests were incubated in 2005 (Plots 2 and 4), differed significantly in the number of RIFA captured in food traps. Plot 2 was more heavily RIFA-infested than Plot 4 during times when bobwhite eggs were present in nests (June: $\chi^2 = 1177.8$, $P < 0.0001$; July: $\chi^2 = 584.0$, $P < 0.0001$; August: $\chi^2 = 167.4$, $P < 0.0001$). The nest in Plot 4, the less ant-infested of the two, hatched twelve eggs successfully. The nest in Plot 2, the heavily, RIFA-infested plot – perhaps, the most infested of all plots in both 2005 and 2006 – did not hatch. As the PI approached the cage on 8 August 2005, the male was discovered walking around the aviary rather than incubating as it had done for approximately 21 previous days. When the PI approached the nest, small holes through the shell and membrane were discovered on the top surface of three eggs near the top layer of eggs in the nest. RIFA were present in the nest, and they filed out the holes in the eggs when the PI disturbed the nest; a fourth egg was missing eggshell, but not membrane, in a ring on the top of the egg. The bobwhite nest was filled with hundreds of RIFA. Curious of the developmental stage of the chick in the eggs, the PI carefully opened one egg that had been breached and filled with ants. A live, fully-developed chick was present inside the attacked egg. The chick died within minutes; no egg successfully hatched from that nest. The male bobwhite that had been incubating the nest during the past several weeks died within days of the nest failure.

Due to damage caused by Hurricane Katrina, two aviaries could not be used in 2006. A third aviary was eliminated from the study after a predator attack that killed all the birds in that cage. All other aviaries, 21 total, were used in the 2006 study, and all those contained nests with eggs laid in the nests. In 2006, twelve nests were incubated over the course of the study. A total of four nests, three on treated plots and one on an untreated plot, hatched successfully. Three of the four nests were hatched by captive-raised Louisiana birds, the fourth by Arkansas birds.

Of the three Louisiana nests, two were hatched on treated plots, whereas the third was on an untreated plot. The Arkansas nest hatched on a treated plot. The mean length of incubation of all successful nests was approximately 24.3 ± 0.5 d as best could be determined using dates of first observed incubation and date of last egg laid. Mean length of incubation did not differ significantly between nests of Louisiana and Arkansas birds ($\chi^2 = 0.002$, $P = 0.96$).

Mean number of nests of Louisiana birds did not differ significantly between treated and untreated plots (1.0 ± 0.0 nests, mean \pm SEM and 1.7 ± 0.7 nests, respectively; $\chi^2 = 0.17$, $P = 0.68$). Mean number of nests of Arkansas birds also did not differ significantly between treated and untreated plots (1.5 ± 0.3 nests, mean \pm SEM and 1.5 ± 0.2 , respectively; $\chi^2 = 0.0$, $P = 1.00$). Mean number of eggs of Louisiana birds was not significantly different between treated and untreated plots (21.2 ± 2.7 eggs/nest, mean \pm SEM and 15.8 ± 2.1 eggs/nest, respectively; $\chi^2 = 0.78$, $P = 0.38$). Mean number of eggs of Arkansas birds also did not differ significantly between treated and untreated plots (17.1 ± 2.8 eggs/nest, mean \pm SEM and 15.1 ± 2.4 eggs/nest, respectively; $\chi^2 = 0.12$, $P = 0.73$). Mean number of chicks of Louisiana birds did not differ significantly between treated and untreated plots (3.5 ± 2.3 chicks, mean \pm SEM, and 4.3 ± 4.3 , respectively; $\chi^2 = 0.07$, $P = 0.79$). Mean number of chicks of Arkansas birds also did not differ significantly between treated and untreated plots (1.7 ± 1.7 chicks, mean \pm SEM, and 0.0 ± 0.0

chicks, respectively; $\chi^2 = 0.76$, $P = 0.38$). Percentage of nests with eggs of Louisiana birds attacked by RIFA differed significantly between treated and untreated plots ($16.7 \pm 16.7\%$, mean \pm SEM and $60.0 \pm 24.5\%$, respectively; $\chi^2 = 24.4$, $P < 0.0001$). Percentage of nests with eggs of Arkansas birds attacked by RIFA also differed significantly between treated and untreated plots ($11.1 \pm 11.1\%$, mean \pm SEM and $66.7 \pm 16.7\%$, respectively; $\chi^2 = 39.7$, $P < 0.0001$; Table 4.1, 4.2).

Table 4.1. Mean number of Northern Bobwhite nests, eggs, and chicks of Louisiana captive origin birds and percentage of bobwhite nests attacked by red imported fire ants, *Solenopsis invicta*, on treated and untreated control plots.

Louisiana Birds	Treated (Mean \pm SEM)	Untreated Control (Mean \pm SEM)	χ^2	P value
Mean Number of Nests	1.0 \pm 0.0	1.7 \pm 0.7	0.17	0.68
Mean Number of Eggs	21.2 \pm 2.7	15.8 \pm 2.1	0.78	0.38
Mean Number of Chicks	3.5 \pm 2.3	4.3 \pm 4.3	0.07	0.79
Percentage of Nests Attacked by RIFA ^a	16.7 \pm 16.7	60.00 \pm 24.5	24.4	< 0.0001
Percentage of Nests Attacked by RIFA ^b	16.7 \pm 16.7	40.0 \pm 24.5	9.6	0.002

^aIncludes nest attacks on nests that might have been previously abandoned.

^bRemoves nest attacks on nests that might have been previously abandoned.

Mean number of nests of Louisiana birds on treated plots did not differ significantly from those of Arkansas birds on treated plots ($\chi^2 = 0.10$, $P = 0.75$). Mean number of nests of Louisiana birds on untreated plots also did not differ significantly from those of Arkansas birds on untreated plots ($\chi^2 = 0.009$, $P = 0.92$). Mean number of eggs of Louisiana birds on treated plots was not significantly different from those of Arkansas birds on treated plots ($\chi^2 = 0.43$, $P = 0.51$). Mean number of eggs of Louisiana birds on untreated plots did not differ significantly

from those of Arkansas birds on untreated plots ($\chi^2 = 0.02$, $P = 0.90$). Mean number of chicks of Louisiana birds on treated plots did not differ significantly from those of Arkansas birds on treated plots ($\chi^2 = 0.47$, $P = 0.49$). Mean number of chicks of Louisiana birds on untreated plots also did not differ significantly from those of Arkansas birds on untreated plots ($\chi^2 = 2.96$, $P = 0.09$). Percentage of nests of Louisiana birds attacked by RIFA on treated plots did not differ significantly from those of Arkansas birds on treated plots ($\chi^2 = 1.1$, $P = 0.29$). Percentage of nests of Louisiana birds attacked by RIFA on untreated plots did not differ significantly from those of Arkansas birds on untreated plots ($\chi^2 = 0.35$, $P = 0.55$; Table 4.3, 4.4).

Table 4.2. Mean number of Northern Bobwhite nests, eggs, and chicks of Arkansas captive origin birds and percentage of bobwhite nests attacked by red imported fire ants, *Solenopsis invicta*, on treated and untreated control plots.

Arkansas Birds	Treated (Mean \pm SEM)	Untreated Control (Mean \pm SEM)	χ^2	P value
Mean Number of Nests	1.5 \pm 0.3	1.5 \pm 0.2	0.0	1.00
Mean Number of Eggs	17.1 \pm 2.8	15.1 \pm 2.4	0.12	0.73
Mean Number of Chicks	1.7 \pm 1.7	0.0 \pm 0.0	0.76	0.38
Percentage of Nests Attacked by RIFA ^a	11.1 \pm 11.1	66.7 \pm 16.7	39.7	< 0.0001
Percentage of Nests Attacked by RIFA ^b	11.1 \pm 11.1	55.6 \pm 17.6	29.7	< 0.0001

^aIncludes nest attacks on nests that might have been previously abandoned.

^bRemoves nest attacks on nests that might have been previously abandoned.

After treatment in June 2006, the mean number of nests on untreated plots was always greater than the mean number of nests on treated plots, although not significantly so (Figure 4.1). RIFA attacks on nests were spread over the sampling period with most attacks occurring later in

the season. Most sampling days when attacks were observed tallied only one or two attacks per day. (Figure 4.1).

Additional scrutiny of data revealed that RIFA might attack nests that have already been abandoned by parents. Abandoned nests would not be incubated and would, therefore, not be able to hatch. Assigning responsibility of potential nest failure in abandoned nests to RIFA may be the equivalent to finding a dead chick covered in ants and assuming RIFA were responsible. Abandoned nests were considered those nests that were (1) obviously abandoned in the field (nest structure collapse, no nest maintenance, etc.), (2) an earlier nest created in an aviary with multiple nests (that is, if a second nest was laid, the first nest was considered abandoned), and (3) filled with eggs, but not incubated within an acceptable timeframe (e.g., if the birds had stopped laying eggs, the nest should be incubated within one week; if not, it was considered abandoned). New figures maintained the significant relationships given above, but the percentages of nests attacked were changed. Percentage of nests with eggs of Louisiana birds attacked by RIFA was significantly different between treated and untreated plots ($16.7 \pm 16.7\%$, mean \pm SEM and $40.0 \pm 24.5\%$, respectively; $\chi^2 = 9.6$, $P = 0.002$). Percentage of nests with eggs of Arkansas birds attacked by RIFA also differed significantly between treated and untreated plots ($11.1 \pm 11.1\%$, mean \pm SEM and $55.6 \pm 17.6\%$, respectively; $\chi^2 = 29.7$, $P < 0.0001$) (Table 4.1-4.4).

Attacks on nests by RIFA came in several varieties. The most common attack usually occurred as RIFA crawling around eggs in nests. Although the ants may not have been observed biting or stinging eggs at that time, all nests with RIFA visits were considered attacked, because those nests were always subsequently abandoned by adult birds. Frequently, RIFA would remain in nests for several consecutive sampling dates during which time, the ants would build debris piles atop the nests. Five nests, all on untreated plots, were buried or partially buried in

this manner (Figure 4.2). A single egg that was attacked by RIFA on a treated plot was partially covered by debris. In this instance, the ants had been working in a circle atop the egg and had built a debris pile in a thin ring around where they had been working. In the most insidious attacks, breached eggs were located within the birds' nests. Eggshell breaches occurred in both new and well-established nests. Eggshell breaching by RIFA will be discussed in greater detail in Chapter Five of this work. Eleven nests were attacked by RIFA in this study. If potentially abandoned nests are removed from the total, nine viable nests were attacked by RIFA.

Table 4.3. Mean number of Northern Bobwhite nests, eggs, and chicks of Louisiana and Arkansas captive origin birds and percentage of bobwhite nests attacked by red imported fire ants, *Solenopsis invicta*, on treated plots.

Treated Plots	LA Origin (Mean ± SEM)	AR Origin (Mean ± SEM)	χ^2	P value
Mean Number of Nests	1.0 ± 0.0	1.5 ± 0.3	0.10	0.75
Mean Number of Eggs	21.2 ± 2.7	17.1 ± 2.8	0.43	0.51
Mean Number of Chicks	3.5 ± 2.3	1.7 ± 1.7	0.47	0.49
Percentage of Nests Attacked by RIFA ^a	16.7 ± 16.7	11.1 ± 11.1	1.1	0.29
Percentage of Nests Attacked by RIFA ^b	16.7 ± 16.7	11.1 ± 11.1	1.1	0.29

^aIncludes nest attacks on nests that might have been previously abandoned.

^bRemoves nest attacks on nests that might have been previously abandoned.

No chicks were found to be attacked by RIFA, although most hatches occurred on treated plots where few RIFA were present. Despite RIFA attacks on other nests in one aviary, one nest, the fourth nest structure built in that aviary, hatched thirteen chicks successfully.

Disappearances and deaths of chicks, in some cases potentially caused by adult birds, precluded behavioral studies of RIFA and chicks. Even in RIFA-infested aviaries, behavior of the chicks

did not appear affected by RIFA, probably because the low number of RIFA in that aviary or, perhaps, because the RIFA were preoccupied attacking abandoned eggs.

Table 4.4. Mean number of Northern Bobwhite nests, eggs, and chicks of Louisiana and Arkansas captive origin birds and percentage of bobwhite nests attacked by red imported fire ants, *Solenopsis invicta*, on untreated control plots.

Untreated Plots	LA Origin (Mean ± SEM)	AR Origin (Mean ± SEM)	χ^2	P value
Mean Number of Nests	1.7 ± 0.7	1.5 ± 0.2	0.009	0.92
Mean Number of Eggs	15.8 ± 2.1	15.1 ± 2.4	0.02	0.90
Mean Number of Chicks	4.3 ± 4.3	0.0 ± 0.0	2.96	0.09
Percentage of Nests Attacked by RIFA ^a	60.0 ± 24.5	66.7 ± 16.7	0.35	0.55
Percentage of Nests Attacked by RIFA ^b	40.0 ± 24.5	55.6 ± 17.6	2.55	0.11

^aIncludes nest attacks on nests that might have been previously abandoned.

^bRemoves nest attacks on nests that might have been previously abandoned.

RIFA were not the only predator observed in aviaries. As in 2005, snakes were major predators of captive quail nests. Seven nest depredation events were probably caused by snakes in 2006, although snakes were never observed in the cages that year. In two cases, > 50% of eggs were removed from two nests, only to be emptied weeks later when the females had re-layed. Due to the construction of the aviaries and the lack of broken eggs (only missing ones), snakes rather than mammals were implicated in 2006. Size of wire openings, 1 inch (2.54 cm) chicken wire, did not exclude some small mammals, but most or all of the depredation events did not indicate small mammals. A nest of small mammals, probably hispid cotton rat, *Sigmodon hispidus*, was found inside an aviary within a meter of an active bobwhite nest, which subsequently hatched successfully.

Breached eggshells were almost certainly due to RIFA, but other ants were also observed in nests. One nest on an untreated plot contained several cracked or broken eggs, possibly caused by a new female in the cage; the nest was soon overrun by hundreds of unidentified acrobat ants, *Crematogaster* sp. It is not likely that eggs were attacked by the *Crematogaster* sp. but scavenged due to the amount of destruction caused to the eggs of that nest.

Predation by vertebrates occurred earlier in bobwhite nesting season than predation by RIFA. Three nests each were depredated presumably by snakes in June and July 2006, and one was depredated in August 2006. One of the nests in July was actually a second attack on the same nest attacked the month before. The one nest of eggs attacked in August was one of the same nests attacked in July. RIFA attacks initiated later in the season with three, five, and three attacks occurring in June, July, and August 2006, respectively. A fourth attack in August 2006 was actually reinfestation of a previously attacked nest. Actual ant attacks could last several days or weeks as nests were frequently buried with debris.

DISCUSSION

Solenopsis spp. have long been considered major predators of nesting bobwhites. Stoddard (1931: 193-194, 1932) reported that 12.5% of bobwhite nests at a Florida plantation were attacked by tropical fire ant, *S. geminata*, and that he witnessed thief ants, *S. molesta*, attacking eggs in several nests. Staller *et al.* (2005) found that 5.2% of bobwhite nests were depredated by fire ants, probably RIFA based on locale. Fire ants ranked third, after mammals and snakes, as the most important bobwhite nest predators. This study finds that RIFA are potentially extremely important nest predators of Northern Bobwhites. In addition, despite frequently cited dogma, RIFA are apparently capable of breaching Northern Bobwhite eggs and

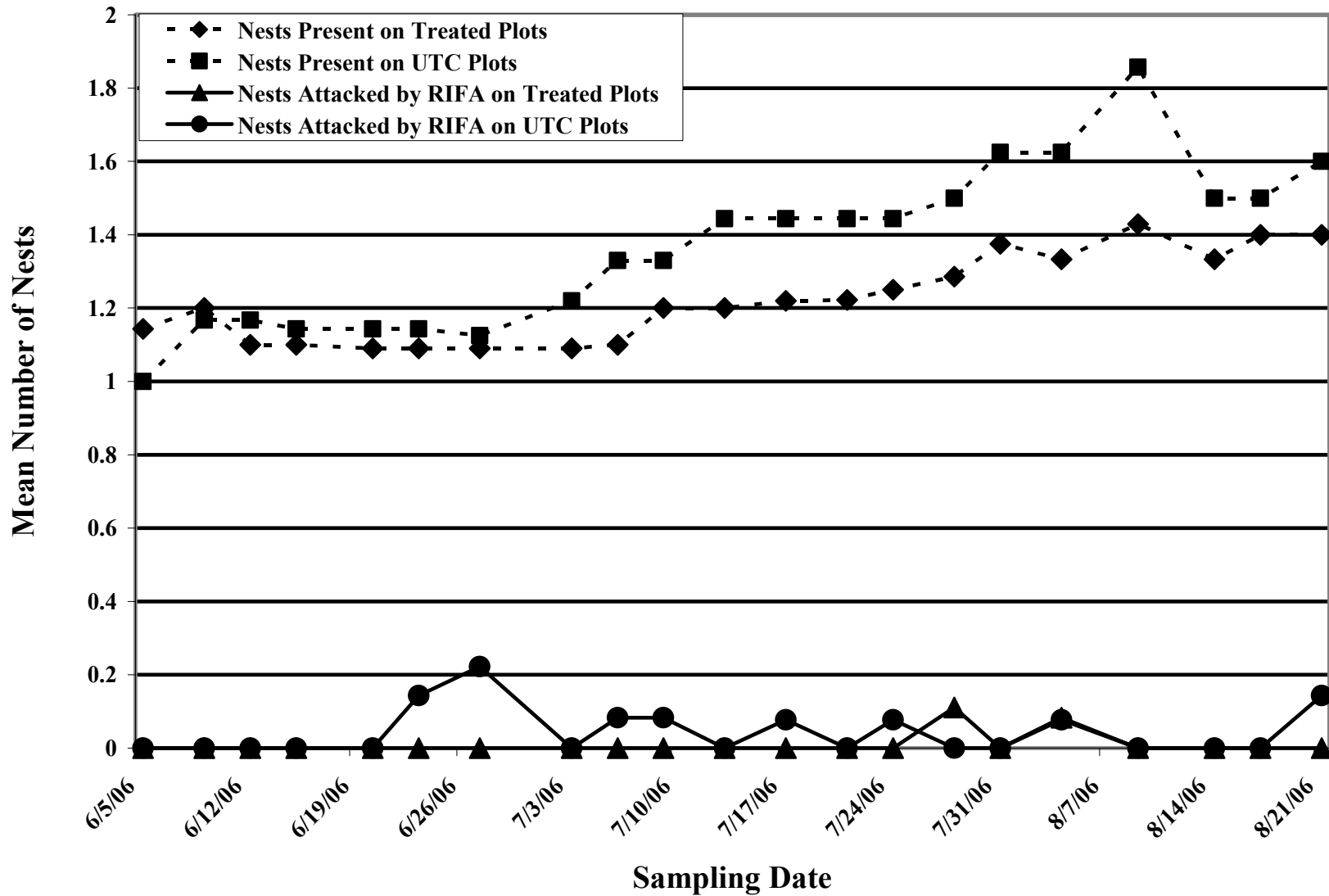


Figure 4.1. Mean number of nests present and attacked by red imported fire ants, *Solenopsis invicta*, on treated and untreated control (UTC) plots at Idlewild Experimental Research Station.

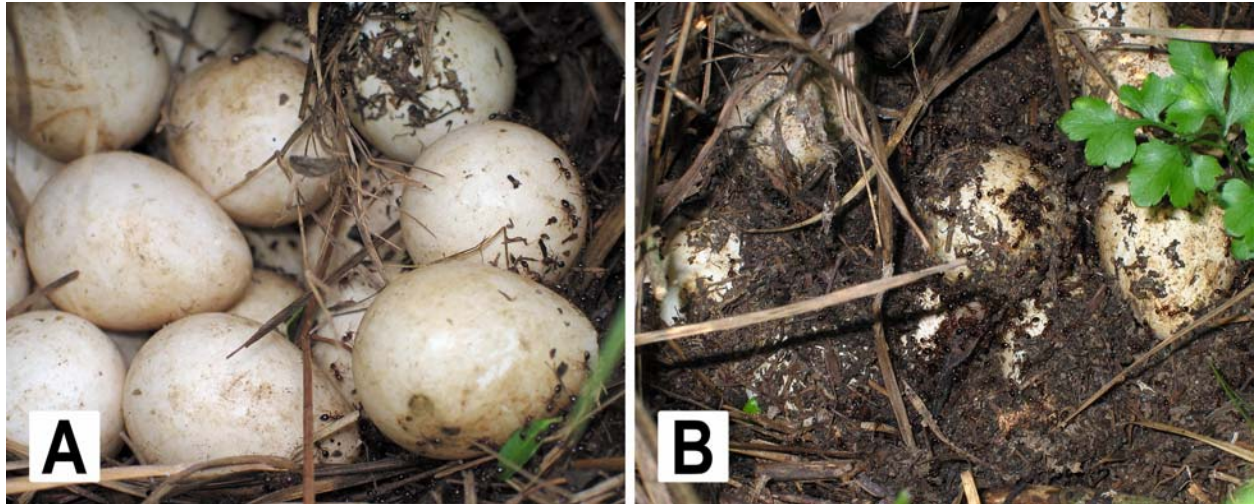


Figure 4.2. Progression of a red imported fire ant, *Solenopsis invicta*, attack on a Northern Bobwhite nest. The first signs of attack may seem as benign as several RIFA crawling around eggs (A); one month later, the nest is buried in debris by RIFA, and some eggs may show signs of breach (B).

do so in a manner similar to eggshell breaching by southern fire ant as shown in Hooper-Bùi *et al.* (2004) in Figure 3 of that work.

High losses of nests were caused by RIFA in this study. Of 29 nests of both Louisiana and Arkansas captive-origin birds on treated and untreated plots tracked during the course of this study, nine (31%) were attacked by RIFA. Method of attack does not change the outcome of attacked nests – all failed in this study, whether simply overrun, buried, or breached. Losses of such magnitude may not occur in nature as several factors were not in the captive birds' favor.

The act of confining the birds to cages, albeit large ones, may have boosted success of ants or even that of the birds in some cases. Captive birds are probably not equivalent in behavior or vigor to actual wild-caught birds. In addition, by placing birds in the cages, birds were forced to nest in areas that may not have been optimal for nesting. Even with successful suppression of RIFA, it may be possible that even a few ants present in a nest could cause abandonment. Although every attempt was made to ensure appropriate habitat for the birds, bobwhites in nature, as has been found in small mammals (Pedersen *et al.* 2003), may alter their

territory in response to RIFA presence. Wild bobwhites, therefore, may actively seek less RIFA-infested areas for nesting. The birds in this study may have experienced unnatural RIFA density if the above scenario is true. Also, because birds were fed *ad libitum*, it is impossible to exclude a constant supply of feed as a major reason for RIFA presence in cages. In fact, RIFA were frequently found in or under feeders on untreated plots. The PI decided not to use pesticide to control ants near feeders, because insecticide near feeders may be dangerous to the birds and may kill other arthropods that may be an important food supplement for nesting birds and chicks. In times of drought, the underside of waterers occasionally harbored some fire ants. The diet provided to the bobwhites was high in mineral content and may have increased eggshell thickness beyond that of wild bobwhite (Maier and DeGraaf 2000), which may further protect eggs from breach.

Seasonality in nest attacks by RIFA may be an important factor that might allow for higher success rates in early season nesting attempts of bobwhites. Bobwhites may nest as early as April (Stoddard 1931: 20). Because the majority of attacks occurred later in the season, particularly when air temperatures were greater, which parallels RIFA activity to a point, early nesting bobwhites may be favored. In fact, RIFA pressure is so great later in the season that even with successful suppression, two nests on treated plots were attacked. Mitigation of fire ant loss later in the season could be as simple (or as difficult) as removing vertebrate predators of bobwhites such as snakes or mesomammals (Staller *et al.* 2005) early in the season. If feasible, treating extremely RIFA-infested bobwhite areas early in the season to suppress RIFA populations later in the season may be beneficial to late nesters. Studies in forested ecosystems suggest successful suppression of RIFA for approximately three months if Amdro® (0.73% hydramethylnon) is used at 1.68 kg/ha (Womack 2006). It is very important to note that treating

some forested areas experiencing less RIFA pressure may be more detrimental in the long run, because treatment may eliminate non-target ants that may be essential predators on founding RIFA queens (See Chapter Three).

After a loss of 47 to 55 days of nesting and incubating, loss of the first nest may have serious consequences for bobwhites (Rosene 1969: 71). Bobwhites forced to renest due to failure of an earlier nest are required to reinvest more energy in nest construction, egg production, and laying and with less available time in the season. Successive broods are smaller than the first, and nests may be less attentively constructed or maintained (Rosene 1969: 73-74). Drier conditions later in summer also mean difficulties in egg emergence, and fewer chicks will hatch from fewer eggs (Rosene 1969: 73). Again, as the season progresses, depredation by RIFA also appears to increase; late-nesters would be triply taxed by weather, predators, and physiology. RIFA attacks on nests early in the first nesting attempt would be less detrimental than attacks later in the season as those early-attacked nests would be abandoned earlier in the nesting attempt; birds would be able to renest with more time left in the season and with less invested in the first nest attempt.

Altricial bird species require more time in the nest for development after hatching than precocial or semiprecocial birds. Confinement to the nest likely increases the risk of a young bird to attack by RIFA. Very few studies of RIFA and altricial bird species have been performed, and none of those involve ground-nesting altricial birds. Several ground-nesting altricial birds are species of conservation concern due to declining numbers. Impacts of RIFA on these species, therefore, should not be overlooked when investigating declines of ground-nesters especially if those birds occur in areas of high RIFA pressure. Even if habitat destruction ranks as the

primary cause of decline, one should not ignore potential exacerbation of decline by invasive, exotic species such as RIFA.

In areas of sympatry, RIFA may exacerbate bobwhite decline by attacking nests prior to or at hatch. At that time, eggs and chicks may be destroyed and nests abandoned causing adult birds to invest greater amounts of energy for renesting attempts. Although not certain how these data translate to real-world bobwhite populations, this work should serve as a warning that red imported fire ants do attack quail nests even prior to pipping. Attacks by fire ants on bobwhite nests have previously been linked only to pipping time, although, Staller *et al.* (2005) also found that fire ants (species not identified) buried bobwhite nests in Georgia prior to hatch. This study is the first to report breaches of bobwhite eggs by RIFA, which indicates no period of nesting is immune to RIFA predation.

CHAPTER 5

EGGSHELL BREACHING BY RED IMPORTED FIRE ANT

Eggshell breaching by red imported fire ant, *Solenopsis invicta* Buren, is deeply entrenched in dogma; all previous studies on the effects of RIFA on nesting birds reiterate that RIFA do not breach bird eggshells. Anecdotal evidence from this research suggests otherwise.

Hooper-Bùi *et al.* (2004) found that southern fire ant (SFA), *Solenopsis xyloni*, is capable of breaching the eggs of California Least Tern, *Sternula antillarum browni*, and Coturnix Quail, *Coturnix coturnix*, eggs in the field. Tern eggs attacked by SFA showed small, irregularly shaped holes. Holes may be shaped like keyholes – a large hole with a narrow branch of removed eggshell and membrane radiating off to one side – or as starburst shaped holes – several irregular points radiating from the center of the breach. In the laboratory, the quail eggs were breached by SFA in 30 min with initial attacks occurring within the first 30 sec. Interestingly, colonies of RIFA in the laboratory did not breach Coturnix Quail eggs (Hooper-Bùi *et al.* 2004).

Two previous studies mention what might have been the first documented cases of eggshell breaching by RIFA. While studying Barn Swallow, *Hirundo rustica*, colonies in Texas, Kopachena *et al.* (2000) discovered one nest that had eggs emptied by RIFA. Unfortunately, the mechanism of this destruction was not discussed in the work; that is, perhaps, these eggs were cracked previously by another predator, for instance. Smith *et al.* (2004) reported observations of nests of Black-capped Vireo, *Vireo atricapilla*, attacked by RIFA. In one case, both adults, hours apart, attempted to remove the ants from the nest via aerial defense displays and actively pecking the ants. The nest was ultimately abandoned due to RIFA. Examination of the eggs revealed a small hole in one egg. This breach was attributed to an eastern woodrat, *Neotoma*

floridana, that had visited the nest during the ant attack, but subsequently left, possibly deterred by RIFA presence. Smith *et al.* (2004) reiterated that RIFA likely do not breach vireo eggs.

Strong circumstantial evidence was obtained during the course of this work that suggests breaching of bird eggshells by RIFA is possible and might be normal protocol for this ant. Of ten active bobwhite nests attacked by RIFA during the 2005 and 2006 captive bobwhite nesting seasons, eight contained eggs with obvious breaches. All nests that were attacked failed, although only one nest was ever incubated. In three instances, RIFA were discovered chewing on the top side of eggs laid in nests. The ants were discovered standing in a circle of six or more individuals with mandibles facing toward the center of the circle atop an egg.

On 31 July 2006, a new nest with one new egg was discovered in one aviary. At 0945 hrs on 4 August 2006, six RIFA were observed standing around in a circle atop the egg and appeared to be chewing on the egg. It was noted that the egg was covered in a fine layer of dust or stained from the nesting substrate except for the spot where the ants had been working, which was pure, glossy white. By 1300 hrs of the same day, ten RIFA were attacking the same spot on the egg in the same manner. Field notes suggest the ants were “definitely making headway” through the eggshell. By 9 August 2006, RIFA were still attacking the egg in the same place and had built a debris ring around the spot where the ants had been working. The egg was collected and brought to the laboratory for documentation (Figure 5.1). Interestingly, even as egg contents were observed seeping through the top of egg where the ants had chewed, no obvious hole was observed (Figure 5.2). Similar ant behavior was observed at an established nest where another egg was breached in 2006 (Figure 5.3).

The most dramatic example of egg breaching occurred during the 2005 study. In this case, a nest was incubated with full expectation of successful hatch during the second week



Figure 5.1. Early stages of eggshell breaching by red imported fire ants, *Solenopsis invicta*, may show a clean, glossy area on the top of the eggshell where ants have been chewing or scratching at the shell. The yellowish orange spot in the photograph above is contents of the egg that are seeping up through a breach in the eggshell caused by fire ants. The debris ring has been washed away by rain. Contrast of the photograph has been increased in Photoshop 6.0 (Adobe Systems, Inc. 1989-2001) to make the area worked by ants more visible in the photograph.

of August 2005. On 8 August 2005, small holes through the shell and membrane were discovered on the top surface of three eggs near the top layer of the nest. RIFA were present in the nest and scurried out the holes in the eggs when the PI disturbed the nest; a fourth egg was missing eggshell, but not membrane, in a ring on the top of the egg. The bobwhite nest was filled with hundreds of RIFA. Curious of the developmental stage of the chick in the eggs, the PI carefully opened one egg that had been breached and filled with ants. A live, fully developed chick was present inside the attacked egg. The chick died within minutes; no egg successfully hatched from that nest (Figure 5.4). Due to the shape of the breaches – keyhole and starburst shaped as in Hooper-Bùi *et al.* (2004) – and due to the egg missing shell but not membrane, it is doubtful that chicks pipping the eggs were the cause of these breaches.



Figure 5.2. It may be difficult to find obvious holes in eggs that have been attacked by red imported fire ants, *Solenopsis invicta*, in the first few days. This egg had been attacked for less than nine days. The droplet of egg content on the surface of the egg has seeped up through a spot on the egg where fire ants had been chewing. This is the same spot on the egg from Figure 5.1 after the egg had been carefully washed.

Despite several laboratory and field experiments using RIFA and eggs of Northern Bobwhite, egg breaches could not be obtained in these artificial tests. It may be possible that RIFA are attracted to bobwhite nests by cues other than simply egg presence. Eggs are frequently covered in trace amounts of feces when laid; as more eggs are laid, the odor of the nest would likely increase. The birds themselves frequently have an odor that may concentrate in the nest with prolonged sitting in or near the nest structure. The microclimate of the nests may also attract ants; heat and humidity may increase in the nest as the bird incubates or shelters eggs. As eggs are warmed, the odor of the nest may diffuse greater distances allowing foraging ants increased success in locating distant quail nests. Finally, as eggs approach hatch, noise and vibration of the nest may also attract foraging ants. The nest attack in 2005 may have been triggered by noise and vibration cues, but the single egg attacked in a brand new nest in 2006 raises even more questions than it answers suggesting multiple cues combined with hungry ants.



Figure 5.3. Red imported fire ants, *Solenopsis invicta*, concentrate effort on small portions on the tops of eggs in nests.

Although no evidence of bird egg breaches by RIFA have been reported prior to this work, warnings from literature suggested the possibility. The closely related SFA breaches bird eggs (Hooper-Bùi *et al.* 2004), and RIFA has been observed consuming intact eggs of a lizard, *Cnemidophorus sexlineatus* (Mount *et al.* 1981). Stoddard (1931: 193-194) reported that thief ants, *Solenopsis (Diplorhoptrum) molesta*, were observed attacking intact eggs, although he never observed breaches. Stoddard (1931: 194) suggested that “it seems probable that [thief ants] sometimes do [breach the shells], for such ant clusters often make noticeable progress in their work on the shell.”



Figure 5.4. With concentrated effort, red imported fire ants, *Solenopsis invicta*, create large holes in eggshells allowing for entry and exit of several workers at a time. Note the keyhole and starburst shaped holes in two eggs. The arrow points to a third egg that is in the process of being breached; note the missing bits of eggshell but intact membrane of the egg.

Elsewhere in the literature, egg breaches by small mammals have been reported. Small mammals may be more readily accepted as egg predators; however, much evidence from the literature suggests their role in quail eggshell breaches to be minimal. DeGraaf and Maier (1996) found that white-footed mouse, *Peromyscus leucopus*, does not breach Coturnix Quail eggs in captivity, but it readily attacks and breaches eggs of the Zebra Finch, *Peophila guttata*. DeGraaf and Maier (1996) suggested that gape of the mouse may be too small to successfully bite a quail egg. Ettel *et al.* (1998) found that cotton rat, *Sigmodon hispidus*, may be overly implicated as a predator on Northern Bobwhite eggs. In laboratory studies, the rats did not breach the quail eggs,

but would consume Zebra Finch eggs. Ettel *et al.* (1998) also suggested that gape prevents the rat from breaching quail eggs. Interestingly, Craig (1998) observed that least chipmunk, *Tamias striatus*, is able to breach Northern Bobwhite eggs placed in artificial nests. The chipmunk is able to overcome the eggshell by restricting its work to the small end of the egg as the egg is held fast against the side of the nest. Therefore, nest structure becomes important in egg breaching by small mammals, and all observed breaches by small mammals, at least those of the size able to squeeze through 1 inch (2.54 cm) chickenwire (the size used in the quail-fire ant study reported here), were restricted to the small end of the egg. No eggs breached in this study were obviously panned to or moved to nest walls for handling, and all breaches occurred on the tops of eggs in almost the widest portion of the egg surface. The PI believes that evidence from the literature combined with that from the field exculpate small mammals.

Laboratory and field studies are currently underway to obtain conclusive proof of eggshell breaches by RIFA. This current report should serve as a red flag to those interested in the protection or management of the ground-nesting or low-nesting birds in the southeastern USA. The previous belief that RIFA attack only pipping chicks is now amended to include even eggs not yet incubated. RIFA attacks on nesting birds may run the gamut from the day the first egg is laid to the second the chicks leave the nest.

CHAPTER 6

CONCLUSIONS

Since its invasion of the USA in the 1930's, the red imported fire ant (RIFA), *Solenopsis invicta* Buren, has spread over most of the Southeast. Its spread has been linked in part to the decline of Northern Bobwhite, *Colinus virginianus*, in parts of its range where the two occur in sympatry (Allen *et al.* 2000). Despite strong evidence that RIFA may affect populations of this bird species, two highly polarized camps have arisen – those who believe the effects of RIFA minimal at best and those who believe RIFA threaten further survival of the species. A call for additional work on the subject was made by Allen *et al.* (1993). This project used captive-origin Northern Bobwhites maintained in large walk-in aviaries built at a research station known for its suitability for upland gamebirds. Amdro® (0.73% hydramethylnon) was broadcast spread over four 4.45 ha plots at 1.68 kg/ha to determine dispersal of the product, efficacy against RIFA, and the effects on non-RIFA ant species. In addition, bobwhites nesting in aviaries on these treated plots could be compared with birds on untreated plots to determine what effect RIFA may have on ground-nesting birds with precocial young.

Dispersal of Amdro® at label rate 1.68 kg/ha by both crop-dusting airplane and helicopter are sufficient for adequate suppression of RIFA in forested settings. The amount of Amdro® reaching the ground in plots is determined by the amount of canopy cover and probably wind with larger particles reaching the ground more readily than smaller, lighter particles. Smaller particles are likely blown away or become trapped in the canopy especially if morning dew is present in the foliage. Number of particles of Amdro® reaching the ground in pastures is not different from number of particles expected to reach the ground based on calculations in the laboratory. The same is not true for areas with canopy, however; significantly fewer particles

reach the target in forested areas. Potentially, canopy acted as a giant sieve through which selected particles could pass. Care should be taken when treating areas with baits designed to kill ants as the shift in particle size composition caused by crop-dusting may alter the amount of insecticide reaching the target organism. In addition, shift in composition and canopy catching of Amdro® particles might make the poison more readily available to non-target species. Those same non-targets, in addition to providing valuable services as ecosystem engineers, may also be important predators limiting the spread of RIFA.

RIFA were significantly less common on treated plots than untreated plots after plots were treated. RIFA prefer particle sizes 2.0 mm or greater, the size most frequently caught on sticky traps after aerial treatment with Amdro®. Although this work should not be used to determine length of efficacy of Amdro® due to the timings of treatments in the study, it appears that suppression in this ecosystem remains evident for at least three months, which agrees with other studies (Womack 2006). Despite morning treatments, photodegradation of the hydramethylnon was not apparent as in Womack (2006). The apparent lack of degradation of the product was likely due to the presence of dense mid- and understory, which shielded the bait particles from sunlight. In addition, presence of RIFA in food traps was seasonal; RIFA were virtually absent in food traps from October through February. Such absence may have important implications to management of this pest. In particular, treatment to remove fire ants may be best applied just after stress from cold temperatures while ant populations are still low in number.

Suppression of RIFA did not allow competitive release for any non-RIFA ant species analyzed in this study. In fact, many ant species actually decreased in number as a result of treatment. Unfortunately, most of the ant species could not be analyzed in this manner due to low trap frequency. *Prenolepis imparis* occurrence in food traps superficially appeared to be

related to RIFA presence as the ant only occurred during months with little RIFA pressure. Because *P. imparis* is more active in cooler weather, it is frequently called the “winter ant.” It is doubtful that this species’ occurrence is directly related to RIFA presence; instead, weather played a key role in its presence.

Among the ants most affected by treatment of plots with Amdro®, the myrmecines appeared to fair the worst. *Aphaenogaster fulva-rudis-texana*, *Crematogaster pilosa*, *Monomorium minimum*, and *Solenopsis (Diplorhoptrum) molesta* were much less commonly collected on treated plots than untreated plots after treatment. This work concurs with that of Zakharov and Thompson (1998) who found myrmecines were wiped out after hydramethylnon treatments. Again, the most important part of determining whether treatment to suppress fire ants is warranted is that one must decide if the ecosystem present is experiencing high fire ant pressure and if treatment will reach non-targets in amounts of sufficient quantity to adversely affect those non-targets. Unfortunately, impacts on ecosystems by RIFA are still not well understood. Eliminating native congeners or other ants may allow RIFA to rebound into empty niches, which they fill with gusto and, frequently, with colony density far greater than that of native ants (Porter *et al.* 1988).

The effect of RIFA on bobwhites is currently a hot topic as the bird is experiencing declines throughout its range, which may be exacerbated by the ant. In caged studies at Idlewild Experimental Research Station, captive-origin bobwhites were found to experience heavy losses to RIFA. In 2005, a nest was destroyed immediately prior to hatching. RIFA entered eggs with live, fully developed chicks inside. Holes in the eggshells of three eggs were believed to be caused by the ants rather than the chicks as some portions of eggshell, but not membrane, were missing off a fourth egg. Presumably, a chick pipping the egg would also puncture membrane.

One nest hatched in 2005 despite its occurrence on an untreated control plot where RIFA were present. RIFA pressure was significantly less on the plot with the successful hatch than the plot with the nest failure.

In 2006, significantly more RIFA nest attacks occurred on untreated control plots than treated plots; forty to sixty-seven percent of nests on untreated plots were attacked by RIFA. Overall, thirty-one percent of nests (nine of 29) were attacked by RIFA and subsequently abandoned. Two additional nests were attacked after they were presumed abandoned. Nest attacks by RIFA may appear as benign as a few ants crawling around eggs to the more insidious eggshell breaches. Five nests were buried or partially buried by RIFA in this study. A sixth “nest,” actually a single egg inside a new nest, was beginning to be buried when the PI pulled the egg for documentation of breaching. The end result of attacks is always the same; the nest fails from abandonment by adults due to ant presence or debris-piling, or both. No chick was discovered to be dead from RIFA attacks, although most chicks hatched on treated plots where RIFA numbers should be minimal.

This work reports the first ever documented cases of probable eggshell breaching by RIFA. Although eggs were not monitored continuously, anecdotal evidence of eggshell breaching is very strong. RIFA were quite literally caught in the act as they chewed or scratched away eggshell with their mandibles. Because the event took place over several days, it is impossible to rule out some other possible candidates for the causes of holes in the eggs. It is unlikely that breaches were caused by small mammals as the eggshell breach caught in the early stages actually showed no obvious hole, which would likely be present from a mammal attack. Interestingly, the ants attacked both brand new and established nests.

The outcome of this research should be of utmost importance to land managers wishing to provide safe harbor for quail on their properties. It is very important to note that the decision to treat an area to suppress RIFA should be well-informed. Treating an area that receives little disturbance or has received little disturbance in the past may be unnecessary, because RIFA prefer disturbed areas. Areas of bobwhite nesting in or adjacent to disturbed habitats may be useful, but extreme care should be taken to apply no more than suggested label rate of the product. As with using any poison, detrimental effects on non-targets is likely, if not probable. Land managers should understand that if non-target ant species are destroyed by treatment, whether treatment is warranted or not, the ecosystem may lose a crucial line of defense offered by predacious non-targets. If a disturbed area within the range of RIFA is not presently colonized by that species, it likely will be after treatment if non-target ant species are eliminated by the treatment. Opportunity costs should be considered as more harm than good may come of unnecessary large-scale treatments of insecticides.

Data from this project suggest that impacts of RIFA on native organisms are still largely understudied with new discoveries still possible. The effects of RIFA on our native bobwhite should not be as quickly dismissed as other researchers have done in the recent past. Studies performed using captive-origin Northern Bobwhite may not be homologous to those on wild strain birds. However, this study should serve as a warning that the possibility of severe losses in bobwhite reproduction may occur in areas of high RIFA density, and those losses may occur at any stage in nesting. The possibility that RIFA affect other ground- or low-nesting birds, especially those with altricial young, deserves more attention.

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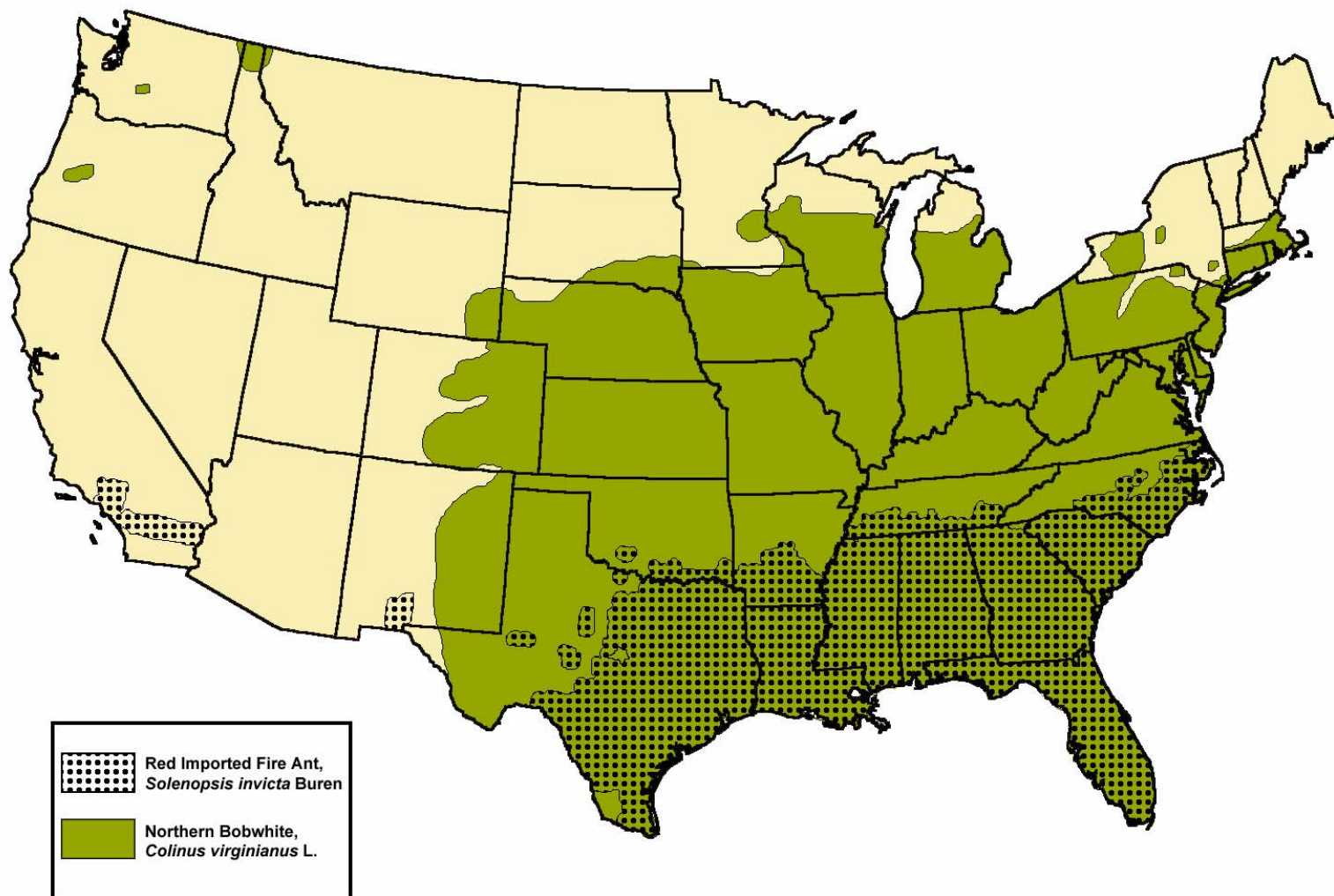
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APPENDIX: RANGE OF NESTING NORTHERN BOBWHITE AND RED IMPORTED FIRE ANT
IN THE CONTIGUOUS UNITED STATES OF AMERICA



Based on maps provided by USGS and USDA, 2000.

A large area of the southeastern USA is colonized by the exotic, invasive red imported fire ant, *Solenopsis invicta* Buren. The native Northern Bobwhite, *Colinus virginianus* L., occurs in sympatry with the ant in much of its range.

VITA

Michael Andrew Seymour was born 7 November 1978 in Baton Rouge, Louisiana. He was the third of three children of Mr. and Mrs. Robert L. Seymour. Living in suburbia strengthened Michael's love of nature as the family home backed up to Jones' Creek, which meanders through neighborhoods and nearby woodland. His brother, Mark, introduced Michael to nature early on taking him fishing, hiking, and collecting arthropods, reptiles and amphibians. One of Michael's first memories of bird observation, in fact, came one afternoon as he and Mark watched a "water turkey" (American Anhinga, *Anhinga anhinga*) as it sunned on a log in the creek.

At age twelve, Michael became curious of "falcons" that were cruising the skies of his neighborhood. Through research, he discovered the birds were actually Mississippi Kites (*Ictinia mississippiensis*) and began actively hawkwatching, counting migrant raptors, with Chuck Feerick and Joe Kleiman on the Mississippi River levee near the LSU Vet School in the early 1990's. Michael's classmates laughed at him for counting for hours what they thought must be ten birds a day; in reality, Michael witnessed one of the most incredible spectacles in nature – the migration of kettles of 1000's of raptors.

Michael became hooked on birding soon after, no longer content with just watching migrant raptors; he traveled the USA in search of new birds to add to his list – visiting Colorado, Utah, California, Texas, Arizona, and New Mexico within a few short years. With the influence of an excellent high school biology teacher, Dr. Marcella Hackney, Michael was more certain than ever that biology, or more specifically zoology, would be his life long passion. In 1994, while a student in Hackney's biology class at Scotlandville Magnet High School for the Engineering Professions, Michael prepared a science fair project on the diets of seven

Neotropical raptor species – a topic that sent him straight to the LSU Museum of Natural Science. The museum and its faculty, staff, and graduate students became like a second home and family to him.

Michael became a student worker for the LSU Museum of Natural Science in 1997 when he started his undergraduate career at LSU and stayed in that position until he graduated. While at the museum, Michael learned techniques to prepare alcohol preserved specimens of both birds and herpetofauna and how to prepare bird study skins and skeletons. He was always excited performing tedious maintenance tasks in the bird collections, because he could steal the time away looking at the thousands of specimens from all parts of the globe.

Michael graduated from LSU in 2001 with a Bachelor's of Science in biological sciences. Michael immediately accepted a contractor position with The Nature Conservancy of Louisiana where he compiled a database of "witness trees" from surveyors' field notes from the early 1800's. In March 2002, Michael accepted a job as research associate in LSU AgCenter's Red Imported Fire Ant Laboratory where he initially worked as a geographic information system specialist.

In fall 2002, Michael began graduate school in the Department of Entomology with Dr. Linda M. Hooper-Bùi as his major advisor. While in graduate school, Michael met Kimberly R. Kennedy through research colleague Kathryn S. O'Brien. Michael and Kimberly were wed on 14 August 2005. Michael will receive a Master of Science degree in entomology in May 2007.