Distribution and Management of the Red Imported Fire Ant, *Solenopsis invicta* Buren in Virginia

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ABSTRACT

Red imported fire ant specimens were first collected in Virginia in 1989 from colonies located in Hampton, Va. Now colonies are established throughout the Hampton Roads area of Virginia. Because the fire ant is a new pest information regarding the distribution, biology, and management of the ant in Virgnia are absent. Therefore studies were conducted to compare the efficacies of four broadcast fire ant control products and an individual mound treatment to control fire ants and to determine the impact of Advion and Top Choice on non-target ant species. Additionally, twenty-six fire ant colonies in Virginia were sampled to determine colony social form and to detect biological control organisms. Lastly, the CLIMEX model was used to predict the potential spread of the fire ant in Virginia.

Field trials showed that combination applications of Advion and Top Choice provided the fastest and greatest reduction in foraging three days after treatment and the longest residual control of fire ant colonies for one year. Field tests documenting the impacts of Advion and Top Choice on non-target ants indicate treatments do supress ant foraging but ant populations will rebound 10-12 months after treatment.

Laboratory PCR assays revealed that colonies in Virginia are primarily polygyne. Also, *Kneallhazia solenopsae*, *Pseudaction* spp. parasitoid decapitating phorid flies, and *Solenopsis invicta* virues (SINV) genetic materials were detected in fire ant samples.

CLIMEX model results indicate that fire ants will be able to spread as far east as the Greater Richmond area and as far north as Alexandria in Virginia.

DEDICATION

This thesis is dedicated to everyone in Johnston, South Carolina. I never thought that my experiences growing up in the “Peach Capital of the World” would be used to shape my career goals. Thanks to Thad, Nate, and Nelson, for allowing me to participate in their many battles against my friend, the red imported fire ant. This thesis is also dedicated to Dr. Peter Schultz, for giving me my first taste of entomological research in 2002. Thanks Dr. Shultz and Go Hokies!

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Attribution

Several colleagues and coworkers aided in the writing and research behind a couple of the chapters presented as part of this thesis. A brief description of their contributions are included here.

Chapter 5: Characterization of *Solenopsis invicta* (Hymenoptera: Formicidae) populations in Virginia: Social form genotyping and pathogen/parasitoid detection.

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Prof. Dini M. Miller- Ph.D (Department of Entomology, University of Florida) is the primary Committee Chair. Prof. Miller co-wrote Chapter 5.

Steven M. Valles- Ph.D (Department of Entomology, University of Florida) is currently a research entomologist with the USDA. Steven contributed his expertise in molecular techniques and co-wrote Chapter 5.

Chapter 6: Predicting the potential range expansion of the red imported fire ant, *Solenopsis invicta*, in Virginia.

Prof. Carlyle Brewster- Ph.D (Department of Entomology, University of Florida) is currently a professor of entomology at Virginia Tech. Prof. Brewster provided ecological modeling expertise.

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Chapter 1 - Introduction

The red imported fire ant, *Solenopsis invicta* Buren, is an extremely aggressive invasive pest ant species that is native to South America. This pest was transported to the U.S. in ship ballast and cargo that was exported and unloaded at seaports located in Mobile, AL during the 1930s (Creighton 1930). Since their introduction, fire ants have gone on to establish colonies throughout the United States. Currently, red imported fire ant (RIFA) colonies can be found throughout the southeastern United States from Texas through Florida, extending as far north as Oklahoma and Virginia. Fire ants have also been transported west into New Mexico and California (Tschinkel 2006, Callcott and Collins 1996). Red imported fire ants are also established in Puerto Rico. Fire ants have become serious pests in the U.S. because they are omnivorous, (feeding on almost any plant or animal material), extremely effective colonizers (Tschinkel 1988b), and can inflict a painful sting when irritiated. As a result, RIFA have caused many serious medical, agricultural, and ecological problems in the U.S. (Porter and Savignano 1990).

Because the fire ant is such a harmful pest, there have been many studies regarding the biology (Tschinkel 2006), management (Williams et. al. 2001, Vinson 1994), and potential spread (Sutherst and Maywald 2004, Korzukhin 1999) of the ant in the U.S. Red imported fire ant colonies exhibit one of two social forms. Monogyne colonies contain one egg laying queen and polygyne colonies contain two or more egg laying queens. In characterizing the large number of U.S. fire ant colonies, researchers have shown that polygyny has played a great role in fire ant colony propagation. Studies have shown that polygyne colonies are more prevalent in the U.S. than in South America (Fritz and Vander Meer 2003). Monogyne and polygyne colonies differ in many aspects, including colony founding behavior, territorial behavior, reproductive strategies, and the features of control efforts (Adams 2003, Deheer 2002). Given that monogyne and poylgyne colonies differ in respect to mound density and colonization methods, it follows that knowing which fire ant colony social form is prevalent in an area assists in predicting future spread of the ant and determining control methods.

Control of the fire ant in the U.S. has proven to be difficult because the most effective treatment is dependent upon the size of the infested area. Fire ant control products are either applied as individual mound treatments (IMT) or broadcast (Williams et. al. 2001, Barr 2002a). Broadcast and individual mound treatment control products are either formulated as baits or granular insecticides. Baits are insecticides that are formulated in a food source that ants will pick up, carry back to the nest and feed to their colony. Granules are insecticides formulated on an inert particle (like corn grit or clay). The ants contact the particles while foraging and transfer the toxicant onto their bodies. IMTs are better suited to be used to treat small fire ant infestations, while products applied in a broadcast manner are better suited to be used to treat larger fire ant infestations (Drees et al. 2006).

Fire ants have been able to spread through much of the southeastern U.S. since their introduction in the 1930s. As a result, interest has been generated in predicting the potential spread of fire ant in the U.S. Several models have been developed that predict the potential range expansion of the RIFA (Pimm and Bartell 1980, Stoker et al. 1994, Killion and Grant 1995, Korzukhin et al. 2001). The most commonly used environmental predictors for fire ant occurrence are ambient and soil temperature (Korzukhin et al. 2001). These variables influence colony metabolism and activity (Porter and Tschinkel 1987). The model developed by Korzukhin et al. (2001), for instance, predicts the expansion of RIFA into many areas in Virginia where temperatures are suitable for colony survival and proliferation. However, these predictions have not been validated.

Although fire ant colonies are widely established in the southeastern U.S., the red imported fire ant is a relatively recent invader of Virginia. Early surveys conducted in 1949-1953 by the Bureau of Entomology and Plant Quarantine in Virginia found no evidence of fire ants within the Virginia state borders (Culpepper 1953). However, RIFA were positively identified in Virginia in 1989. These first RIFA specimens were collected in Hampton, Virginia. Since 1989, the Virginia Department of Agriculture and Consumer Services (VDACS) has documented all fire ant incidences within the state. VDACS reported that the number of RIFA mounds in Virginia has increased each year since 1989 (Frank Fulgum, VDACS Plant and Pest Services, Program Manager, personal communication). The greatest numbers of reported mounds have been found in the developing cities of Chesapeake (3000), Norfolk (1700), and Virginia Beach (2400) (Gina Goodwyn, VDACS Plant and Pest Services, Regional Supervisor, personal communication). The fact that *S. invicta* is so prevalent in these expanding southeastern coastal communities of Virginia is not surprising, because King and Tschinkel (2008) determined that there was a positive correlation between increased human activity, environmental disturbance, and fire ant colony establishment. In response to the increasing fire ant population, the United States Department of Agriculture-Animal Plant and Health Inspection Services (USDA-APHIS) and the Virginia Department of Agriculture and Consumer Service (VDACS) have implemented the Imported Fire Ant Quarantine in the Tidewater area of Virginia. In 2009, the cities of Chesapeake, Hampton, Newport News, Norfolk, Poquoson, Portsmouth, Suffolk, Virginia Beach, and Williamsburg and the counties of James City and York were placed under Imported Red Imported Fire Ant Quarantine. The single greatest impact that the quarantine will have on residents of the quarantine areas is that VDACS will no longer be responsible for treating fire ant mounds. Fire ant control will now be the responsibility of those citizens living and working in the quarantine locations.

Fire ant colonies in California (Greenberg et al. 2003), Louisiana (Milks et. al. 2007), Georgia (Gardner et al. 2008), Florida (Tschinkel 2006), and Texas (Drees and Frisbie 2001) have been well studied. However, to date, no studies have documented the distribution, management, or biology of the fire ant in Virginia. Based on the increases in *S. invicta* incidents reported to VDACS and the recent implementation of the quarantine by the USDA, it is apparent that there is need to better understand *S. invicta* populations in Virginia.

Therefore, the goals and objectives of this research project were:

1) To compare the efficacy of three broadcast fire ant control products and an individual mound treatment method to control fire ants:

2) To determine the impacts of Advion® fire ant bait and Top Choice granular on non-target ant foraging in Virginia:

3) To assess the prevalence of monogyne and polygyne fire ant colonies in Virginia and to determine if any biological control agents were present in Virginia *S. invicta* colonies:

4) To determine the potential spread of red imported fire ants in Virginia using the CLIMEX modeling system.

Chapter 2 - Literature Review

2.1 History of Imported Fire Ants in the United States

In 1918, the Black Imported Fire Ant (BIFA), *Solenopsis richteri* Forel, was transported from South America to the United States in ship ballast and cargo. The ants were first observed near seaports located in Mobile, AL (Creighton 1930). In the 1930s, a second species, the red imported fire ant (RIFA), *Solenopsis invicta* Buren, was also introduced from South America into Mobile, Al (Buren 1972). The two imported fire ant species were originally identified as *Solenopsis savevissima richteri*, "dark phase" and "light phase" (Wilson 1952), until Buren (1972) named the light phase ant *S. invicta*. Since their introduction, many control efforts have been aimed at eliminating, or at least, slowing the spread of imported fire ants (IFA). However, in spite of management efforts, IFAs have continued to establish colonies throughout the United States, and now range from Florida to California.

Although both imported fire ant species originated in South America, the ants differ in their native distribution and range. Populations of *S. richteri* occur in Uruguay, central eastern Argentina, and southern Brazil (Tschinkel 2006) whereas *S. invicta* has a much larger distribution inhabiting areas in Argentina, Uruguay, along the Pantanal River basin, and in Brazil (Allen et al. 1974, Tschinkel 2006, Folgarit et al. 2008).

Currently, not much is known about the exact origin or initial geographic spread of IFAs in South America. However, the origin and spread of imported fire ants in the U.S. has been well documented (Canter 1981, Callcott and Collins 1996). IFAs initially spread out from Mobile, Al at rate of one mile per year between 1918 and 1932 (Callcott and Collins 1996) into the surrounding counties via mating flights and human transport. Between 1932 and 1950, the

IFAs rate of spread from Mobile, AL reportedly increased to three miles per year (Callcott and Collins 1996).

2.1.1 Control History and Current Distribution

In response to the rapid spread of RIFA throughout the southern states, the United States Department of Agriculture (USDA) instituted a $2.4 million federal and state fire ant eradication and control program in 1957 (George 1958, Lockley and Collins 1990). The goals of the program were to survey and identify imported fire ant infested areas and to treat these infested areas with government approved pesticides (Williams et al. 2001).

The first active ingredients deemed appropriate for fire ant control were dieldrin, heptachlor, and chlordane (George 1958). As part of the fire ant eradication program the Plant Pest Control Division of the USDA, and Southern Plant Board developed an extensive spray regimen using these residual pesticides (George 1958). Initially, heptachlor and dieldrin were applied aerially or on the ground at a rate of 2 lbs of active ingredient per acre. However, these applications caused a great deal of damage to the environment and also caused significant mortality in bird and mammal populations (Tschinkel 2006). As a result, in 1959, the USDA lowered the application rate of these pesticides to 1.25 lbs of active ingredient per acre. The aerial applications of heptachlor and dieldrin were terminated in 1962 (Williams 1983) following the U.S. Food and Drug Administration’s discovery of epoxide residues in meat and milk. By this time, $15 million dollars had been spent to control the fire ant (Williams et al. 2001, Tschinkel 2006). Yet unfortunately, the spray control program was a complete failure because the fire ant managed to increase its range from 8 million to 12 million hectares of land during the three year control period (1959-1962) (Tschinkel 2006).

Before usages of heptachlor and dieldrin were halted, researchers from the USDA and several institutions began developing chemical baits for fire ant control. Researchers wanted to develop bait that could be easily formulated of low cost materials, was easily applied, was not easily degraded by rain, and was non-toxic to non-target organisms. The first bait resulting from this research was Mirex. Mirex contained the active ingredient mirex (nerve poison) formulated in soybean oil and carried on corncob grit. In 1961, Mirex took the place of heptachlor as the premiere fire ant control product (Lofgren and Vander Meer 1986). That same year, aerial and ground applications of Mirex began. Initially, Mirex was applied at a rate of 11.2 kg/hectare, but by 1965 this amount was reduced to 1.4 kg/hectare because lower application rates of mirex still provided desirable control results (Tschinkel 2006). In early studies conducted by Lofgren et al. (1964) mirex showed great promise. Lofgren et al. (1964) reported that Mirex could provide 90-100% control in research plots. Based on this study, the USDA believed that imported fire ants could, in fact, be eradicated from the U.S. To test the efficacy of Mirex in the field, the USDA established research sites in Savannah, GA, Tampa-St. Petersburg, FL, and Columbus-Starkville, MS (Banks et al. 1973). Two treatment blocks of variable size were constructed in each city, and Mirex was applied three times aerially at a rate of 2.5 lb/acre or 1.25 lb/acre. Banks et al. (1973) reported near 100% colony mortality in all treatment plots except two. One surviving colony was found in a research plot at the Savannah, GA research site and several colonies were found in a research plot located in Tampa-St. Petersburg, FL. However, even in locations where Mirex provided near complete control, the fire ants were able to reinfest the treated areas within 3-6 months after treatment. Although, the ants quickly re-colonized in the treated areas, Banks et al. (1973) still argued that eradication of the fire ant in the U.S. was possible. Therefore, approximately 800,000 lbs of Mirex was dispersed over 140 million of acres of land between

1962 and 1978 (Williams et al. 2001). However, as with heptachlor, Mirex was found to be a severe environmental contaminant. In 1978, the EPA cancelled Mirex's registration after high levels of mirex residues were discovered in the environment, fresh water fish, and other non-target organisms (Kaiser 1978).

In spite of these two massive chemical efforts to eradicate imported fire ants, the ants continued to expand their range within the U.S. By 1975, IFAs could be found in over 70 million hectares of land in North Carolina, South Carolina, Texas, Louisiana, Florida, Georgia, Mississippi, Arkansas, and Alabama (Callcott and Collins 1996). The failed eradication efforts combined with the negative environmental impacts of the chemical control options convinced the USDA that eradication was most likely impossible. Therefore, IFA control efforts after 1976 focused on limiting IFA spread rather than species elimination.

As part of the original eradication program, the USDA also implemented a fire ant quarantine program. Under the quarantine, commercial trade articles such as soil, plants with roots, grass sod, hay straw, mechanized soil moving equipment, and other items capable of harboring ants had to be inspected for fire ants before being transported into an uninfested location (Canter 1981). Items that were inspected and found to be infested with *S. invicta* also had to be treated with government approved control products, or washed with water, or cleaned with air pressure gun (Canter 1981). However, many of the recommended chemical control products especially calcium cyanide dust, were considered to be lethal to the environment and wildlife. In response to these issues, the EPA halted the usage of these chemicals in the 1970s. After use of these chemicals was stopped, the USDA recommended that fire ant infested soil, and other items be treated with chlorpyrifos and methyl bromide (Canter 1981).

The RIFA quarantine is still in effect today. Currently, Puerto Rico and 14 states (Alabama, Arkansas, California, Florida, Georgia, Louisiana, Mississippi, New Mexico, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Virginia) are under the Federal fire ant quarantine. As of 2010, the active ingredients used for treating fire ant infested materials under the quarantine are bifenthrin, fipronil, hydramethylnon, methoprene, pyriproxyfen, and tefluthrin (USDA 2010). These active ingredients have low mammalian toxicity ranging from 5,000 mg/kg to 32,500 mg/kg but are effective for controlling RIFA. The current quarantine inspections and treatment efforts have done much to slow the spread of fire ants, but RIFA continues to increase their range. RIFA can now be found in areas outside of the quarantine such as Arizona, Maryland, Delaware, and New Mexico (Vander Meer et al. 2003).

2.1.2 Current Range in the U.S.

The range expansion of IFAs can mainly be attributed to the red imported fire ant, because the black imported fire ant, *S. richteri* has not been able to increase its U.S range beyond northern Mississippi, northwestern Alabama, and southern Tennessee (Lockley and Collins 1990, Street et al. 2006). Currently, IFAs infest over 310 million acres of land in Alabama, Arkansas, Arizona, California, Florida, Georgia, Louisiana, Mississippi, New Mexico, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Puerto Rico (Kemp et al. 2000, Buren 1982).

In South America, the BIFA and RIFA are reproductively isolated (Ross and Shoemaker 2005). In fact, alkaloids found in the venom produced by *S. invicta* and *S. richteri* can be used to distinguish the two species. However, in the United States the two imported fire ants have been able to interbreed. Vander Meer et al. (1985) provided the initial evidence of fire ant hybridization.

Vander Meer et al. (1985) collected fire ant specimens from an *S. richteri* rich area in Starkville, Mississippi. The ant specimens possessed morphological features characteristic of *S. richteri* but the author wanted to confirm that the ants were indeed black imported fire ants. They compared the venom profiles from the Starkville, Mississippi ants to standard *S. invicta* and *S. richteri* profiles. Results from the venom analysis revealed that the collected ant specimens were a hybrid species, because the venom produced a blended *S. richteri* and *S. invicta* gas chromatograph profile. The *S. invicta* x *S. richteri* hybrid currently occurs in twenty-seven Mississippi counties, 43 counties in Georgia, western and southern Tennessee, western North Carolina, and northern Alabama (Streett et al. 2006, Gardner et al. 2008).

2.2 Imported Fire Ant Morphology and Biology

2.2.1 Morphology

Imported fire ants are small polymorphic ants ranging in size from 1.6 - 6.6 mm (1/16 - 1/4") in length. Their antennae are 10 segmented with a two segmented club. The ants have a two segmented pedicel, they lack spines on the propodeum, and have a stinger on the distal end of the gaster. The clypeus has three distinct teeth on the clypeus and four teeth on each mandible (Trager 1991). The characteristics listed above are conserved among *S. invicta*, *S. richteri*, and the *S. invicta* x *S. richteri* hybrid. However, there are a few key morphological characteristics that can be used to distinguish between the three IFA species. See descriptions below.

Solenopsis invicta (Buren, 1972)
Common Name: Red Imported Fire Ant
Other names: *Solenopsis wagneri*

The head and thorax of these ants is reddish brown, and the gaster is black. However, RIFA have a brownish red spot on the first gaster segment. Queen ant color is identical to that of worker ants but the gaster of the queen ant is much larger due to the reproductive organs (Trager 1991). Male reproductives have small heads and are completely black in color.

Solenopsis richteri (Forel)
Common Name: Black Imported Fire Ant

BIFA are uniformly darker than RIFA, however this characteristic alone should not be used to distinguish the two ant species. One of the most distinguishing characteristics of BIFA is the presence of a golden patch on the dorsal side of the gaster.

Solenopsis invicta x *Solenopsis richteri* hybrid

Hybrid fire ants are morphologically similar to BIFA. Consequently, venom alkaloids and cuticular hydrocarbons must be analyzed by gas chromatography in order to distinguish hybrid fire ants from *S. invicta* and *S. richteri* (Streett et al. 2006).

2.2.2 Monogyne RIFA Colony Development and Production

S. invicta is a very aggressive ant species with colonies that can contain either one egg-laying queen (monogyne) or several egg-laying queens (polygyne); (Glancey et al. 1973). Variation in queen number is directly related to a single Mendelian factor, the *Gp-9* (general protein-9) allele as reported by Keller and Ross (1999). Although monogyne and polygyne ants remain morphologically the same with respect to taxonomy, they differ in phenotypic characteristics of the colony (Ross and Shoemaker 1993). For example, monogyne and polygyne ant colonies differ in the number of wingless queens per nest, reproductive fecundity of wingless females, mode of colony founding, worker size, and number of new reproductives produced (Ross and Keller 1995).

The establishment of a new monogyne red imported fire ant colony begins with a mating flight. Mating flights occur between the spring and fall, at midday on a warm (24°C) sunny day following a rain (Tschinkel 2006). Six to eight flights occur each year with each flight consisting of up to 4,500 alates (Porter and Tschinkel 1987). Mating occurs during flight and the males die soon after mating with females.

Once mating has occurred, a newly impregnated queen will fly 2-50 m until she finds a suitable habitat in which to land. Once on the ground, she removes her wings and begins excavating a small chamber 2 to 10 cm deep in the soil. After the chamber is completed, she seals herself in (Markin et al. 1972, Tschinkel 2006). The excavated nest is a claustral chamber where the first brood will be reared. The queen begins laying eggs within 1-3 days of creating the claustral chamber. By the third day, the queen will have laid between 15-20 eggs. She will typically lay 30-70 eggs within the first week of making her chamber (Markin 1972 et al., Tschinkel 2006). Worker ant larvae hatch from the eggs in about 22 days (Markin et al. 1972). The larvae that emerge from these first eggs will develop into “minim” workers. These “minim” workers are the smallest ants that will ever be produced by the queen. The queen produces “minim” workers as opposed to larger workers, in order to maximize the production of workers from her limited stored body resources (Porter and Tschinkel 1986). Because foraging worker ants are not yet available, the queen depends on stored nutritional reserves and unembryonated eggs to feed the first “minim” worker brood (Tschinkel 1996, Holldobler and Wilson 1990). The queen feeds the larvae by regurgitating nutrients stored in her crop.

Once the claustral period is completed, the “minim” worker ants open the colony and begin to perform regular colony functions such as caring for new brood and foraging. The chief responsibilities of the “minim” workers are brood rearing and brood raiding (Tschinkel 2006). Brood rearing involves caring for the developing brood in the parent colony. Brood raiding is a non-violent competition between neighboring colonies where workers from one colony steal the brood of another colony until the raiding colony has amassed enough worker brood to defend itself from other raiding colonies in the future (Tschinkel 1992b). In most cases, the "winning" colony has more workers at the onset of the raid giving that particular colony a considerable advantage over the opposing colony (Tschinkel 1992b). Interestingly, some workers from the losing colony will join the larger "winning" colony, and may even assist the winning colony in raiding more brood from their own parent colony.

Queen ants from raided colonies often exhibit peculiar behavior. The abandoned queens will follow the raiding colony, in an attempt to displace the queen from the winning colony (Adams and Tschinkel 1995). The probability of successful queen usurpation depends on the number of related daughters she has within the new colony. Queens entering into colonies where the majority of workers are the daughters of another queen are attacked and killed (Adams and Tschinkel 1995). However, if the colony contains a large number of workers produced by the queen attempting usurpation, inclusion into the colony may occur. The ultimate result of brood raiding by claustral colonies is that many incipient monogyne colonies coalesce into a single large colony, greatly improving the survival and reproductive capabilities of the colony.

As the monogyne colony ages and grows in size, the queen stops producing minim workers, and begins producing major and minor workers (Tschinkel 1988a). These workers are larger than the minims and they are also polymorphic (Wood and Tschinkel 1981). Larger larvae develop into major workers and the smaller larvae develop into minor workers (Wheeler 1991).

The rate of ant development from egg to adult is dependent on temperature and the caste to which it will belong (Porter 1988a). When temperatures are held constant at 32°C, worker ants can develop within 26 days (Porter 1988a). Alate brood completes development in 35 days when temperatures are held at 32°C.

After a monogyne colony amasses ~23,000 workers, or is about a year old, the queen begins producing alates (reproductives) (Markin et. al 1973, Tschinkel 1993). After the colony begins to produce alates, reproductive rates and changes in colony size begin to follow a seasonal pattern (Markin and Dillier 1971, Tschinkel 1993).

During the fall the worker hatch rate within a monogyne colony surpasses the death rate. The increase in worker hatch rate results in a worker population that reaches its peak at the onset of winter (Tschinkel 2006). However, low temperatures throughout the winter will cause a decrease in both brood production and foraging (Porter and Tschinkel 1987). Worker death and birth rates become similar during this time, so the number of active workers in the colony is steady throughout the winter. Moreover, by decreasing brood production the colony is able to offset decreases in foraging, and use its metabolic reserves to support the existing colony during the winter (Tschinkel 2006).

When spring begins, the monogyne queen devotes 50% of her reproductive capabilities to producing alates (Tschinkel 1993). Consequently, the number of workers being produced is reduced, and the total population of ants within the colony declines (Tschinkel 1993). According to Tschinkel (2006), this trade-off in production from workers to reproductives in the spring increases the chances of successful colony founding, because the earlier a new monogyne queen leaves the parent colony the earlier she can began producing workers and join the seasonal population growth cycle. Also, during the spring, worker death rate exceeds the birth rate and by midsummer the size of the colony is half of its pre-winter maximum. Because worker numbers and metabolic reserves are low during the spring, worker ants spend most of their time foraging for food. Thus, by the end of the summer the worker ants will have replenished their metabolic reserves and can begin the fall egg production cycle (Tschinkel 1993).

2.2.3 Pleometrosis

Monogyne fire ant colonies are typically started with a single newly mated queen (haplometrosis) but sometimes multiple queens will assist each other in colony founding (pleometrosis) (Tschinkel and Howard 1983). Pleometrosis usually occurs when high numbers of newly mated queens land in the same area, and crowd together. The underlying factors involved in the queens' selection of a nest location are still unknown (Tschinkel 2006). However, queens typically choose partially vegetated, non-shaded fields as nesting sites (Tschinkel 2006). As more queens land at a particular site the possibility of coming into contact with one another increases, so does the possibility of foundress association (Tschinkel and Howard 1983).

Pleometrosis is associated with a higher production of workers during the initial stages of colony growth. Increased worker numbers allow the colony to execute brood raids more efficiently (Tschinkel 1998), and ensure colony founding success (Tschinkel and Howard 1983, Tschinkel 1993). Similar to haplometric (single colony founding queen) colonies, pleometric colonies begin with the digging of a claustral chamber by one queen. However, other fire ant queens landing in the area enter the chamber before the founding queen closes the entrance. Once inside the chamber, all of the queens begin producing minim workers. However, once the claustral period has passed, many of the queens in a pleometrotic colony are executed by the workers, or by other queens (Tschinkel and Howard 1983, Tschinkel 1998), until only a single queen is left. The surviving queen is usually heavier in weight than the other queens. The difference in weight allows the queen to either survive worker attacks or take possession of the colony's brood pile (Tschinkel 1998).

2.2.4 Monogyne Colony Foraging and Territorial Behavior

Monogyne fire ant colonies are territorial. A colony's territory can be defined as a continuously defended area that is used for foraging (Wilson et al. 1971, Tschinkel 1995). Initially, researchers believed that the seasonal fluctuations in worker and alate production (biomass) played a role in of territory size (Wilson et al. 1971). However, this idea has been rejected by Tschinkel et al. (1995) and Adams (2003). Adams' (2003) experimental results suggested that the size and location of a monogyne fire ant colony's territory is related to colony size (worker number) and the colony's intraspecific competitive neighborhood. Tschinkel et al. (1995) observed that the acquisition of territory was not linearly proportional to the seasonal increase in population experienced by a colony during the winter. During the winter as in the spring, colony territory size remained the same and colony territories held by smaller numbers of workers were often taken over by colonies containing a larger number of workers (Tschinkel et al. 1995). Adams (2003) was able to show that worker numbers within a colony were positively correlated to territory size. When workers were removed from a RIFA colony, the total territory size shrank considerably, but rose again when workers were placed back into colony (Adams 2003).

2.2.5 Discovery of Polygyny in U.S. Fire Ant Colonies

Imported fire ant colonies in the U.S were thought to be all single queen colonies until 1971 when Glancey and his associates collected 22 fire ant queens from two separate colonies (Glancey 1973). When the spermathecae were removed from these collected queens, it was discovered that each queen had mated. This study represented the first observed case of polygyny in imported fire ant colonies. Since the Glancey et al. (1973) study, polygyne fire ant colonies have been found throughout the southeastern U.S (Fritz and Vander Meer 2003).

However, the polygyne form of *S. invicta* is still considered to be less prevalent in the U.S than the monogyne form (Macom and Porter 1996, Mescher et al. 2003).

Polygyne colonies in the U.S. are typically distributed in a mosaic pattern separated from one another by monogyne colonies (Mescher et al 2003). This mosaic pattern is also observed in the fire ants' native South America, where the monogyne social form is more common than the polygyne form (Mescher et al. 2003).

2.2.6 Origins of Polygyny and Colony Founding Behavior

According to Holldobler and Wilson (1990), three different behaviors are responsible for polygyny in ant colonies. The three behaviors are: multiple queen colony founding (pleometrosis); colony fusion; and the inclusion of inseminated queens into an established colony. Red imported fire ant colonies are capable of utilizing all three of these methods, though only two of these methods are used on a regular basis. Normally, polygyne red imported fire ant colonies use budding as their primary mode of colony founding. However, newly mated queens are sometimes accepted into established colonies (Glancey and Lofgren 1988) but queen acceptance rates into new colonies are considerably low (Tschinkel 2006). Moreover, polygyny and queen acceptance into a colony, are not a function of queen and worker choice but to a specific set of alleles (Keller and Ross 1999, Ross and Keller 2002).

The general protein-9 (*Gp-9*) allele is responsible for social form in fire ant colonies. Ross and Keller (1998) conducted a study to determine the effects of the Gp-9 on colony social form. They reported that queens possessing the homozygous $Gp\text{-}9^{BB}$ allele were heavier than queens possessing the heterozygous $Gp\text{-}9^{Bb}$ allele. This discovery suggested that queens possessing the dominant allele (BB) were monogyne and queens possessing the recessive allele (Bb, bb) were polygyne. These findings also determined that colony founding type was directly correlated to the *Gp-9* allele. Monogyne queens are heavy and produce a large number of eggs (Tschinkel 2006) which are characteristics of independent colony founding. On the other hand, polygyne queens have a lower fat content and produce a smaller number of eggs which are characteristics that promote budding (Vargo and Fletcher 1989, Keller and Ross 1995, Goodisman and Ross 1996, Keller and Passera 1989, Tschinkel 2006). However, in some cases queens exhibiting genotypes/phenotypes common to polygyne queens do attempt to found colonies independently (Deheer 2002).

The *Gp-9* gene also affects the phenotype of male and worker fire ants (Goodisman et al. 1999). Male and worker ants are from polygyne colonies are considerably smaller than male and worker ants found in monogyne colonies (Goodisman et al. 1999). Males and worker ants in monogyne colonies possess the dominant allele (B), and the polygyne males and workers possess the dominant (B) or recessive allele (b).

2.2.7 Budding

Although polygyne queens have the ability to participate in mating flights, the colony founding success rates are quite low (Tschinkel 1998). Therefore, polygyne *S. invicta* colonies usually spread by means of budding (Vargo and Porter 1989). During the budding process, workers from an established colony leave their original nest with one or more fecund queens, to found a new colony in a different location (Holldobler and Wilson 1990, Walin et al. 2001). Budding fire ant colonies are able to multiply quickly. One mound has the potential to split off into as many as eight individual or satellite mounds in less than five months (Vargo and Porter 1989). Fire ant mounds produced by budding colonies are typically in close proximity with one another (Vargo and Porter 1989, Walin et. al 2001). The close associations of mounds within a defined area allow the fire ants to quickly monopolize a habitat.

2.2.8 Polygyne Colony Foraging and Territorial Behavior

Workers produced by polygyne queens are considerably smaller and less aggressive towards non-nestmate conspecifics than workers in monogyne colonies (Goodisman et al. 1999, Fritz and Vander Meer 2003, Vander Meer and Alonso 2002). This lowered aggression between the ant workers results in a lack of territoriality between colonies (Holldobler and Wilson, 1990). Territoriality in monogyne fire ants is caused primarily by competition between neighboring RIFA colonies (Adams 2003). The competition results in a small number of colonies being able to coexist in a particular area. However, the non-territorial nature of polygynous fire ants colonies often results in a high density of mounds in an area. In some cases these colonies can even be interconnected (Byron and Hays 1986). As a result, RIFA workers originating from different polygyne colonies frequently forage within the same territory (Tschinkel 2006).

2.2.9 Polygyne Colony Production

The production of alates and workers in polygyne colonies differs from that of monogyne colonies. Individual polygynous fire ant queens produce fewer eggs than monogyne queens (Vargo and Fletcher 1989), and as a result produce fewer reproductives and workers. However as a collective, polygyne queens produce more eggs than monogyne colonies (Vander Meer et al.1992).

The proportion of eggs in polygyne colonies developing into workers and reproductives also differs from that of monogyne colonies. The number of reproductives produced decreases as the number of queens in a polygyne colony increases (Vargo and Fletcher 1987). Queens in polygyne colonies cause female larvae to develop into workers, while male eggs are eaten by workers. Polygyne queens allowed to develop to maturity are replacement queens (Fletcher and Blum 1981). Inhibition pheromones produced by existing queen ants prevent the replacement queens from being able to dealate and develop their ovaries. Additionally, male alates produced by polygyne queens are usually diploid and sterile (Hung et al. 1974, Vargo and Fletcher 1987). Because alate production is suppressed in polygyne nests, queens spend most of their reserves on the production of workers (Tschinkel 1998). As a result, polygyne colony growth does not follow the seasonal growth trend as closely as a monogyne colony. Because there is no specific seasonality to the worker production period, polygyne colonies have the ability to develop faster with greater numbers of workers than monogyne colonies.

2.3 Ecological Impacts

Because of their ability to rapidly establish colonies, fire ants are considered to be one of the world's most successful invasive species (Tschinkel 1988b). As a result of their global spread and release from the pressure of native natural enemies, fire ants oftentimes become the dominant ant species within non-native habitats (Allen et al. 2004). The aggressive nature and shear abundance of RIFA in non-native habitats have had a profound effect on many ecological communities (Porter and Savignano 1990).

2.3.1 RIFA Impacts on Arthropod Biodiversity

RIFA are omnivorous and opportunistic feeders. They gain a substantial portion of their nutrients from consuming other invertebrates (Wojcik 2001). In fact, fire ants are known to significantly decrease native arthropod populations immediately after their introduction into a new habitat (Porter and Savignano 1990). Porter and Savignano (1990) conducted a 5 year study at the Brackenridge Field Laboratory in Austin, Texas documenting the introduction and impact of polygyne RIFAs on the native ant and arthropod community. They discovered that in the presence of polygyne fire ant colonies, arthropod species richness decreased by 40%. Twelve years after the study by Porter and Savignano (1990), Morrison (2002) conducted a follow-up study, documenting the long-term effects of polygyne fire ant invasion. Morrison's (2002) results indicated that after the initial fire ant invasion, native ant and arthropod species diversity, as well as abundance, rebounded, and in some cases surpassed, the preinvasion levels. Morrison (2002) collected forty-nine ant species, including 7 new ant species that were not documented by Porter and Savignano (1990). In addition, several new Coleopteran, Dipteran, Hemipteran, and Hymenopteran species were also collected. These results suggest that fire ant effects on arthropod communities were only significant immediately after invasion (Morrison 2002).

However, monogyne fire ant colonies may not affect arthropod species richness and abundance to the same extent as polygyne colonies. Morrison and Porter (2003) conducted studies in North Central Florida using pitfall traps to evaluate the associations between monogyne RIFA colony density and the species richness of other ant species and non-ant arthropods in the area. Surprisingly, as fire ant densities increased so did arthropod diversity. Morrison and Porter (2003) found 37 different ant species from 16 genera and 19 other arthropod species in their pitfall traps over the course of the study. These findings with regard to monogyne invasions were different than those reported in the study on polygyne species, which suggested that polygyne RIFA colonies suppressed other ant and arthropod populations. Morrison and Porter's (2003) results suggested that native ant and arthropod communities may be able to survive or coexist with monogyne fire ant invasion, and that the biotic and abiotic factors that typically affect native arthropod populations in an area may also help regulate RIFA density. However, further investigation will be needed to fully characterize the specific impacts of polygyne fire ants on arthropod diversity and abundance.

2.3.2 RIFA Impacts on Arthropod Pests and Beneficial Arthropods

Some studies have shown that fire ants do indeed have the ability to reduce arthropod pest populations (Wojcik 2001), such as lone star ticks in northern Louisiana (Burns and Melancon 1977), sugarcane borers (Cacienne 1959), and boll weevils (Fillman and Sterling 1983). However, in many of these field studies the arthropod pests were used as live bait or were confined to reduce mobility.

Because, RIFA are not selective feeders they are known to prey on beneficial insects (Eubanks 2001, Eubanks et al. 2002). Eubanks et al. (2002) conducted greenhouse and field studies documenting the effects of fire ants on beneficial cotton arthropods. Eubanks et al. (2002) documented that fire ants reduced populations of two lady beetle species (*Coccinella septempunctata* L., and *Hippodamia convergens* Guerin-Meneville) by 50%. The fire ants also reduced green lacewing larvae (*Chrysoperla carnea* Stephens) survival by 38% (Eubanks et al. 2002).

Although RIFA has been observed suppressing many arthropod species, they have had a very obvious impact on established fire ant ant communities in the field and have been responsible for the displacement of native fire ant species. Upon its introduction into the U.S., RIFAs competitively displaced two native fire ant species, the southern fire ant, *Solenopsis xyloni* (McCook), and the tropical fire ant, *Solenopsis geminata* (Forel) (Tschinkel 2006). According to Tschinkel (2006), *S. xyloni* "simply disappeared" from areas it previously inhabited as *S. invicta* expanded into its range. Presently, *S. xyloni* can only be found in Texas, New Mexico, Arizona, and California (Jacobson et al. 2006). Although the exact range of *S. geminata* is not currently known, it has been documented in locations ranging from Florida west to Texas. However, after the introduction of *S. invicta*, populations of *S. geminata* decreased in the southeastern U.S (Tschinkel 2006). Currently, tropical fire ant populations co-occur in zones within the RIFA range but the two species do not overlap (Tschinkel 1988b). Porter and Savignano (1990) determined that, fire ants do not include many ant species in their diet, so species displacement is probably a result of interspecific competition.

2.3.3 RIFA Impacts on Birds

S. invicta has also been documented as having negative impacts on wildlife both directly and indirectly (Wojcik et al. 2001, Allen 1994). Fire ants nest in any type of soil and are therefore particularly dangerous to other ground nesting animals like birds, reptiles, or mammals (Allen 2004). Although the adult animals are rarely attacked due to their ability to escape, the immature nestlings are particularly vulnerable to fire ant attacks.

Fire ants are well known predators of newly hatched chicks (Dickinson 1995). Pipping (newly emerging) nestlings are extremely vulnerable to predation because young birds are confined to the nest, making escape from fire ants almost impossible. Large scale studies describing the interactions between fire ants and northern bobwhite quails (*Colinus virginianus*) were conducted in Texas where Allen et al (1995) discovered that bobwhite quail abundance was lower in counties infested with fire ants than in counties that were not infested. Allen et al. (1995) obtained *C. virginianus* population data (1966-1992) from annual Christmas bird counts conducted in 15 Texas counties infested with *S. invicta*. The *C. virginianus* abundance data from the infested counties was compared with abundance data from 16 uninfested Texas counties. The comparison indicated that bobwhite quail abundance decreased in the presence of fire ants.

In another study, Allen et al. (2000) examined the abundance of northern bobwhite quail before and after fire ant invasion in South Carolina, Florida, and Georgia. Quarantine maps were used to determine the first year of fire ant infestation in each state. Christmas bird counts for bobwhite quails were conducted during one 24h period, within a 24-km diameter sampling site. Allen et al. (2000) reported that bobwhite quail abundance was greater in South Carolina and Florida prior to the fire ant invasion.

Fire ants also have had negative impacts on swallows. Kopachena et al. (2000) reported that 25% of barn swallows (*Hirundo rustica*) from a Northern Texas colony were preyed upon by RIFAs. However, nests from a Southern colony were not attacked by RIFA. Nests from the northern colony were shaded and nests were spaced nearly 10 apart. Nests from the southern colony were located in direct sunlight and were spaced nearly 200 m apart. Kopachena et al (2000) concluded that the risk of fire ant predation decreases when nest densities are low and located in direct sunlight. Similarly, Sikes and Arnold (1986) documented that RIFA influenced the nesting success of cliff swallows (*Hirundo pyrrhonota*) in cement drainage culverts located in the Texas counties of Burleson and Washington. Cliff swallow nesting success in fire ant free culverts was 74.9%. Nesting success was reduced to between 34.4 to 40.4% in culverts containing fire ants. Fire ants in the culverts were observed entering pipped eggs and stinging newly hatched cliff swallows.

2.3.4 RIFA Impacts on Reptiles

Introduced species like the fire ant have been listed as one the factors causing the global decline of amphibians and reptiles (Gibbons et al. 2000). Amphibians and reptiles are vulnerable to fire ant predation because of their underground egg laying behavior (Allen 1994). Mount et al. (1981) compiled a list documenting direct evidence of fire ant predation on reptile eggs and young. Fire ants were observed preying on soft-shell turtle (*Trionyx sp.)* eggs, gopher tortoise (*Copherus polyphemus*) hatchlings, chicken turtles, and many other turtle species. However, these observations were not quantified (Mount et al. 1981). Allen et al. (2001), conducted laboratory experiments in which fresh water turtle, *Pseudemys nelsoni*, hatchlings were exposed to foraging fire ants. Based on laboratory results, Allen et al. (2001) reported that 70% of the exposed turtle hatchlings were killed by fire ant predation and stings. Wetterer and Moore (2005) also conducted a field study where the presence of RIFA at gopher tortoise (*Gopherus polyphemus*) burrows was documented. Tuna bait was placed near the entrance of 154 tortoise burrows and after two hours, high numbers of RIFA were present at 33% of the burrows. Wetterer and Moore (2005) suggested that RIFA may be potential threats to gopher tortoises and other native species living in tortoise burrows.

In addition to anapsids, RIFA have also been documented as having indirect impacts on alligators. American alligators (*Alligator mississippiensis*) nest in open habitats, which are also suitable nesting sites for fire ants (Allen et al. 1997). In a survey conducted by Allen et al. (1997), 20% of the American alligator nests in Florida marshes contained established RIFA colonies. According to Allen et al. (1997), fire ants are attracted to the cracked eggs of pipping alligators. As a result, young alligators may be stung as they emerge from the eggs (Allen et al. 1997). Allen et al. (1997) also documented the effects of fire ant envenomization on neonatal American alligators. Alligators that experienced RIFA stings as new hatchlings had a lower average body mass than alligators that had not been stung.

Reagan et al. (2000) conducted a study observing the nesting success of female alligators in the presence of RIFA. Where fire ants were present, young alligators were constantly stung and maternal alligators were less inclined to open nests. Therefore, RIFA presence decreased nesting success by 14.6%.

2.3.5 RIFA Impacts on Mammals

Prior to the 1980's studies evaluating mammalian response to fire ant invasions had not been conducted on a large scale. However, since the 1980's numerous reports have been published, with most focusing on rodent populations. Lechner and Ribble (1996) observed the impact fire ants had on the foraging behavior of three rodent species *Baiomys taylori*, *Peromyscus leucopus*, and *Sigmodon hispidu*s. Lechner and Ribble (1996) conducted both field and laboratory studies to observe changes in rodent behavior when fire ants were present. The three species were placed into Y-shaped mazes with crushed fire ants located in one of the arms. *B. taylori* avoided the arm of the maze containing fire ants 71% of the time. Conversely, *S. hispidus* and *P. leucopus* did not avoid the fire ant infested arms of the maze.

Lechner and Ribble (1996) also conducted field studies to determine whether or not rodent populations decreased in the presence of fire ants. Six 150-m transects were constructed in a fire ant infested field. Rodent trapping stations were positioned 10-m apart along transects. Lechner and Ribble (1996) reported that the number of rodents confined to traps did not decrease as fire ant mound density increased suggesting that rodents did not decrease their foraging to avoid fire ant colonies.

Holtcamp et al. (1997) documented the influence of RIFA on the foraging behavior of deer mice (*Peromyscus maniculatus*). Comparisons were made by measuring mouse foraging activity in rich seed patches (15 unhusked sunflower seeds) and poor (6 husked sunflower seeds) seed patches. The mice spent more time foraging in rich patches when fire ants were present, but spent the same amount of time in rich and poor patches when fire ants were absent. These results suggested that fire ants caused deer mice to become more active foragers and to forage longer under threats of competition. In a similar rodent foraging study, Orrock and Danielson

(2004) used foraging trays filled with seed to observe changes in old field mouse (*Peromyscus polionotus*) foraging patterns in fire ant infested fields. The trays were also set up to simulate settings or scents associated with predation risk or success (predator urine, moonlight, precipitation, and shelter). However, Orrock and Danielson's (2004) results differed from the results presented by Holtcamp et al (1997). When RIFA were present, the mice spent less time foraging. In fact, deer mouse foraging decreased more in the presence of *S. invicta* than under normal predation risks (predator urine, moonlight, precipitation, and shelter) tested in the study.

The influence of fire ants on the behavior and ecology on large wild mammals has not been studied extensively. Those studies documenting interactions between RIFA and large mammals have focused primarily on white tailed deer (*Odocoileus viginianus*) (Allen et al. 1997, Mueller 2001). Allen et al. (1997) constructed ten 202 hectare plots and treated 5 of the plots with Amdro (Am. Cyanamid Co., Wayne, N. J.) fire ant bait. When fire ant populations were suppressed in the treated areas the fawn: doe ratio was found to be significantly greater in the treated plots than in the untreated plots. Allen et al. (1997) revisited the sites after one year, and reported that fire ant colonies had rebounded in the treated plots, and that the fawn: doe ratio's were no longer significantly different in the treated and untreated plots. These results suggest that RIFA presence may affect deer reproductive success by reducing fawn survival.

2.3.6 RIFA Impacts on Cattle

Although RIFA may affect many types of livestock, their impacts are best documented in cattle. The USDA estimates that RIFAs are responsible for $38 million dollars in production losses annually in the U.S. (Dorough 2006). These losses are the results of RIFA causing blindness and death in newborn cattle, the disruption of cattle foraging, and the reduced availability of (non-fire ant infested) foraging pastures (Jetterer et al. 2002, Dorough 2006).

According to Jetterer et al. (2002), penned animals and livestock are at the greatest risk for fire ant attacks. Barr and Drees (1994) conducted a survey of Texas veterinarians documenting the impact of RIFA on livestock. Barr and Drees (1994) reported that RIFA stings on cattle accounted for 49.6% of fire ant related veterinarian visits, and cattle blindness accounted for 20.1%. In addition to health problems resulting from stings, the presence of RIFA in pastures significantly reduces livestock feeding. Cattle can become malnourished. Compounding the problem cattle may refuse to feed from food in troughs of hay bales intended to supplement their diet, if the troughs or hay bales are infested (Jetterer et al. 2002).

2.3.7 RIFA Impacts on Crops

Wilson and Eads (1949) conducted one of the first documented surveys regarding the fire ant's impact on agricultural crops. In Wilson and Eads' (1949) survey, 174 farmers were interviewed from two fire ant infested counties in Alabama. The farmers claimed that fire ants most commonly damaged corn, soybeans, and cabbage. Prior to 1972, many scientists and farmers argued that the fire ant was a serious agricultural field crop pest (Adams et al. 1976). However, these reports were based on personal accounts, and lacked any quantitative data (Adams et al. 1976). Adams et al. (1976) was the first to quantify the economic damage caused by fire ant feeding on soybeans. Adams et al. (1976) estimated that fire ants could cost farmers between $178,537 and $1,000,000 in losses if 10 to 75% of the soybean crop was infested. Adams et al. (1983) conducted a subsequent study quantifying the fire ant impact on soybean crop yield. The fire ants reduced the soybean yield by feeding on germinating seeds and on the roots of the young soybean plants. The data indicated that soybean yields were 400 to 575 kg less per ha in fields where fire ants were present than in fields with no fire ants.

In addition to soybeans, fire ants have been documented to reduce the yield in many other agricultural crops. These crops include potatoes (Adams et al. 1988), corn (Glancey 1979) and citrus trees (Banks et al. 1991). Banks et al. (1991) determined that citrus tree mortality was 5.5 to 6.6 times greater in areas where fire ants were present, than in locations that were fire ant free.

2.3.8 RIFA as Potential Biological Control Organisms

Although fire ants have been known to cause crop damage, they are not generally considered to be crop pests. In many cases they may be considered beneficial because they feed on a host of insect pests that feed on crops. In Texas, fire ants are the primary insect predators of cotton field pests (Diaz et al. 2004), such as the bollworm, *Helicoverpa zea*, and beet armyworm, *Spodoptera exigua*. Fire ants in cotton fields have been found to reduce bollworm and beet armyworm eggs by 20-30% (Diaz et al. 2004), thus reducing the overall population during mid and late growing season. Fire ants also prey upon cotton boll weevils, *Anthronomus grandis*. Agnew and Sterling (1981) determined that fire ants were responsible for consuming 44% of boll weevils in Texas cotton fields. In addition to preying on cotton pests, fire ants are also documented predators of the sugarcane pest, *Diatraea saccharalis,* (Bessin and Reagan 1993), and the soybean pest, the velvetbean caterpillar larvae, *Anticarsia gemmatalis* Hubner (Lee et al. 1990).

Because the fire ant is such an effective predator of many agricultural insect pests, researchers have begun to evaluate fire ants as potential biological control agents (Way and Khoo 1992). Fire ants have been used as effective biological controls for both diamondback moth larvae, *Plutella xylostella*, and leaf beetles in collard crops (Harvey and Eubanks 2004). Fire ants have been shown to reduce leaf beetle densities in collard crops by 45%. In fact, in field plots containing high numbers of RIFA, collard leaf damage was 33% lower than in plots containing lower RIFA numbers. Consequently, collard crop yield increased by 28% in plots containing high RIFA numbers (Harvey and Eubanks 2004).

Hood et al. (2003), used fire ants as biological controls for the greater wax moth, *Galleria mellonella*, and the lesser wax moth, *Achroia grisella*, in stored beehives supers with drawn comb. Hood et al. (2003) reported that moth mortality increased in the presence of RIFA and consequently resulted in decreased of wax comb damage.

However, the fire ant's role as a biological control agent is disputed, as the ant is also known to prey on beneficial insect species (Eubanks et al. 2002). Eubanks et al. (2002) conducted a study documenting the influence of fire ants on beneficial cotton insects. In this study, fire ants were found to be responsible for significantly reducing the survival of green lacewing larvae, *Chrysoperla carnea* (Stephens), and two ladybird species, *Coccinella septempunctata* L., and *Hippodamia convergens* (Guérin-Méneville), both of which are considered beneficial insect predators in cotton.

Quantifiying the positive and negative impacts of RIFA on agricultural crops is difficult because RIFAs are omnivourous, sometimes damaging crops directly or consuming beneficials while preying on crop pests. The combination of positive and negative RIFA impacts also makes it difficult to assign some dollar amount to their potential for damage. However, it is clear the *S. invicta* does have an impact on agricultural crops and more study is needed to determine if their overall impact on any particular crop is either positive or negative.

2.3.9 RIFA Impacts on Urban Environments

Fire ants are also of economic importance in urban environments. Fire ants are regarded as urban pests because they sting people and because of the unattractive mounds they produce in peoples' yards and recreational areas. They are also known to occasionally invade structures where they have caused damage electrical circuits the resulted in fires. According to Pereira (2003), fire ants cost the American public $6 billion annually in the of structural damage repair, control efforts, and medical bills resulting from fire ant sting reactions.

Fire ants typically flourish in highly disturbed habitats (Tschinkel 1988b), where human activities have greatly modified the natural environment (Pereira 2003). These are usually urban environments. Therefore, urbanization of the U.S. has significantly increased the spread of the fire ant (DeShazo et al. 1999). According to Williams et al. (2001) the transportation of nursery stock, fill dirt, turf and other landscape products related to construction and residential development are largely responsible for the spread of the red imported fire ant (RIFA) in the U.S (Williams et al. 2001).

In urban environments, fire ants typically build their nests outdoors in open sunny areas. These areas may be lawns, sports fields, golf courses, housing developments, or road medians (Kemp et al. 2000). Fire ant mounds and their associated underground foraging tunnels have been known to cause damage to the foundations of sidewalks and driveways (Miller et al. 2000). Fire ants are also known to occasionally nest in electrical equipment (Slowik et al. 1997) because they are attracted to AC and DC fields of increasing voltage (MacKay 1992). In fact, fire ant colonies in the highly infested metropolitan areas of Texas have been known to cause over $100 million in damage to electrical equipment (Reinert and Maranz 2001).

Fire ants do not typically invade human structures, but in some cases, fire ants will invade structures opportunistically and will sting anyone attempting to remove them. When fire ants attack, they clasp their victim's skin with their mandibles, and insert their stinger into the skin releasing a venom composed of alkenyl disubstituted piperidines and proteins (Haight and Tschinkel 2003). These compounds are allergenic and can cause different types of reactions depending on the immune response of their victim. Human reactions to fire ant stings include the development of sterile pustules, edema, dermal necrosis, and in rare cases death from anaphylactic shock (Adams and Lofgren 1991, Haight and Tschinkel 2003).

More than 20,000 patients in the U.S. are treated annually for fire ant stings (Tracy et al. 1995). Most of these patients are children (Kemp et al. 2000). In a survey conducted by Clemmer and Sterling (1975) it was reported that 55% of the children in New Orleans experienced fire ant stings annually. While deaths from fire ant stings are not common (Vinson 1997), there have been more than 80 fire ant sting related deaths recorded in the U.S. prior to 1989 (Rhoades et al. 1989).

Considerable amounts of money have been spent on fire ant prevention and control (Varlamoff et al. 2001). Miller et al. (2000) conducted telephone surveys in South Carolina to quantify the cost of fire ant control per household. The surveys indicated that South Carolina homeowners spent an average of $83.37 on fire ant control annually. Varlamoff et al. (2001) conducted a telephone survey to quantify homeowner pesticide usage in Georgia. Varlamoff et al. (2001) determined that 64% of homeowners applied insecticides to their landscape and 76% of those residents regularly purchased insecticides specifically for fire ant control.

2.4 Current Control Methods

2.4.1 Broadcast Control Methods/Baits

After the Mirex bait registrations were phased out by the EPA in 1978. The only chemical products labeled for fire ant control were contact insecticides that were either applied as a mound drenches or applied directly to the mound surface. However, all of these contact insecticides required that each mound be treated individually (Williams et al. 2001).

In 1979, the EPA granted the first experimental usage permit for a new fire ant bait AC 217,300, also known as hydramethylnon (American Cyanamid Company, Princeton, NJ) (Mallipudi et al. 1986, Williams et al. 2001). The following year, the EPA granted American Cyanamid Company of Princeton, NJ a conditional registration for hydramethylnon (Amdro) to be used for fire ant control in pastures, lawns, turf, and nonagricultural land. Williams (1983) conducted field tests in Florida and Mississippi to determine the efficacy of hydramethylnon. Baits containing hydramethylnon (0.75%) applied at a rate of 1.4 kg/ha provided 95% control of fire ant colonies located in Mississippi 26 weeks after treatment. However, baits containing 0.375% hydramethylnon applied at a rate of 2.4 kg/ha provided the best results in field tests conducted in Florida. Hydramethylnon formulated in a lower concentration and applied at a higher volume reduced fire ant colonies by 96% (Williams 1983). The development and refinement of hydramethylnon as a bait formulation was extremely important to the pest control industry. At last there was an effective fire ant bait that did not have disastrous environmental consequences.

Currently, several broadcast products are being produced and marketed for fire ant control. Individual mound treatments are still available for use but, broadcast application has become the preferred RIFA control method. This is because broadcast application does not require the location of all mounds within a treated area. Additionally, many of the active ingredients in broadcast products are formulated as baits. Baits are effective because they take advantage of the ants' natural foraging and feeding behavior. Because ants orally transfer food between colony members, the active ingredient contained in bait, is naturally spread throughout the colony (Williams et al. 2001). There are now several different active ingredients in fire ant bait formulations including hydramethylnon, abamectin, indoxacarb, spinosad, and methoprene (Drees et al. 2002).

Of these active ingredients, hydramethylnon and indoxacarb have proven to be the most efficacious. However, in field tests, the sodium channel blocker indoxacarb has performed slightly better than hydramethylnon (Barr 2002b). Barr (2002b) conducted field trials comparing the efficacy of several formulations of indoxacarb (0.025%, 0.05%, and 0.1%) to several other broadcast baits including those containing hydramethylnon (0.73%), s-methoprene (0.5%), and bifenthrin (0.2%). Barr's (2002b) results indicated that all formulations of indoxacarb reduced RIFA mound density faster than the other active ingredients. Although, mound density within some of the other treated plots decreased as well, indoxacarb provided quicker mound suppression, and residual activity for four months (Barr 2002b).

Oi and Oi (2006) conducted a similar field test to compare the ability of indoxacarb (0.045%), spinosad (0.015%), and hydramethylnon (0.73%) to suppress fire ant populations. Results from these field tests indicated that indoxacarb (0.045%) provided 100% colony mortality within three days, whereas hydramethylnon (0.73%) produced only 60% colony mortality after eleven days. Seventeen percent of colonies treated with Spinosad (0.015%) were killed within three days. However, after 3 days no additional mortality was observed in the spinosad treated plots (Oi and Oi 2006).

Many chemical manufacturers in the 1970's were developing and evaluating insect growth regulators (IGRs) specifically juvenile hormone analogues (JHAs), as potential active ingredients in baits. Cupp and O'Neal (1973) were the first to document the effects of juvenile hormones (JH) on the development and behavior of RIFA. Two juvenile hormone analogues, Ethyl 3, 7, 11-trimethyldodeca-2, 4-dienoate (hydroprene) and Isopropyl 11-methoxy-3, 7, 11-

trimethyl-dodeca-2, 4-dienoate (methoprene) were evaluated in the study. Brood and worker ants were exposed IGRs using 3 different methods: surface contact, *per os* administration (manual oral application), and contact via trophallaxis. These tests demonstrated that both hydroprene and methoprene were capable of preventing pupation in fire ants, but only methoprene worked consistently using all three exposure methods. Banks *et al*. (1978) also evaluated IGRs as active ingredients for fire ant baits between 1974 and 1976. During that time, twenty six IGRs were administered to fire ant colonies in peanut butter baits. Fourteen of the IGRs tested caused a shift in caste differentiation. Specifically, queens stopped producing worker ants and began producing alates. Banks *et al.* (1978) concluded that IGRs could be used in field situations to control fire ants by interfering with colony structure.

Wendel and Vinson (1978) reported that the EPA was initially reluctant to register any IGRs labeled for fire ants, because many IGR studies produced inconsistent field results. In addition, IGRs were metabolized and excreted rapidly by worker ants before worker ants could transfer the active ingredients to larvae (Wendel and Vinson (1978). However, in 1983 the EPA granted the first registration for RIFA IGR, fenoxycarb which was sold under the trade name Logic or Award (Novartis Crop Protection, Greensboro, NC). Although, fenoxycarb is a carbamate it does not act as a cholinesterase inhibitor but as an IGR by disrupting the egg laying of fire ant queens, and disrupting brood development (Banks et al. 1988). In field tests conducted by Banks et al. (1988) baits containing fenoxycarb eliminated 60% of the ant colonies in test plots and reduced the total fire ant population by 67-99%, within 12-13 weeks after treatment.

2.4.2 Granular Formulations

While many of the active ingredients used for fire ant control are formulated as baits, the active ingredient fipronil is formulated as a granule. Fipronil is a contact insecticide that causes nerve hyperexcitation in the insect by blocking the passage of chloride ions through GABA mediated chloride channels (Greenberg et al. 2003). In field tests conducted by Barr and Best (1999), fipronil granules were found to be the most efficacious contact insecticide fore fire ants, and provided the longest residual control. Barr and Best (1999) evaluated the efficacy of several different broadcast formulations in the field. These included fipronil granules (0.143%), bifenthrin (0.2%) granules, hydramethylnon (0.73%), and s-methoprene bait (0.5%). Fipronil (0.143%) suppressed the number of active RIFA mounds to < 3.25 five weeks after treatment, and after six weeks RIFA colonies were absent. Colonies did not re-appear in the treated plots until three months after treatment. Fewer mounds were found in fipronil (0.143%) treated plots 12 months after treatment than in plots treated with other formulations.

2.4.3 Individual Mound Treatments (IMTs)

While baits can be used for IMTs, many formulations use contact insecticides to control active mounds. These contact insecticides can be formulated as dusts, liquids, granules, or aerosols. Contact IMTs are applied around the immediate perimeter of the mound, so that non-target ant populations aren't affected by foraging near the treated mound surface. Contact IMTs usually provide quick control of fire ant mounds because ants pick up a lethal dose simply by walking across the insecticide.

2.4.4 Combination Treatment Methods

Baits containing hydramethylnon and indoxacarb can suppress fire ant colony activity in one month (personal observation). Other baits containing IGRs (fenoxycarb and methoprene) generally take 3-5 months to suppress populations (Barr et al. 2002). Contact IMTs can control mounds within a few days or weeks depending on the active ingredient. However, after the initial suppression of RIFA populations in the field, the ants are often capable of reestablishing colonies within the treated areas (Barr et al. 2002). To avoid recolonization in treated areas researchers have begun to evaluate combinations of different products applied as "hopper blends". Barr and Best (2002) compared the efficacy of four insect growth regulators (abamectin, pryriproxyfen, 2-methoprene, and fenoxycarb) baits mixed with a hydramethylnon bait (0.73%) to control RIFA. In all tests IGR/hydramethylnon blends performed better than the IGRs when used alone.

In addition to combining bait formulations for fire ant control, scientists have also found other ways to combine different RIFA treatment methods. The Texas Cooperative Extension Service created the two-step method which calls for the application of a broadcast bait in late summer or early fall, followed by a liquid, bait, dust, or granular application directly to the mound a few weeks after the initial bait treatment. Jones et al. (1997) conducted one of the first trials to evaluate the effectiveness of the two step method using a fenoxycarb (Logic®/Award®) bait followed by an acephate (Orthene® 75S) mound drench. Jones et al. (1997) chose field sites north of the Federal Imported Fire Ant Quarantine border in Arkansas, Oklahoma, and Tennessee. Field plots varied in size, but fenoxycarb (1.7 kg/ha) and acephate (45 cc) were applied at the label rate in all plots. Within two months, fire ants were eradicated in all field plots, except those located at the Bryant, AR location.

2.4.5 Biological Control Agents/Fungi

When chemical control efforts associated with the IFA eradication program began to decline in 1978, USDA officials began taking trips to South America in hopes of discovering some natural enemies of fire ants to use as biological control agents (Jouvenaz 1983). The organisms discovered on these trips provided the initial evidence that natural pathogens and parasites of IFAs did exist in South America (Jouvenaz 1983). Since Jouvenaz's (1983) initial trip, numerous biological control agents for IFAs have been discovered including viruses, arthropod parasites, bacteria, and nematodes (Williams et al. 2003). However, only a small number of these organisms have been evaluated for RIFA control in the U.S., and even fewer have performed well in field trials.

Beuaveria bassiana (Balsamo) is a fungus that infects the insect integument, digestive tract, and tracheae (Broome et al. 1976). Broome et al. (1976) were the first to conduct studies documenting the routes of entry and dosage effects of *B. bassiana* on *S. richteri* larvae. To determine the route of entry of *B. bassiana*, the fungus was topically applied to 100 alate larvae. One hundred and worker larvae were treated per orally. Both treatment groups were exposed to varying concentrations of fungal spores. LD_{50} values were then calculated for each treatment group. The results indicated that *B. bassiana* spores were able to infect both through the cuticle of the alate larvae and the digestive tract of the worker larvae. The maximum spore germination occurred within 48 hours of treatment and 79-84% mortality was observed in all trials. The LD_{50} value for spore applied topically to larvae was 3.6×10^1 spores/larva. The LD_{50} value for spore applied perorally was 3.9×10^2 spores /larva. While these laboratory evaluations looked promising, Callcott et al. (1988) and Oi et al. (1994) determined that *B. bassiana* did not provide acceptable levels of colony mortality in the field.

2.4.6 Viruses

Valles et al. (2004) were the first to discover a positive strand RNA virus capable of infecting RIFA workers and colonies. The virus, known as *Solenopsis invicta* virus 1 or SINV-1, was found to be capable of infecting all members of the colony during any developmental stage. However, infected ants did not display any disabling symptoms, which is a common trait of most RNA viruses infecting insects. In 2008, Valles and Hashimoto discovered a second fire ant virus. However, like the SINV-1 virus, ants infected with SINV-2 did not exhibit any morbidity as a result of the infection. Although studies have shown that SINV-1 and SINV-2 are capable of infecting all the ants within a colony, not much is known about the ability of these viruses to have any negative effects on infected colonies.

2.4.7 Fire Ant Decapitating Phorid Flies

The first arthropod biological control agents considered for widespread RIFA suppression was a group of South American fly species in the genus *Pseudacteon* (Diptera: Phoridae). These flies were first identified by Borgmeir (1963) as potential *Solenopsis spp.* parasites (Williams 1973). Later studies conducted by Williams (1973) and Porter (1995) confirmed that female flies in this group would use their ovipositor to deposit a single egg into the fire ant's thorax. The fly's lifecycle was very unusual in that within a couple of days of the egg hatching, the maggot emerged, from the egg and traveled to the ant's head where it crawled inside the head capsule. The maggot then developed inside of the ant's head for two to three weeks. Prior to pupation, the maggot consumed all of the material inside of the ant's head capsule and released an enzyme that caused the ant's head to separate from the thorax. A single adult fly emerged from the separated head after two to three weeks of development, depending on the temperature (Porter *et al*. 1995).

Since the Borgmeir (1963) discovery, more than 20 different phorid fly species that parasitize fire ants have been identified in South America. However, only *P. tricuspis*, *P. litoralis*, and *P. curvatus* have been released as biological controls in the United States (Porter and Gilbert 2005). Each *Pseudacteon* species released in the U.S is known to exhibit a specific parasitizing behavior that contributes to the overall biological control effort. For example, *P. tricuspis* parasitizes medium size worker ants while *P. curvatus* attacks small worker ants, and *P. litoralis* parasitizes medium size-medium/large worker ants (Porter and Gilbert 2005).

Pseudacteon tricuspis was the first species to be released in the U.S. *P. tricuspis* was initially released by Gilbert in 1995 at the University of Texas' Brackenridge Field Laboratory (Vasquez *et al*. 2006). However, the flies were unable to establish because an insufficient number of flies were released and because ambient temperatures were not suitable for survival. The first successful release and establishment of *P. tricuspis* was conducted by Porter and his associates in Florida during the summer of 1997 (Porter *et al*. 2004). After the first success in Florida *P. tricuspis* has since been released in several other states including Arkansas, Alabama, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Tennessee (Porter *et al*. 2004). The second *Pseudacteon* species released for fire ant control in the U.S., was *P. curvatus*. *P. curvatus* was released near Gainesville, Florida in 2003 by Vasquez et al. (2006). Two different *P. curvatus* biotypes have been since released in the U.S. The *P. curvatus* biotype released in Alabama, Mississippi, and Tennessee only parasitize black and hybrid fire ants, while the *P. curvatus* biotype flies released in Florida, South Carolina, and Texas only parasitize RIFA (Porter and Gilbert 2005). *P. litoralis* is currently established in Alabama.

Laboratory studies have confirmed that *Pseudacteon spp.* flies have a negative impact on RIFA behavior, for example RIFA decrease their foraging activity in the presence of the *Pseudacteon* flies (Morrison 2000). However, field studies documenting the effects of fire ant decapitating flies on RIFA populations over large spatial and temporal scales have been less conclusive (Morrison and Porter 2005).

2.4.8 Microsporidia

Kneallhazia solenopsae is an intracellular microsporidian parasite that infects all castes and life stages (Sokolova and Fuxa 2008) of *S. invicta* (Allen and Buren 1974) and *S. richteri* (Briano et al. 1995). Allen and Buren (1974) isolated *K. solenopsae* spores from Brazilian RIFA colonies in 1973. The first report of *K. solenopsae* in U.S. fire ant colonies was produced by Williams et al. (1998) in Gainesville, Florida. Williams et al. (1999) were also the first to inoculate field colonies with *K. solenopsae*. In an effort to determine if *K. solenopsae* could be artificially transferred to field colonies of *S. invicta* Williams *et al*. (1999) attempted to inoculate 5 polygyne field colonies with *K. solenopsae*. Forty-eight weeks after colony inoculation with *K. solenopsae*, the overall RIFA population in these colonies was decreased by 30%. Further colony observations revealed that four colonies had been successfully infected. The Williams et al (1999) study determined that while *K. solenopsae* can be transferred to RIFA colonies artificially, but that *K. solenopsae* may not be able to completely suppress individual colonies in the field.

2.4.9 Areawide Suppression Program

Many current fire ant control products are not labeled for use over large areas or pastures. RIFA products labeled for use in pastures aren't specific for fire ants, and often end up killing non-target insects (Pereira 2003). In response to the need for a viable fire ant suppression program for pastures, the USDA-ARS, USDA-APHIS, and the USDA's Imported Fire Ant and Household Insects Research Unit partnered with several universities to create and implement an areawide fire ant suppression program. The purpose of this program was to reduce the number of viable fire ant colonies in pastures using biological control agents (*Pseudacteon spp*. flies and *K. solenopsae*), and to a lesser extent, toxic baits (Aubuchon and Vander Meer 2008).

As of 2009, only five states (Florida, Mississippi, Oklahoma, South Carolina, and Texas) have been selected to participate in the areawide fire ant suppression program (Vander Meer *et al*. 2007). These states were chosen because they represented the diversity of habitats in the U.S. that currently support RIFA populations (Pereira 2003). Two demonstrations sites were established in each state; one site for the release of biological control agents, and another site as a control where no biological control agents would be released. The sizes of the sites have ranged between 60-120 hectares. All sites were treated first with bait mixtures (1:1) containing hydramethylnon (0.73%) and methoprene (0.5%). Baits were aerially applied at a rate of 1.7 kg per hectare at each treatment site (Pereira 2003, Vander Meer *et al*. 2007). After the bait application, 4000-6000 adult *P. tricuspis and P. litoralis* flies and laboratory-parasitized ants were released at each of the biological control sites by first disturbing a fire ant mound and releasing the flies or ants over the mound.

Prior to releasing the flies, researchers had documented that *K. solenopsae* was already present in some Florida, Oklahoma, and Texas RIFA colonies. However, *K. solenopsae* did not occur in South Carolina or Mississippi RIFA colonies. Therefore, *K. solenopsae* inoculations were only conducted in Mississippi and South Carolina to establish the parasite within those states.

Preliminary results of the area-wide suppression program have shown that the decapitating flies and *K. solenopsae* have become established in all of the release sites (Vander Meer et al. 2007). As a result, RIFA populations have declined at least 20% in all sites where bait and biological control agents were released.

The area-wide suppression program was designed specifically for control of fire ants but, the applications of hydramethylnon (0.73%) and methoprene (0.5%) had the potential to affect non-target ant species. However, applications of hydramethylnon (0.73%) and methoprene (0.5%) did not have a significant effect on non-target arthropods. Baits only reduced non-target ant species abundance at the Florida site. Additionally, species richness of non-target ant species did not differ between treated and control plots at all sites. In the future, the USDA hopes to expand the areawide RIFA control program to areas other than pastures such as parks, poultry farms, hunting clubs, natural areas, etc. in hopes of reducing national RIFA populations by 40-45% (Vander Meer *et al*. 2007).

2.5 Predicting the Spread

Red imported fire ants (RIFA) possess many of the characteristics necessary to survive in a non-native habitat. Good dispersal ability, high reproductive rates, the ability to start new colonies by budding, polygyny, and the aggressive defense of the colony against predation have allowed for RIFA to expand its range in almost all areas where the ant has invaded (Moller 1996, Tsuisui et al. 2003). Although, the biology and ecology of RIFA has been extensively studied the global range potential of this insect is still not fully understood.

RIFA colonies in the U. S. are currently established across most of the southeastern states. However, RIFA have been found in several other states outside the southeastern U. S. In addition, RIFA populations have been discovered in other non-native countries. These include China (Zhang et al. 2007), Taiwan, Australia (McCubbin and Weiner 2002), and New Zealand. The ability of RIFA to become established and proliferate in these non-native environments makes us speculate as to the potential range limits of this species.

Many researchers agree that the fire ant's potential range will be limited by climate (Porter and Tschinkel 1993), specifically temperature and rainfall. Soil temperature is integral to the development of fire ant brood, and fire ant mating flights are stimulated by rainfall (Porter

1988b, Markin et al. 1971). Many simulation models have used temperature and/or rainfall as variables to predict the fire ant's potential range (Pimm and Bartell 1980, Killion and Grant 1995, Korzukhin et al. 2001). However, the results produced by these range expansion models have varied. Nonetheless, even a cursory understanding the range potential of the red imported fire ant can help to improve future quarantine and control efforts throughout the world.

One of the first predictive fire ant range tools was created by Pimm and Bartell (1980). This particular model used RIFA incidence data obtained from the USDA's Cooperative Economic Insect Reports to document the spread of the ants in the U.S. from 1965-1976. The Pimm and Bartell (1980) model also used weather data obtained from U.S. Weather Bureau Reports to define the relationship between RIFA spread and climatic conditions in Texas. Mean monthly rainfall, the mean number of days below 0^{o}C (cold days), and the mean number of days where the temperature exceeded 32^{o}C (hot days) in the states of Alabama, Arkansas, Florida, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, and Texas were used as parameters in the Pimm and Bartell (1980) model. The results of the model suggested that RIFA would not readily establish in areas where the climate was typically dry and temperatures were consistently below 0^{o}C. Furthermore, Pimm and Bartell (1980) correctly concluded that RIFA would not increase their northern range in Texas, but would spread to 18 new counties in western and central Texas by 1990.

Stoker *et al.* (1994) also developed a fire ant range model that predicted range expansion and colony survivorship as functions of temperature and precipitation. The model was used to simulate colonization under three different climate conditions considered to be either favorable (Austin, Texas), too dry (Yuma, Arizona), or too cold for colony survival (Fargo, ND). Five 7-year simulations were run for each location. The mean number of workers, total number of mating flights, mean number of alates per flight, and the total number of new colonies produced were predicted for each climate condition used by the model.

The Stoker et al. (1994) model suggested that parent colonies at the two extreme climate sites (Yuma, AZ and Fargo, ND) would be unable to produce new colonies. However, twenty-six new colonies would be produced and able to survive in the Austin, Texas location where the conditions were favorable. The model also made predictions about the number of workers that would be produced at each site. In Fargo, ND the mean number of worker ants was only 70,273, which is approximately half the mean number of workers that would be produced at the Texas and Arizona sites. The model predicted that adequate numbers of alates for successful nuptial flights would be produced in both the Arizona (dry) and Texas (favorable) sites. However, conditions at the dry site (AZ) did not favor mating flights, because mating flights needed to be initiated by rain. The model also predicted that inadequate numbers of alates would be produced at the cold site (Fargo, ND), diminishing any chances of a successful mating flight ever taking place.

The Stoker et al. (1994) model was also used to simulate fire ant range expansion in Texas. Colonization was simulated at 13 sites along a line running northwest through Texas, from Burnet to Sherman county, each site having different climate conditions. Five simulations, 3,000 days in length were run for each site. The mean and minimum number of new colonies, number of mating flights, number of workers and alates produced, were predicted for each site. Unfortunately for Texas, the model predicted that RIFA colonization and establishment could occur in all of the 13 simulation sites.

The Pimm and Bartell (1980) model as well as the Stoker et al. (1994) models were designed to be specific for the state of Texas. Killion and Grant (1995) however, decided to develop a model capable of simulating the potential range expansion of RIFA for the entire U.S. Killion and Grant's (1995) model used variables such as temperature driven developmental rates, and the longevity of worker ants to simulate colony growth and range expansion. Temperature data was taken from three locations, College Station, Texas, Oklahoma City, Oklahoma, and Wichita, Kansas. These three sites were selected to represent different climatic conditions. College Station was an area where RIFA was well established. Oklahoma City represented an area where the climate should support RIFA colonies. Wichita represented an area where the climate would be detrimental RIFA colony survivorship. Results from the model suggested that the first worker ants would appear 38 days after colonization in College Station, after 46 days in Oklahoma City, and after 47 days in the Wichita. The model also predicted that the Wichita colony would only amass 16,484 workers, and that the entire colony perished on Julian date 252. This simulation supported the hypothesis that RIFA colony expansion was limited by the -17.8ºC January isotherm, which was at that time (1995) thought to be the northernmost limit of RIFA range expansion.

Korzukhin et al. (2001) developed one of the most widely accepted RIFA range expansion models for the U.S. The model utilized minimum and maximum soil temperatures, alate production, and territory size as determinants of colony growth. In the Korzukhin et al. (2001) model, soil temperature instead of ambient temperature was a predictive variable, because soil temperature has a greater influence on subterranean colony activity than ambient temperature. Soil temperature data were collected from the National Ocean and Atmosphere Administration CD-ROMs which contained weather data compiled from 4,537 meteorological stations in the United States. Soil temperature data was obtained for 15 states (Alabama, Arizona, Arkansas, California, Florida, Georgia, Louisiana, Mississippi, New Mexico, North

Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Virginia) where fire ants were known to be established. Soil temperature data was also obtained for 9 states (Delaware, New Jersey, Nevada, Oregon, Kansas, Kentucky, Maryland, Missouri, and Washington) where fire ants had not yet been reported.

A colony establishment model was predicted for 4,537 meteorological sites located within the aforementioned states. The predicted number of female alates produced by colony was recorded and results from model were used to create a potential fire ant range map (Figure 1). Sites that produced more than 3,900 alates were represented by a red circle and designated as areas that could "certainly" support fire ant colony establishment. Green circles were used to represent areas that produced at least 2,100 female alates, and that could "possibly" support fire ant colony establishment. Dark blue circles were representative of habitats capable of producing less than 2,100 female alates. These sites were labeled as sites that had not yet demonstrated their ability to support fire ant colony establishment. Light blue circles were indicative of areas where fire ant establishment was "improbable". The model predicted that no female alates could be produced at the improbable sites. Areas currently under the federal fire ant quarantine are shaded grey on the map (Figure 1). The fact that all of the grey areas are covered with red dots, confirmed that the model did successfully predict locations where fire ant colonies were "certain" to become established. The data represented on the map also suggested that *Solenopsis invicta* has the potential to expand from its current range into western Texas, southern New Mexico, southern Arizona, California, Oregon, and coastal areas of Virginia.

Morrison et al. (2005) created a model similar to Korzukhin et al. (2001) that used colony growth and alate production as determinants of colony establishment under a global warming scenario. Climate change data from the Vegetation-Ecosystem Modeling and Analysis Project

(VEMAP) were used to simulate global warming trends. The model predicted that any increase in *S. invicta*'s range would be first observed in the states of Oklahoma, Arkansas, Tennessee, and Virginia. The climate change model also predicted that RIFA would expand its current range by 5% within the following 40-50 years. Moreover, by 2100, RIFA were predicted to have increased their total range in the U.S. by 21%.

Many of the RIFA range expansion models were created specifically for the U.S. However, several models have been developed to predict the spread of RIFA throughout the world (Morrison *et al*. 2004, Sutherst and Maywald 2005). Similar to Korzukhin (2001), Morrison *et al*. (2004) used soil temperatures and female alate production to predict fire ant potential spread in nations outside the U.S. However, in the Morrison et al. (2004) model, locations producing a minimum of 1,500 female alates (instead of 2,100 like Korzukhin (2001)) were labeled as "possible" fire infestation zones. Locations with colonies producing less than 1,500 female alates were designated as "unlikely" to support RIFA. Rainfall was also included in the Morrison et al. (2004) model. The model predicted that many locations in Mexico, Central America, the Caribbean, and Northern South America were susceptible to fire ant establishment, as well as, some countries in Europe, Asia, and Africa. All locations receiving less than 510 mm of precipitation were presumed to be uninhabitable by RIFA.

Sutherst and Maywald (2005) used the CLIMEX program to model the effects of temperature and moisture on RIFA colony survival. The CLIMEX model was developed to describe the response of an organism to climate conditions in the U.S and Australia (Sutherst and Maywald 2005). Temperature (hot and cold limits), population growth, and moisture (minimum and maximum limits) were used as parameters for the CLIMEX fire ant model. The CLIMEX model predicted that RIFA could significantly expand its current range north beyond Virginia on the east coast and f north beyond California on the west coast of the U.S. The model also predicted that fire ants would be able to successfully reproduce in the Australian cities of Brisbane, Sydney, and Perth. However, temperatures in Melbourne are too low during the winter months to support RIFA survival. Additionally, on a larger scale, model results suggested that fire ants could produce alates everywhere in Australia except the extreme southeast and high altitude areas.

RIFA have been able to establish themselves outside of their native range in a wide variety of habitats with variable climates. RIFA colonies can now be found in countries on four of the world's continents. According to many species distribution models, RIFAs have the ability to continue their spread as long as temperatures and rainfall within these non-native areas are adequate for survival. The development of species distribution models and continued research to predict the potential RIFA range limits will continue to be important in our attempts to manage this noxious pest.

Chapter 3 - Comparison of Three Broadcast Fire Ant Control Products and an Individual Mound Treatment for Control of Fire Ant Colonies in Virginia

3.1 Introduction

Prior to 2009, all reported red imported fire ant (RIFA), *Solenopsis invicta* Buren, infestations in Virginia were documented and managed by the Virginia Department of Agriculture and Consumer Services (VDACS). However in spite of VDACS' efforts, RIFA infestations within the state continued to spread. Therefore, in 2009 the United States Department of Agriculture (USDA) in conjunction with VDACS implemented the Imported Fire Ant Quarantine in the following areas of Virginia: the counties of James City and York, and the cities of Chesapeake, Hampton, Newport News, Norfolk, Poquoson, Portsmouth, Suffolk, Virginia Beach and Williamsburg. Consequently, VDACS is no longer responsible for treating RIFA mounds in the quarantined areas. Fire ant control in the quarantined counties/cities is now the responsibility of homeowners, nurseryman, and pest management professionals. However, VDACS is still responsible for managing RIFA infestations in cities outside of the designated quarantine areas.

The VDACS standard control method for treating RIFA mounds in Virginia was to apply MaxForce® fire ant bait (1.0% hydramethylnon) (Bayer CropScience, Kansas City, MO) around each active mound. Bait applications were followed six weeks later by an acephate mound drench. Although effective, these individual mound treatments (IMT) required that all target mounds be located before application. IMTs are labor intensive and time consuming, but the direct application of the chemical to the mound enhances the chance of insecticidal contact with colony members (Barr and Best 1999).

Baits and liquid insecticides are the typical formulations used for IMTs, but aerosols, granules, and dusts can also be used. IMTs are useful when 20 or fewer mounds are present in an acre of land (Barr and Best 2002). IMTs are also beneficial because they are only applied to visible RIFA mounds, thus preventing native ant mortality. However, because IMTs are only applied to visible mounds, fire ant recolonization can easily occur in treated areas where small mounds are not obvious. Quite often multiple applications are necessary to control all mounds in a particular area.

In contrast to IMTs, broadcast insecticide treatments for RIFA do not require individual mounds to be located. These products are generally applied at locations where mound densities exceed 30-40 per hectare. Therefore, application time and labor is decreased when broadcast products are used. Broadcast fire ant control products are currently formulated as either granules or baits.

The active ingredient in fire ant bait is often combined with soybean oil or some other food matrix that is attractive to foraging fire ants (Williams et al. 2001). Broadcast baits are applied using either a hand or tractor mounted spreader (Drees et al. 1996). Once the ants transport the bait inside the colony, the ants transfer the active ingredient throughout the colony by trophallaxis. Because the active ingredients found in baits are spread throughout the colony by the worker ants, several weeks to months may pass before significant colony mortality occurs (Drees et al. 2006). Although colony suppression may take longer using broadcast baits, studies have indicated that individual mound treatments are more costly than and not as effective as some of the more novel broadcast bait products (Barr and Best 1999).

Broadcast fire ant products have been used to treat fire ants in Virginia, but they have been infrequently used, due to VDACS' preference for individual mound treatments. Because

VDACS relinquished their responsibility to treat infestations in quarantined counties and cities, residents in these locales will have to learn to manage fire ants on their own. With the quarantine implementation, the need for broadcast RIFA product evaluations and other control recommendations are vital to Virginia.

Two of the leading broadcast fire ant control products are Advion® fire ant bait (Indoxacarb 0.045%; DuPont, Wilmington, DE) and Top Choice granular (Fipronil 0.143%; Bayer Environmental Sciences, Cary, North Carolina). Advion is a fast acting bait (Furman and Gold 2006) that contains the active ingredient, indoxacarb, which belongs to the oxadiazine chemical class. Oxadiazines block sodium channels in the nerve axon. Immediately after bait ingestion, feeding begins to decrease and target individuals usually succumb to death within 48 hours (Barr 2002). Top Choice contains the active ingredient fipronil which belongs to the phenylpyrazole chemical class. Fipronil is a nerve poison that blocks the passage of chloride ions through GABA receptor and glutamate-gated chloride channels causing nerve hyperexcitation in target insects (Kolaczinski and Curtis 2001).

Previous studies have shown that Advion significantly reduced fire ant foraging 24 hours after treatment (Barr 2004) and eliminated > 95% of colonies after one week (Hu and Song 2007) after application. Top Choice has a longer residual activity than Advion but is much slower acting. Barr and Best (2004) reported that Top Choice® reduced the mean number of active fire ant mounds by 80% five weeks after treatment and greater than 90% control was observed 52 weeks later.

In this study, field applications of Advion, Top Choice, a combination application of Advion and Top Choice, and an IMT treatment using MaxForce were evaluated for efficacy against established RIFA populations. After one year test, the products were re-applied to evaluate the rapidity of foraging reductions

3.2 Materials and Methods

Study Area

Research plots were established within Fun Junktion Park, a converted landfill located in Elizabeth City, NC. The study was conducted from 5 August 2008 to 26 July 2009. Elizabeth City is located on the northeast coast of North Carolina in Pasquotank County (36°17’44’N; 76°13’30’W). Average monthly temperatures range from a low of 32.3°F during the winter months to a high of 89.2°F during the summer months. The city receives about 122 centimeters of rainfall annually.

Research Plots

Fourteen 30 x 30 m (900 m^2) research plots (Figures 3.1 and 3.2) were established within three different locations within the park. Eight plots were located on a driving range that was covered with grass and mowed weekly. Four plots were established in a grass covered field located near an artificial lake. Two plots were located in a weed covered field that was not mowed. An 7.6 m untreated buffer zone separated each plot to reduce ant foraging between research plots. Plots were randomly assigned to different treatments so that each of the four insecticide treatment had three replicates. The two remaining plots served as untreated controls.

Treatment Products

The broadcast products evaluated in the study were Advion® Fire Ant Bait (0.045% indoxacarb; DuPont, Wilmington, DE.), Top Choice® Insecticide (0.0143% fipronil; Bayer Environmental Science, Research Triangle Park, NC) and MaxForce® Fire Ant Killer Granular Bait (1.0% hydramethylnon; Bayer Crop Science, Kansas City, MO). Advion and Top Choice were also used in a combination treatment where they were applied together in the same plot. All products were applied at the label rate (Advion: 1.68 kg/hectare (1.5 lbs./acre), Top Choice: 209 kg/hectare (85 lbs./acre), MaxForce: 14-28g/mound (0.5-1.0 oz./mound), Combination:Advion/Top Choice) with Scotts Handy Green II hand spreaders (Scotts International B.V., Scotts Professional, Geldermalsen, The Netherlands) except for MaxForce which can be applied as a broadcast or as an individual mound treatment. However, for the purposes of this study MaxForce was used as an IMT and was applied directly to individual mounds from the product container. Treatment applications were made on 12 July 2008 between 5:00 p.m. and 7:00 p.m. Each broadcast treatment was applied to three plots. MaxForce was applied to 7 active fire ant mounds located in experimental plots.

Sampling Regimen to Quantify Foraging Activity

Prior to treatment applications, hot dog baits were used to quantify foraging activity in each of the plots. Pre-treatment bait counts were taken on 11 July 2008 between 5 and 7:00 p.m. Eight beef hot dog (Gwaltney, Smithfield VA) slices (0.5 cm thick) were placed in each plot. The hot dog slices were arranged in two rows of four with each row spaced 7 m apart. Hot dog slices were left in place for one hour after which photographic images were taken of each slice with a Sony Cybershot digital camera (Sony Electronics Inc., San Diego, CA). All images were downloaded onto a computer so that the species and number of ants in each hot dog photograph could be counted and recorded.

Post-treatment ant sampling with hot dog slices was conducted between 5:00 p.m. and 7:00 p.m. at 3, 7, 14, and 30 days after treatment and every month thereafter for one year. During the initial study, post-treatment data collected on 7, 14, 30, and 60 days were lost. In addition, sampling was not conducted during the winter months (121 and 239 days after treatment) because temperatures were too low to support ant foraging.

Product Reapplication to Determine Time to Knockdown

All products were reapplied after one year. Treatment applications were made on 21 July 2009 between 5:00 p.m. and 7:00 p.m. The sampling regimen to determine the number of RIFA foragers pre- and post-treatment was conducted as described above. MaxForce was applied to 5 mounds. Control plots (2) were left untreated. Post-treatment sampling was conducted between 5:00 p.m. and 7:00 p.m. Samples were conducted at 3, 7, 14, 30, 60, and 90 days after treatment to determine the time to knockdown for all treatment products.

Statistical Analysis

The mean number of foraging fire ants collected per treatment on each sampling date was calculated by adding the total number of ants foraging on all 8 hot dogs in each treatment plot, and dividing that total by number of plots per treatment. To determine if the treatment applications had any effect on the mean number of foraging ants, data were transformed by ((√(x +$^{3/8}$) (Zar 1984)) and subjected to repeated-measures multivariate analysis of variance (MANOVA) with post treatment date as the repeated measure. Repeated-measures MANOVA was also used to determine if the residual activities of each treatment were significantly ($\alpha = 0.05$) different from one another.

Differences in the mean number of RIFA collected in each treatment on each sampling date was determined using two by one way repeated-measures ANCOVA, with the mean number of foraging ants collected on DAT-0 as a covariate. Significant differences among treatment means on each post treatment sampling date were separated by Tukeys HSD test ($\alpha = 0.05$). LS Means produced in the ANCOVA were used to calculate percent change in the mean number of

RIFA foraging ants after treatment relative to the initial number of foraging ants collected on DAT-0 (Vickers 2001). Separate repeated-measures MANOVA and ANCOVA analyses were conducted on initial application and re-treatment data.

3.3 Results

Product Efficacy Tests (2008)

Repeated-measures MANOVA was used to determine whether product applications had any effect on the mean number of foraging ants collected in plots. Results of the repeated-measures MANOVA indicated that there was a significant overall treatment effect on the mean number of foraging fire ants ($F = 72.0$; df = 9, $P < 0.0001$) (Figure 3.3). Contrast comparison tests between the mean number of foraging fire ants collected from treatment plots and control plots indicated that the mean numbers of foragers collected from each treatment plot were significantly lower ($P < 0.05$) than that collected in the controls. In addition, contrast comparison tests revealed that the mean number of ants collected in each of the chemical treatment plots was significantly different from one another.

The ANCOVA was conducted to compare the mean number of foraging ants in each treatment on each sampling day. LS means calculated by the ANCOVA were used to calculate the percent change in the mean number of active foragers on each post treatment sampling date (Table 3.1). Three days after treatment the mean number of foraging fire ants in the Advion, MaxForce, and Advion/Top Choice combination plots was significantly lower than that in the untreated controls ($P < 0.05$). The greatest percent reduction in foraging three days after treatment was observed in Advion/Top Choice combination plots (82.7%) followed by Advion (79.5%), MaxForce (68.4%), and Top Choice (6.6%). All active fire ant mounds in all treatment plots were determined to be inactive three days after treatment. When post-treatment sampling resumed on DAT-90 there were significantly fewer ants collected from the Advion (355.5), Top Choice (38.2), and Advion/Top Choice (0.0) plots than in the MaxForce (995.6) and control plots (1369.1). The greatest percent reduction in foraging at DAT-90 was observed in the Advion/Top Choice combination plots (100.0) followed by Top Choice (96.4), Advion (61.2), and MaxForce (27.5). For the remainder of the test (DAT-90 – DAT-360), fewer fire ants were collected in combination and Top Choice treatment plots than in all of the other experimental plots. At the conclusion of the test on DAT-360, there were significantly fewer ants collected in Advion (777.7), Top Choice (972.8), and combination plots (596.2) than in the control plots (1257.8) (df 13, $F = 8.3$, $P < 0.05$). However, the mean number of ants collected from MaxForce treatment plots was not significantly different from controls ($P > 0.05$).

Overall, the results suggest that the Advion/Top Choice combination, and the Advion treated plots had the greatest reductions in ant foraging by day 3, causing foraging reductions of 82.7 and 79.5 percent respectively. However, Advion, Top Choice, and the Advion/Top Choice combination treatment also provided the longest lasting control with significant reductions in foraging at 360 days.

Product Reapplication to Determine Time to Knockdown

Repeated-measures MANOVA results indicated that the insecticide products had a significant overall treatment effect on the mean number of foraging fire ants ($F = 76.1$; df = 9, $P < 0.0001$) (Table 3.2). Contrast comparison tests between the mean number of foraging fire ants collected from insecticide treated plots and control plots indicated that the mean number of foragers collected from insecticide treated plots were significantly lower ($P < 0.05$) than that of the controls. Additionally, contrast comparisons also indicated that the greatest reductions in the number of active foragers occurred in the Advion (82.9%), MaxForce (79.6%), and Advion/Top

Choice (85.7%) combination plots were far greater than reductions observed in Top Choice (17.5%) and control plots.(0.9%).

ANCOVA results indicated that throughout the test the mean number of ants collected in all chemical treatments was significantly lower ($P < 0.05$) than the mean number of ants collected in control plots on each sampling date. Additionally, from DAT-3 to DAT-30 the mean numbers of ants collected in chemical treatment plots were significantly lower ($P < 0.05$) than those from control plots from the control mean but not one another. However on DAT-60, the mean number of foraging ants increased in all plots except those treated with Top Choice. While Advion and MaxForce still had significant reductions in foraging at day 60, the reductions were significantly less than those of Top Choice and the Advion/Top Choice combination at 60 days. At the conclusion of test on DAT-90, percent reductions in foraging were greatest in the Advion/Top Choice combination and Top Choice treated plots. At 90 days, MaxForce had the least reduction in foraging but this reduction was not significantly different from that in Advion or Advion/Top Choice combination plots. Overall, the knockdown of foragers was the most rapid and complete in the Advion/Top Choice treated plots on day 7 (100%). However all insecticide treatments produced between 90-100% knockdown in 7-14 days. The Advion/Top Choice combination and Top Choice treatments had the longest lasting effect, suppressing foraging by 89-93% for 90 days.

3.4 Discussion

Results obtained from the year long field efficacy test indicated that the Advion/Top Choice combination treatment provided the most rapid control of fire ants and provided the greatest residual control of fire ants. In the 90 day knockdown evaluations the Advion/Top Choice combination provided the most complete and rapid results by day 7, causing foraging reductions of 100 percent. However, this reduction in foraging was not significantly different from that of the other chemical products on day 7 (75.6 - 95.9%).

The data provided in these studies support the hypothesis that broadcast fire ant control products would provide longer residual control that may slow the spread of colonies into untreated areas better than individual mound treatments (Williams 2001, Banks 1990). In both the year long, and 90 day tests Advion and Top Choice provided faster, longer lasting control than MaxForce mound treatments. Studies evaluating Advion conducted by Barr (2002a, 2002b) reported similar results. Similarly, studies evaluating Top Choice found that the product provided longer residual fire ant control than other products tested by Barr and Best (2004).

Barr (2002a, 2002b) conducted two tests, one in the summer and one in the fall, to evaluate the efficacy of indoxacarb to control RIFA colonies. Both tests were conducted at an airport located in Yoakum, Texas. In both tests, Barr compared the efficacies of three different concentrations of indoxacarb (0.025%, 0.05%, and 0.1%), Amdro® fire ant bait (0.73% hydramethylnon; Ambrands, Atlanta, GA), Extinguish® fire ant bait (0.5% s-methoprene; Wellmark International, Schaumburg, IL), and Talstar® 2G (0.2% bifenthrin; FMAC Professional Solutions, Philadelphia, PA) for fire ant control. Results from the summer test (Barr 2002a) indicated that the three indoxacarb formulations provided faster control of RIFA colonies than the other RIFA products tested. One week after treatment, the mean number of active mounds in all of the indoxacarb treated plots ranged from (0.25 – 1.25) while the mean number of active mounds in plots treated with Amdro was 4.0; Extinguish 16.25, and Talstar was 3.25. However, 6 weeks after treatment the number of active colonies in all the indoxacarb treated plots began to increase. The number of active mounds found in the other treatment plots also began to increase, however fewer active colonies were documented in Extinguish treatment plots. Mound density in plots treated with indoxacarb continued to increase for the remainder of the test. During the test, significant differences in the observed mean number of active mounds were not documented between the three indoxacarb treatments. Barr (2002b) replicated the test in the fall and the results were similar to the summer test, with the three indoxacarb formulations providing more rapid control than the other fire ant control products.

Barr and Best (2004) conducted a test to evaluate the efficacy of two granular formulations of fipronil (0.0143%; 0.00015% fipronil) Amdro Ant Bait (0.73% hydramethylnon, Ambrands, Atlanta, GA), and Talstar 2G (0.2% bifenthrin, FMC, Philadelphia, PA). Results presented by Barr and Best (2004) demonstrated that fipronil provided greater long-term control than the other fire ant control products. Five weeks after treatment, granular applications of 0.0143% fipronil caused a 83% - 98% decrease in the number of active mounds. By week 52 the number of active mounds began to rebound in all treatment plots except those treated with the granular 0.0143% fipronil.

Because the combination treatment was comprised of Advion (one of the fastest acting baits on the market) and Top Choice, (which provides long residual control) I expected the combination treatment to outperform the other products. However, the use of these products in all situations may not be ideal. Broadcast products are more expensive than IMTs costing $7-10 per hectare to apply. Treating mounds individually may cost 25 cents per mound (Drees et. al. 2006).

The overall result of this study shows that broadcast fire ant control products are more effective at providing greater control of fire ant colonies when compared to IMTs. Presently, VDACS manages fire ant infestations outside of Virginia’s fire ant quarantined areas while homeowners and pest control operators are responsible for treating infestations within quarantine borders. VDACS currently treats all fire ant mounds outside of the quarantine using an individual mound treatment. Given the evidence provided in this study, it is reasonable to assume that all active fire ant mounds in an area may not be visible and therefore an IMT may not provide adequate control. Therefore, the methods used by VDACS may not slow the spread of the ant in Virginia. Broadcast products are more expensive than products used as IMTs and because VDACS is a government agency, budget constraints may be issue. However, broadcast applications require very little labor. Thus, VDACS may actually save money on the man hours required to apply individual mound treatments. Additionally, these methods can be utilized by home owners and pest management professionals for fire ant control in the quarantined areas of Virginia.

Table 3.1 Least square means and mean percent change in the number of foraging fire ants before and after treatment application

	Day After Treatment (DAT)								
Treatment	DAT-0	DAT-3	DAT-90	DAT-120	DAT-240	DAT-270	DAT-300	DAT-330	DAT-360
Top Choice									
LS Mean (± SE)	1064.3	994.0^{a} (± 51.1)	38.2^{b} (± 108.7)	-3.3^{b} (± 56.7)	28.5^{c} (± 52.6)	8.7^{c} (± 63.8)	345.0^{bc} (± 101.2)	752.4^{b} (± 70.3)	972.8^{bc} (± 83.9)
Percent Change	-	(6.6)	(96.4)	(100.0)	(97.3)	(99.2)	(67.6)	(29.3)	(8.6)
Advion									
LS Mean (± SE)	916.7	188.1^{bc} (± 52.2)	355.5^{b} (± 111.1)	260.1^{ab} (± 58.0)	186.3^{bc} (± 53.7)	577.3^{b} (± 65.2)	510.2^{ab} (± 103.5)	734.3^{bc} (± 71.8)	777.7^{c} (± 85.8)
Percent Change	-	(79.5)	(61.2)	(71.6)	(79.7)	(37.0)	(44.3)	(19.9)	(15.2)
Max Force									
LS Mean (± SE)	1372.3	433.4^{b} (± 53.7)	995.6^{a} (± 114.3)	239.7^{ab} (± 59.6)	352.7^{ab} (± 55.3)	668.7^{b} (± 67.0)	723.6^{ab} (± 106.4)	1189.4^{a} (± 73.9)	1318.3^{ab} (± 88.2)
Percent Change	-	(68.4)	(27.5)	(82.5)	(74.3)	(51.3)	(47.3)	(13.3)	(3.9)
Advion/Top Choice Combination									
LS Mean (± SE)	944.0	63.3^{c} (± 51.9)	-24.4^{b} (± 110.4)	-15.2^{b} (± 57.6)	3.9^{c} (± 53.4)	-18.2^{c} (± 64.8)	78.0^{c} (± 102.8)	396.5^{c} (± 71.4)	596.2^{c} (± 85.2)
Percent Change	-	(82.7)	(100.0)	(100.0)	(99.6)	(100)	(91.7)	(58.0)	(36.8)
Untreated Control									
LS Mean (± SE)	1240.5	943.7^{a} (± 63.1)	1369.1^{a} (± 134.3)	519.6^{a} (± 70.0)	508.9^{a} (± 65.0)	1155.3^{a} (± 78.8)	1076.7^{a} (± 125.0)	1029.6^{ab} (± 86.8)	1257.8^{a} (± 103.7)
Percent Change	-	(23.9)	10.4	(58.1)	(59.0)	(6.9)	(13.2)	(17.0)	1.4
F	-	58.6	19.1	9.5	11.7	38.1	8.5	12.6	8.3
Df	-	13	13	13	13	13	13	13	13
P	-	<0.0001	0.0003	0.003	0.001	<0.0001	0.0046	0.0013	0.005

Means within a column followed by the same letter are not significantly different using the Tukeys HSD mean separation test (α = 0.05).

Table 3.2 Least square mean and mean percent change in the number of foraging fire ant worker ants before and after treatment reapplication.

	DAT (Days After Treatment)						
Treatment	DAT-0	DAT-3	DAT-7	DAT-14	DAT-30	DAT-60	DAT-90
Top Choice							
LS Mean (± SE)	972.8	809.0^{b} (± 64.4)	239.0^{b} (± 53.4)	83.0^{b} (± 46.0)	75.0^{b} (± 49.3)	68.3^{c} (± 46.7)	107.6^{c} (± 106.5)
Percent Change		(17.5)	(75.6)	(91.5)	(92.4)	(93.0)	(89.0)
Advion							
LS Mean (± SE)	777.7	149.1^{c} (± 70.0)	70.4^{b} (± 58.0)	-21.5^{b} (± 50.0)	39.2^{b} (± 53.5)	349.7^{b} (± 50.7)	441.4^{bc} (± 115.6)
Percent Change		(82.9)	(91.9)	(100)	(95.5)	(59.9)	(49.4)
Max Force							
LS Mean (± SE)	1318.3	264.2^{c} (± 97.7)	53.3^{b} (± 81.0)	187.0^{b} (± 69.7)	120.3^{b} (± 74.7)	429.7^{b} (± 70.7)	989.6^{ab} (± 161.4)
Percent Change		(79.6)	(95.9)	(85.5)	(90.7)	(66.8)	(23.5)
Advion/Top Choice Combination							
LS Mean (± SE)	596.2	90.2^{c} (± 106.7)	-18.5^{b} (± 88.5)	-85.1^{b} (± 76.1)	44.0^{b} (± 81.7)	116.5^{bc} (± 77.3)	44.3^{bc} (± 176.4)
Percent Change		(85.7)	(100)	(100)	(93.0)	(81.6)	(93.0)
Untreated Control							
LS Mean (± SE)	1257.8	1227.9^{a} (± 99.5)	1489.1^{a} (± 82.5)	1414.8^{a} (± 71.0)	1005.8^{a} (± 76.1)	1039.0^{a} (± 72.0)	1460.7^{a} (± 164.4)
Percent Change		(0.9)	20.1	14.1	(18.9)	(16.2)	17.8
F	-	36.7	79.5	91.3	45.5	59.0	20.3
Df	-	13	13	13	13	13	13
P	-	<0.0001	<0.0001	<0.0001	<0.001	<0.0001	0.0002

Means within a column followed by the same letter are not significantly different using the Tukeys HSD mean separation test (α = 0.05).

Figure 3.1. Advion (Adv), Top Choice (TC), MaxForce (MF), combination (Com) and untreated control (Con) plots located at Fun Junktion Park, Elizabeth City, NC (Google Earth 2010)

Figure 3.2. Advion (Adv), MaxForce (MF), and untreated control (Con) plots located at Fun Junktion Park, Elizabeth City, NC (Google Earth 2010)

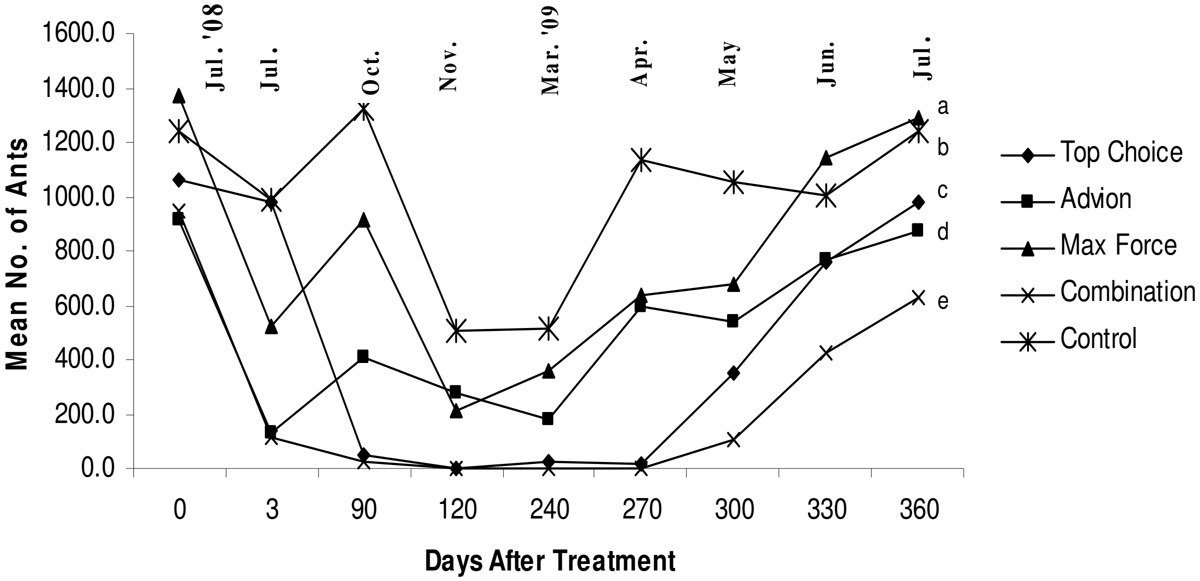

Figure 3.3 Mean number of foraging fire ants in experimental plots before and after product applications. Trend lines followed by the same letter are not significantly different ($\alpha = 0.05$).

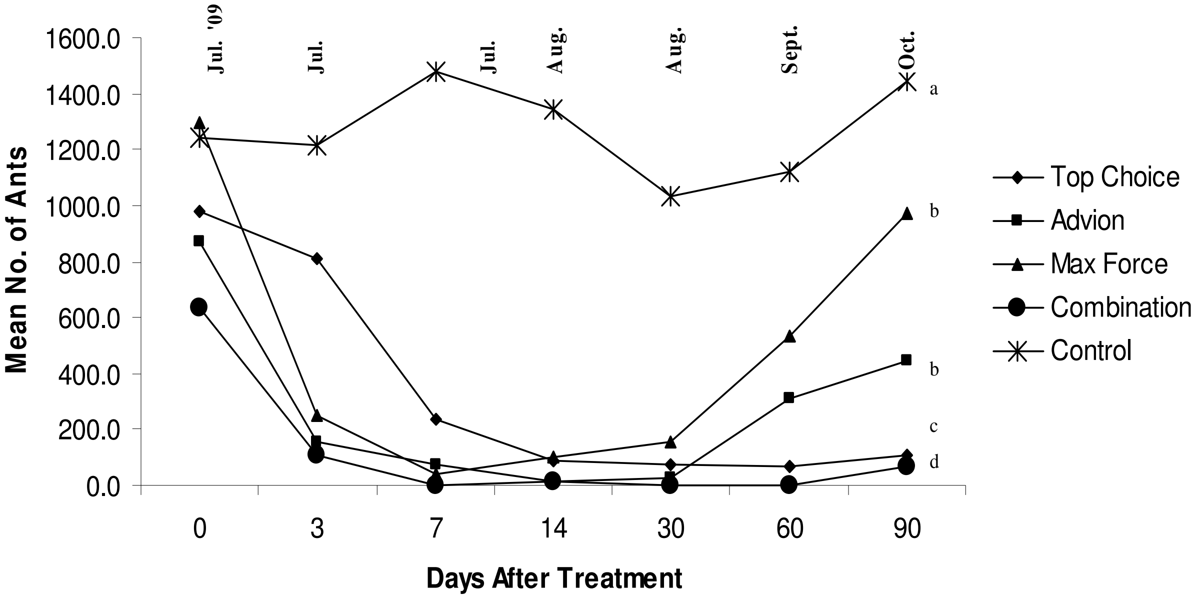

Figure 3.4 Mean number of foraging fire ants in experimental plots before and after product reapplications. Trend lines followed by the same letter are not significantly different ($\alpha = 0.05$).

Chapter 4 - Effects of Two Broadcast Fire Ant Control Products on Non-target Ant Species in Virginia

4.1 Introduction

The red imported fire ant (RIFA), *Solenopsis invicta* Buren, is a major agricultural and public health pest in the southeastern United States and in southern California. Because this ant is such a noxious pest, control options are extremely important. In the field, fire ant control products are applied either directly to individual mounds (individual mound treatment or IMT) or by broadcasting the products over a large area to target many different mounds at once. Both treatment methods are effective, but broadcast fire ant control products are relatively less expensive than IMTs and require less labor to apply (Furman and Gold, Hu and Song 2007).

Two of the most widely recommended broadcast fire ant control products are Advion® Fire Ant Bait (0.045% indoxacarb; DuPont, Wilmington, DE.) and Top Choice®, a granular formulation (0.0143% fipronil; Bayer Environmental Science, Research Triangle Park, NC). Advion provides quick control of fire ant colonies but long term control may not be achieved without re-application (Barr and Best 2004). However, the granular broadcast fire ant control product, Top Choice can provide long-term residual control of fire ants (Barr and Best 2004).

IMTs and broadcast treatment methods have both been used to treat fire ant infestations in Virginia. However, the use of broadcast fire ant control products in Virginia has been limited, due to VDACS' reliance on IMTs. However, the number of fire ant reports in Virginia continues to increase annually and the need for broadcast fire ant control products has become evident. Because Advion and Top Choice have performed well in other studies (Barr 2002, 2002, Barr and Best 2004, and Oi and Oi 2004) field trials were conducted in Virginia to compare the efficacy of Advion and Top Choice to an individual mound treatment control product (MaxForce

Fire Ant Bait, 1.0% hydramethylnon, MaxForce, Bayer Crop Science, Kansas City, MO) typically used by the Virginia Department of Agriculture and Consumer Services, Plant And Pest Division for RIFA control. Test results indicated that Advion provided faster control than the MaxForce IMT and Top Choice provided longer residual control in the field tests. While the product efficacy tests were focused solely on controlling fire ants, these products may also have had an impact on non-target ant species. Because these broadcast fire ant control products are applied over a large area, homeowners and pest control operators using these products may unknowingly cause mortality in non-target terrestrial arthropods particularly non-target ant species (Vogt et al. 2005).

The effects of fire ant control product on non-target species is a concern because reductions in non-target ant populations as a result of fire ant control product applications have was documented almost immediately after the development of the first fire ant bait, Mirex (1974). Markin et al. (1974) reported that Mirex applications caused population reductions in several non-target ant species including, *Solenopsis xyloni* (McCook), *Pogonomyrmex badius* (Latreille), and *Monomorium minimum* (Buckley) ant populations. Hydramethylnon, the second active ingredient registered for use in fire ant baits (Williams 2001), also caused population declines in non-target ant species (Edmonson 1982). Edmonson (1981) reported that hydramethylnon suppressed *Iridomyrmex pruinosum*, *Conomyrma spp*., and *Pheidole spp*. ant populations by 50-80% over a six month period after broadcast treatments. In addition, Vogt et al (2005) reported that after broadcast applications of Seige Pro (0.73% hydramethylnon, BASF Corporation, Research Triangle Park, NC) the number of *Monomorium minimum* worker ants collected in pitfall traps decreased significantly. Studies regarding the impacts of relatively new broadcast products like Advion on non-target ant species have not been conducted. Although, Ipser and

Gardner (2010) did evaluate the effects of broadcast applications of fipronil, they reported that the applications did not negatively affect native ant species in Georgia.

Fire ants are becoming an increasing problem in Virginia and the use of broadcast products to control fire ants could greatly assist control efforts in the state. However, these products may negatively affect native ant populations. Because Advion® and Top Choice® have never been evaluated in Virginia the potential impacts of these products on non-target ant species are unknown. The purpose of this study was to evaluate the effects of Advion® and Top Choice® on non-target ant species populations in Virginia.

4.2 Materials and Methods

Study Site

Research plots were established on the campus of the Hampton Roads Agricultural Research Extension Center (HRAREC) located in Virginia Beach, VA (Latitude 36.75° N; Longitude 76.05°W). Average monthly temperatures in Virginia Beach range from a low of 0.3–2.5°C to a high of 9.4 – 30.5°C depending on season. Hampton Roads receives 113 cm of rain annually with the majority of precipitation occurring in July and August (www.weather.com/weather/wxclimatology/monthly/USVA0797).

Research Plots

Eight 10 x 10 m (100 m^2) research plots (Figures 4.1 and 4.2) were established in open lawn areas on the HRAREC campus that were fire ant free. During the study, research plots were mowed weekly. All plots were covered in tall fescue grass (*Festuca urundimacea*) and several different weed species.

Treatment Products

The broadcast fire ant control products evaluated in the study were Advion® Fire Ant Bait (0.045% indoxacarb; DuPont, Wilmington, DE.) and Top Choice® Insecticide (0.0143% fipronil; Bayer Environmental Science, Research Triangle Park, NC). Both products were applied at the label rate (Advion: 1.68 kg/hectare, Top Choice: 209 kg/hectare) with Scotts Handy Green II hand spreaders (Scotts International B.V., Scotts Professional, Geldermalsen, The Netherlands). Plots were treated on August 6th 2007 between 5:00 p.m. and 6:00 p.m. Each product was applied to three randomly selected plots. Control plots (2) were left untreated.

Sampling Regimen

Prior to treatment applications sugar water baits were used to quantify ant species richness and foraging activity in the plots. Sugar water baits consisted of a cotton rope (5.6 mm Wellington Medium Load Braided Cotton Multi-Purpose Clothesline; Wellington Cordage, Madison, GA) cut into 4.3 cm segments. Cotton rope segments were saturated in a 20% sucrose solution (Kroger Granulated Sugar; Kroger Co., Cincinnati, OH). After soaking, excess sugar water was removed and an individual rope segment was transferred into an open glass vial (2 dram; Acme Glass and Vial Co., Paso Robles, CA). Pre-treatment bait counts were taken on 5 August 2007 between 5 and 6:30 p.m.

Each plot was divided into 9 subplots. Five vials with sugar water baits were placed in the center of 5 subplots, one in the center of each of the four corner subplots and one in the center subplot. The vials containing baits were collected after one hour. Upon collection, caps were placed on the vials to prevent any ants from escaping. Collected sample vials were transferred into boxes labeled with collection data (date of collection, name of treatment). Upon returning to the HRAREC, ethanol (90-95%) was added to each vial to kill and preserve ant samples. Ant samples were transported to the Dodson Urban Pest Management Laboratory in Blacksburg, Virginia where the collected ants were identified, and their numbers were recorded.

Post-treatment ant sampling with sugar water baits was conducted between 5:00 p.m. and 6:30 p.m. at 3, 7, 14, and 30 days after treatment, and every month thereafter for one year. Sampling was not conducted during the winter months (121-239 days after treatment) because temperatures were too low to support ant foraging.

Species Identification

Ant samples were identified to genus using dichotomous ant keys (Ross et al. 1971, Fisher and Cover 2007). Ant specimens were identified to species by Eric Day, Manager of Insect Identification Lab, Virginia Tech, Department of Entomology.

Statistical Analysis

The mean number of non-target foraging ants collected per treatment on each sampling date was calculated by adding the total number of foraging ants (5 sample vials) collected in each treatment plot and dividing it by number of plots per treatment. To determine if product treatment applications had any effect on the mean number of foraging ants, data were transformed $\{\sqrt{(x + 3/8)}\}$ (Zar 1984) and analyzed using repeated-measures multivariate analysis of variance (MANOVA) with post treatment date as the repeated measure. Repeated-measures MANOVA was also conducted to determine differences in product residual activity.

Differences in the mean number of ants collected in treatment plots on each post treatment sampling date were determined using repeated-measures ANCOVA with the mean number of ants collected on DAT-0 (day after treatment) as a covariate. Significant differences among treatment means at each post treatment sampling date were separated by Turkey's HSD test ($\alpha = 0.05$). LS Means produced in the ANCOVA were used to calculate percent change in the mean number of non-target foraging ants after treatment relative to the initial number of foraging ants collected on DAT-0 (Vickers 2001).

Species Specific Treatment Effects on Non-target Ant Foraging Activity

Treatment effects on all ant species collected were analyzed by repeated-measures multivariate analysis of variance (MANOVA) with post treatment date as the repeated measure to determine if there was a significant treatment and species interaction. Before analysis, foraging data were transformed $\{(\surd(x + {}^{3}\!/_{8}))$ (Zar 1984). Repeated-measures analysis of variance (MANOVA) was also conducted to determine treatment effects on the foraging of the three most abundant ant species collected, *Lasius neoniger*, *Monomorium minimum*, and *Pheidole megacephala*.

Treatment differences on the mean number of *Lasius neoniger*, *Monomorium minimum*, and *Pheidole megacephala* collected in treatment plots on each post treatment sampling date were determined using repeated-measures ANCOVA where the mean number of ants collected on DAT-0 (day after treatment) was a covariate for each individual species. Significant differences among treatment means on each post treatment sampling date were separated by Tukey's HSD test ($\alpha = 0.05$). LS Means produced in the ANCOVA were used to calculate percent change in the mean number of ants after treatment relative to the initial number of foraging ants collected on DAT-0 for each ant species (Vickers 2001). All statistical analyses were conducted using JMP Statistical Discovery Software (SAS Institute 2003).

4.3 Results

Sample Species Composition

Over the course of the study a total of 520 ant samples were collected. The ant samples contained 15,561 individual ants representing four different genera. The four genera represented were *Monomorium*, *Paratrechina*, *Pheidole*, and *Lasius*. Five different species were identified among the genera. Of the five ant species collected, *Pheidole megacephala* (Fabricus), *Lasius neoniger* (Emery), and *Monomorium minimum* (Buckley) were the most abundant.

Treatment Effects on the Mean Number of Non-Target Foraging Ants

Repeated-measures MANOVA was used to determine whether the product applications had any effect on the mean number of foraging ants in the plots. Results indicated that there was a significant overall treatment effect on the mean number of foraging ants ($df = 2, 21$, $F = 4.59$, $P = 0.02$). Contrast comparison tests indicated that there were significantly fewer foraging ants in Advion ($df = 1, 21$; $F = 7.23$, $P = 0.01$) and Top Choice ($df = 1, 21$; $F = 7.48$, $P = 0.01$) treated plots than in untreated control plots (Figure 4.3). However, the mean number of non-target ant foraging ants collected in the Advion and the Top Choice treatment plots were not significantly different (df = 1,21, $F = 0.0027$, $P > 0.05$) from one another (Figure 4.3).

The ANCOVA results, the LS means for the non-target foraging ants collected per treatment plot, and percent change from day 0 in the number of foraging ants after product application on each sampling date are shown in Table 4.1. Three days after treatment there were no significant differences in the mean number of foragers between treatments ($df = 3, 7$; $F = 3.61$, $P = 0.12$). Although post treatment foraging means were not significantly different between treatments on DAT-3, the mean number of foraging ants in Advion treatment plots had decreased by 53.3 percent while foraging in untreated control and Top Choice plots increased by 14.6 percent and 10.1 percent respectively. By DAT-7 foraging activity in plots treated with Advion and Top Choice had decreased by 67.7 percent and 46.7 percent respectively. However, the mean number of foraging ants collected in treatment plots was still not significantly different from those in the controls ($df = 3,7$; $F = 1.47$, $P = 0.35$). Significant differences in the mean number of non-target foraging ants were not observed between treatment and control plots until DAT-14 (*df* = 3, 7, *F* = 6.88, *P* = 0.05). The Tukey's HSD means separation test ($\alpha = 0.05$) results indicated that the mean number of foraging ants collected in Top Choice plots was significantly less than that of the controls but not significantly different from that of the Advion plots. However, on DAT-30, the mean number of non-target foraging ants collected in Advion and Top Choice treatment plots were both significantly less than in the untreated controls (*df* = 3,7; *F* = 17.75, *P* = 0.0009). After DAT-30, the mean number of foraging ants in the control and the treatment plots began to decrease. This overall reduction in ant foraging continued into DAT-120 (December). On DAT 120, the mean number of foraging ants in Advion, Top Choice, and untreated control plots were 100.0, 99.9, and 81.1 percent lower than pretreatment means respectively. These near complete reductions in ant foraging were attributed to reductions in ambient temperature. After DAT-120 post-treatment sampling was halted because ambient temperatures were too low to support non-target ant foraging (Holldobler and Wilson 1990).

Post treatment sampling resumed on DAT-240 (April 2008) and the mean number of foraging ants collected in the Top Choice treated plots was significantly lower than the mean number collected in the Advion or control plots (*df* 3, 7; *F* = 6.28, *P* = 0.05). After DAT-240 the mean number of foraging ants began to increase in all plots. This increasing trend continued for the remainder of the test and at the conclusion of the test (DAT-360), the mean numbers of foraging ants in Advion and Top Choice plots were nearing pre-treatment levels. The mean number of ants collected in control plots were 40.3 percent higher than pre-treatment numbers, both the Advion and the Top Choice treated plots had foraging ant numbers near pre-treatment levels.

Treatment Effects on Individual Ant Species

Repeated measures MANOVA was conducted to determine if the test products had any species specific effects on non-target ants. The repeated measures MANOVA results indicated that was not a significant treatment and species interaction (*df* = 4, 15; F = 0 .407, P = 0.8). The results suggested that the effects of the Advion and Top Choice applications did indeed suppressed foraging in *L. neoniger*, *M. minimum*, and *P. megacephala* populations, the overall trend was that the products produced similar results in all species.

The MANOVA results for *L. neoniger* indicated that the overall mean number of ants collected in the Advion and Top Choice treated plots, or the controls were not significantly different (*df* = 2, 5; F = 3.41, *P* = 0.11) (Fig. 4.4). The ANCOVA results, least square means, and the mean percent change in the mean number of *L. neoniger* workers before and after the treatments are presented in Table 4.2. Neither broadcast treatment appeared to have a significant effect on ant foraging for 30 days. However, the mean number of *L. neoniger* ants in Advion plots was lower than controls at DAT-60 (*df* = 7, 15; F = 7.10, *P* = 0.04). At DAT-120 (December) the number of foraging *L. neoniger* in the Top Choice and Advion plots was significantly different from the control plots (*df* = 7, 15; F = 1470.7, *P* < 0.05). After the winter months, *L. neoniger* foraging activity in Advion treated plots had rebounded completely (DAT-300). In fact, the mean number of foraging ants at DAT-300 was 24.2 percent higher than pretreatment levels. At the conclusion of the test on DAT-360, foraging activity of *L. neoniger* in Advion plots was 69.1 percent higher than it had been on DAT-0. These results suggest that Advion does suppress *L. neoniger* foraging but the ants return to pretreatment levels.

Unlike Advion, Top Choice suppressed *L. neoniger* foraging during the entire test period. Significant differences between the mean number of *L. neoniger* collected in Top Choice and control plots were observed at DAT-60 (*df* = 7, 15; F = 7.1, *P* = 0.04). While Top Choice appeared to suppress *L. neoniger* for the entire test period the % reduction was not always significantly different from that in the controls. However, foraging activity never completely rebounded in Top Choice plots and on DAT-360 *L. neoniger* foraging was still 67.9 percent less than it had been on DAT-0. These results suggest that Top Choice applications may cause severe population reductions in non-target species and these populations may not be able to recover one year after the initial application.

Analyses evaluating the product effect on *M. minimum* indicated that overall mean number of ants collected between treatments were significantly different from one another (df = 2, 5; *F* = 5.86, *P* = 0.05) (Figure 4.5). The mean contrast comparison tests also revealed that the mean number of ants collected in Advion (df = 1, 5; F = 6.04, P = 0.05) and Top Choice (df = 1, 5; F = 11.43, P = 0.01) treated plots during the test period were both significantly lower than that of the controls. However, the mean number of ants collected in the Advion and Top Choice treated plots were not significantly different from each other (*df* = 1, 5; F = 1.06, *P* = 0.35).

ANCOVA results, least square means, and the mean percent change in the mean number of foraging *M. minimum* worker ants before and after treatment are presented in Table 4.3. Three days after the product applications, the mean numbers of ants collected between treatments were not significantly different for any of the three treatments (*df* = 7, 15; F = 1.04, *P* = 0.46) (Table 4.3). However, Advion applications caused *M. minimum* foraging reductions of 99.4 percent at DAT-3 and 100% by DAT-30. The percent reduction of *M. minimum* collected in Advion treated plots was at or near 100% from DAT-90 through DAT-120. *M. minimum* continued to be suppressed significantly in the Advion plots through DAT-270 (*df* = 7, 15; *F* = 7.3, *P* = 0.04).

In the Top choice plots, the mean number of *M. minimum* collected had decreased by 100 percent by DAT-3 (Table 4.3). However, the number of foraging ants collected in the Top Choice plots was still not significantly less than those in the control plots at DAT-3. In fact, M. minimum were not significantly reduced in the Top Choice plots until 90 days after treatment (*df* = 7, 15; *F* = 8.4, *P* = 0.03). *M. minimum* populations in the Top Choice plots remained suppressed relative to the controls through DAT-270 when populations began to rebound.

MANOVA results for *P. megacephala* indicated that the broadcast products did have a significant effect on non-target ant foraging populations (*df* = 2, 5; *F* = 11.20, *P* = 0.01) (Fig. 4.6). Mean contrast comparison tests indicated that there were significantly fewer foraging *P. megacephala* ants over the test period in the Advion (*df* = 1, 5; F = 20.55, *P* = 0.006) and Top Choice (*df* = 1, 5; F = 14.53, *P* = 0.01) plots when compared with control plots. However, the mean number of ants collected in the Advion and Top Choice treated plots were not significantly different from each other (df = 1, 5; F = 0.65, P = 0.46).

ANCOVA results, least square means, and the mean percent change in the mean number of foraging *P. megacephala* worker ants before and after treatment are presented in Table 4.4. ANCOVA results show that there were not any significant mean differences between treatments on any particular post-treatment sampling date during the duration of the study.

Three days after treatment foraging in Advion plots had decreased by 69.7 and remained suppressed DAT-3, foraging in Advion plots for the remainder of the study.

In Top Choice treated plots, the foraging activity of *P. megacephala* foraging had been reduced by 87.4% on DAT-7. This trend was observed for the remainder of the test and post-treatment foraging means in Top Choice plots were consistently lower than pre-treatment means until DAT-330. On DAT-330, *P. megacephala* foraging in Top Choice treated plots was 5.5

percent higher than pre-treatment levels. Additionally, on DAT-360, *P. megacephala* foraging numbers had rebounded and the post-treatment mean on this day (103.6± 21.4) was 76.5 percent higher than the pre-treatment mean (58.7).

4.4 Discussion

Overall, both broadcast products did cause a decrease in the mean number of non-target ants in the treatment plots. Previous studies such as those conducted by Furman and Gold (2006) have shown that Advion can be used to control pest ant species other than RIFA but studies documenting the impact of Advion on non-target ant species have not been conducted. However, the effects of Top Choice on non-target ant activity observed in this study differ from those presented by Ipser and Gardner (2010), who conducted a similar study comparing the effects of several broadcast fire ant control products on non-target ants in Georgia. Ipser and Gardner (2010) conducted a year long study (June 2003 – January 2004), in which they collected seventeen different ground nesting ant species in plots treated with RIFA control products. They reported that there was no significant difference in the mean number of non-target ants collected in (0.0103%) fipronil treated (Over-n-Out™, Bayer Corp., Cary, NC), hydramethylnon treated (Amdro, EPA Reg. No. 73,342-1), and untreated control plots.

Because non-target ant foraging was initially suppressed in broadcast control plots and foraging increased in control plots, I attributed the foraging reductions to the broadcast treatments. However, in addition to foraging reductions due to broadcast treatments, foraging activity in untreated control plots had decreased by 11.2 percent and 49.8 percent on DAT-60 and 90 when compared to pre-treatment levels. Foraging declines observed on DAT-60 (October) may have been the result of colony movement but low temperatures may have contributed to the foraging reductions observed on DAT-90. Ant foraging behavior is highly dependent upon temperature (Holldobler and Wilson 1990) and low temperatures ambient temperatures may have contributed to the low number of foraging ants collected on post treatment day 90 (November) (13.3°C), 120 (December) (13°C), and 240 (April) (17.2°C). After the month of April, temperatures and the numbers of foraging ants began to increase but pre-treatment foraging levels were observed in control plots. Ultimately, these results show that broadcast applications of Advion and Top Choice may negatively affect non-target ant populations especially during the winter months, but populations will rebound at the onset of spring when temperatures begin to increase.

When determining whether there was a significant treatment effect on an individual ant species, MANOVA results indicated the relationship between treatment and species was not significant ($P = 0.08$). However, Advion provided the greatest decline of foraging activity three days after treatment as the mean number of *M. minimum* foraging workers had decreased by 99.4 percent (Table 4.3). *M. minimum* foraging remained suppressed until DAT-120. After DAT-120, foraging numbers began to rebound and by DAT-360 the number of active foragers was only 18.7 percent lower than pre-treatment numbers. Advion applications did not affect *P. megacephala* foraging activity as quickly as *M. minimum* populations were affected. However, after Advion treatments *P. megacephala* populations were not able to rebound. At the conclusion of the test, the mean number of *P. megacephala* foragers was still 82.9 percent lower than pre-treatment counts. These data indicate that Advion had a greater residual activity on *P. megacephala*. These results are not surprising because *P. megacephala* are listed on the Advion label as potential target ant species.

Similar to Advion, Top Choice applications reduced *M. minimum* foraging activity by 100 percent three days after treatment and remained suppressed for the remainder of the test.

However, pre-treatment means for *M. minimum* in Top Choice treatment plots were relatively low, therefore inflating reduction levels. Thus, I cannot assume that Top Choice applications had any significant effect on *M. minimum* populations. Foraging populations of *P. megacephala* were not reduced until DAT-7. Foraging populations remained reduced until DAT-330 when foraging began to rebound. Fourteen days after Top Choice applications, *L. neoniger* foraging populations had decreased by 91.8 percent. *L. neoniger* foraging remained suppressed for the duration of the test and by DAT-360 foraging levels were still 31.7 percent lower than pre-treatment levels. Furthermore, Top Choice applications suppressed *L. neoniger* faster and longer than the other two ant species.

This study was conducted at a location where imported fire ant colonies are absent. Previous studies have shown that fire ants can out compete native ant species for food, thus there is a possibility that results of this study may vary if conducted in the presence of active fire ant colonies (Porter and Savignano 1990, Vinson 1997). Because Top Choice has a granular formulation, results would probably be similar in subsequent tests. However, Advion is formulated as a bait and competitive interactions between fire ants other ant species for bait might limit exposure to the active ingredient lessening non-target mortality.

To provide a better assessment of the effect of these two products on non-target ant species in Virginia further studies need to be conducted in multiple locations including those infested with fire ants. However, the results of this study indicate that broadcast applications of Top Choice and Advion did suppress the foraging activity of non-target ant species in Virginia, but overall populations were able to rebound after a year.

Table 4.1. Least square mean and mean percent changes in non-target foraging ants before and after treatment application

	Day After Treatment (DAT)						
Treatment	DAT-0	DAT-3	DAT-7	DAT-14	DAT-30	DAT-60	DAT-90
Advion							
LS Mean (± SE)	281.7	131.6^{a} (± 58.0)	91.1^{a} (± 62.6)	170.9^{ab} (± 28.0)	150.1^{b} (± 22.0)	37.1^{b} (± 30.8)	50.8^{b} (± 11.0)
Percent Change		(53.3)	(67.6)	(39.3)	(46.7)	(86.8)	(82.0)
Top Choice							
LS Mean (± SE)	259.7	285.6^{a} (± 57.8)	138.3^{a}(± 62.3)	93.5^{b} (± 27.9)	54.6^{b} (± 21.9)	83.6^{ab} (± 30.6)	65.2^{ab} (± 11.0)
Percent Change		10.1	(46.7)	(63.9)	(78.9)	(67.8)	(74.8)
Untreated Control							
LS Mean (± SE)	244.5	280.1^{a} (± 71.0)	276.0^{a} (± 76.6)	266.8^{a}(± 34.3)	284^{a} (± 26.9)	219.9^{a} (± 37.6)	77.5^{a} (± 13.5)
Percent Change		14.6	12.9	9.1	16.2	(10.1)	(68.3)
F	-	3.61	1.47	6.88	17.75	4.94	6.63
Df	-	7	7	7	7	7	7
P	-	0.12	0.35	0.047	0.01	0.08	0.05

Means within a column followed by the same letter are not significantly different using the Tukeys HSD mean separation test (α = 0.05).

Table 4.1 (cont.) Least square mean and mean percent changes in non-target foraging ants before and after treatment application

	Days After Treatment (DAT)						
Treatment	DAT-0	DAT-120	DAT-240	DAT-270	DAT-300	DAT-330	DAT-360
Advion							
LS Mean (± SE)	281.7	-0.2^{b} (± 0.4)	64.5^{ab} (± 15.4)	41.1^{b} (± 21.0)	225.4^{a} (± 67.0)	180.9^{a} (±53.6)	203.7^{b} (±30.0)
Percent Change		(100)	(77.1)	(85.4)	(19.9)	(35.7)	(27.7)
Top Choice							
LS Mean (± SE)	259.7	0.1^{b} (± 0.4)	49.2^{b} (± 15.3)	34.6^{b} (± 21.0)	156.1^{a} (± 66.7)	199.6^{a} (±53.4)	239.8^{a} (±29.9)
Percent Change		(99.9)	(81.0)	(86.7)	(39.8)	(23.1)	(7.6)
Untreated Control							
LS Mean (± SE)	244.5	46.3^{a} (± 0.5)	149.9^{a} (± 18.9)	197.9^{a} (± 25.8)	342.2^{a} (± 82.0)	338.2^{a} (± 65.6)	343.1^{a} (± 36.8)
Percent Change		(81.1)	(38.7)	(19.1)	39.9	38.3	40.3
F	-	19.57	6.28	10.18	2.65	1.54	4.84
Df	-	7	7	7	7	7	7
P	-	0.0008	0.05	0.02	0.19	0.33	0.08

Means within a column followed by the same letter are not significantly different using the Tukeys HSD mean separation test ($\alpha = 0.05$).

Table 4.2. Least square mean and mean percent changes in *L. neoniger* non-target foraging ants before and after treatment application

	Day After Treatment (DAT)						
Treatment	DAT-0	DAT-3	DAT-7	DAT-14	DAT-30	DAT-60	DAT-90
Advion							
LS Mean (± SE)	79.3	116.0^{a} (± 61.3)	67.2^{a} (± 26.6)	151.3^{a} (± 35.8)	94.2^{a} (± 56.0)	42.0^{a} (±14.2)	48.5^{a} (± 8.3)
Percent Change		46.3	(15.3)	90.8	18.8	(47.0)	(38.8)
Top Choice							
LS Mean (± SE)	173.0	114.0^{a} (± 75.7)	91.3^{a} (± 32.8)	14.1^{a} (± 44.2)	39.0^{a} (± 69.1)	55.3^{a} (± 17.5)	55.6^{a} (± 10.2)
Percent Change		(34.1)	(47.2)	(91.8)	(77.4)	(68.0)	(67.9)
Untreated Control							
LS Mean (± SE)	188.0	105.4^{a} (± 64.4)	101.8^{a} (± 28.0)	142.8^{a} (± 37.6)	161.3^{a} (± 58.8)	101.0^{a} (± 14.9)	58.8^{a} (± 8.7)
Percent Change		(44.0)	(45.8)	(24.0)	(14.2)	(46.3)	(68.7)
F	-	0.89	5.60	2.89	0.72	7.10	0.29
Df	-	7	7	7	7	7	7
P	-	0.52	0.06	0.17	0.60	0.04	0.83

Means within a column followed by the same letter are not significantly different using the Tukeys HSD mean separation test (α = 0.05).

Table 4.2. (cont.) Least square mean and mean percent changes in *L. neoniger* non-target foraging ants before and after treatment application

	Days After Treatment (DAT)						
Treatment	DAT-0	DAT-120	DAT-240	DAT-270	DAT-300	DAT-330	DAT-360
Advion							
LS Mean (± SE)	79.3	35.9^{b} (± 0.4)	32.8^{a} (± 4.2)	37.3^{b} (± 17.5)	98.5^{a} (± 68.0)	68.5^{a} (± 32.0)	134.1^{a} (± 49.7)
Percent Change		(54.7)	(58.6)	(53.0)	24.2	(13.6)	69.1
Top Choice							
LS Mean (± SE)	173.0	-0.05^{b} (± 0.5)	32.5^{a} (± 5.2)	23.4^{b} (± 21.6)	81.0^{a} (± 84.0)	136.8^{a} (± 39.6)	118.1^{a} (± 61.4)
Percent Change		(100)	(81.2)	(86.5)	(53.2)	(20.9)	(31.7)
Untreated Control							
LS Mean (± SE)	188.0	0.1^{a} (± 0.4)	54.5^{a} (± 4.4)	144.5^{a} (± 18.4)	181.3^{a} (± 71.4)	214.5^{a} (± 33.7)	140.7^{a} (± 52.3)
Percent Change		(99.9)	(71.0)	(23.1)	(3.6)	14.1	(25.2)
F	-	1470.69	54.15	7.34	0.43	2.63	0.04
Df	-	7	7	7	7	7	7
P	-	<0.0001	0.001	0.04	0.74	0.19	0.99

Means within a column followed by the same letter are not significantly different using the Tukeys HSD mean separation test ($\alpha = 0.05$).

Table 4.3. Least square mean and mean percent changes in *M. minimum* non-target foraging ants before and after treatment application

	Day After Treatment (DAT)						
Treatment	DAT-0	DAT-3	DAT-7	DAT-14	DAT-30	DAT-60	DAT-90
Advion							
LS Mean (± SE)	80.3	0.5^{a} (± 7.7)	35.0^{a} (± 27.7)	10.0^{a} (± 26.5)	-1.9^{a} (± 21.7)	-0.07^{a} (± 7.5)	2.8^{b} (± 7.9)
Percent Change		(99.4)	(56.4)	(87.5)	(100)	(100)	(96.5)
Top Choice							
LS Mean (± SE)	28.0	-0.4^{a} (± 7.6)	6.7^{a} (± 27.5)	50.7^{a} (± 26.2)	20.5^{a} (± 21.5)	7.7^{a} (± 7.4)	9.7^{b} (± 7.8)
Percent Change		(100)	(76.1)	(81.1)	(26.8)	(72.5)	(65.3)
Untreated Control							
LS Mean (± SE)	43.5	18.4^{a} (± 9.2)	56.0^{a} (± 33.0)	56.4^{a} (± 31.6)	50.0^{a} (± 25.8)	39.0^{a} (± 9.0)	59.8^{a} (± 9.4)
Percent Change		(57.7)	28.7	29.6	14.9	(10.3)	37.5
F	-	1.04	0.52	0.71	0.78	4.05	8.40
Df	-	7	7	7	7	7	7
P	-	0.46	0.69	0.59	0.56	0.10	0.03

Means within a column followed by the same letter are not significantly different using the Tukeys HSD mean separation test ($\alpha = 0.05$).

Table 4.3. (cont.) Least square mean and mean percent changes in *M. minimum* non-target foraging ants before and after treatment application

	Days After Treatment (DAT)						
Treatment	DAT-0	DAT-120	DAT-240	DAT-270	DAT-300	DAT-330	DAT-360
Advion							
LS Mean (± SE)	80.3	0^{a} (± 0.0)	47.0^{a} (± 6.3)	0.7^{b} (± 3.5)	76.3^{a} (± 22.2)	70.0^{a} (± 10.1)	65.3^{a} (± 16.8)
Percent Change		(100)	(41.5)	(99.1)	(5.0)	(12.8)	(18.7)
Top Choice							
LS Mean (± SE)	28.0	0^{a} (± 0.0)	9.7^{b} (± 6.2)	12.4^{b} (±3.4)	34.0^{a} (± 22.0)	30.8^{a} (± 10.0)	12.4^{a} (± 16.6)
Percent Change		(100)	(65.4)	(55.7)	21.4	10.0	(55.7)
Untreated Control							
LS Mean (± SE)	43.5	0^{a} (± 0.0)	72.4^{a} (± 7.5)	42.8^{a} (± 3.4)	65.1^{a} (± 26.4)	32.3^{a} (± 12.0)	86.6^{a} (± 20.0)
Percent Change		(100)	66.4	(1.6)	49.6	(25.7)	99.1
F	-	-	54.15	7.34	0.43	2.63	0.04
Df	-	7	7	7	7	7	7
P	-	-	0.001	0.04	0.74	0.19	0.99

Means within a column followed by the same letter are not significantly different using the Tukeys HSD mean separation test (α = 0.05).

Table 4.4. Least square mean and mean percent changes in *P. megacephala* non-target foraging ants before and after treatment application

	Day After Treatment (DAT)						
Treatment	DAT-0	DAT-3	DAT-7	DAT-14	DAT-30	DAT-60	DAT-90
Advion							
LS Mean (± SE)	122.0	37.0^{a} (± 37.5)	19.4^{ab} (± 14.2)	6.1^{a} (± 15.4)	37.9^{a} (± 23.8)	11.8^{a} (± 11.8)	0.3^{a} (± 3.6)
Percent Change		(69.7)	(84.1)	(95.0)	(69.0)	(90.3)	(99.7)
Top Choice							
LS Mean (± SE)	58.7	157.3^{a} (± 34.1)	7.4^{b} (± 12.9)	2.5^{a} (± 14.0)	3.3^{a} (± 21.6)	13.0^{a} (± 10.8)	-0.1^{a} (± 3.3)
Percent Change		168.0	(87.4)	(95.7)	(94.4)	(77.8)	(100)
Untreated Control							
LS Mean (± SE)	13.0	145.6^{a} (± 45.3)	92.4^{a} (± 17.1)	65.7^{a} (± 18.7)	90.1^{a} (± 28.8)	66.3^{a} (± 14.3)	7.6^{a} (± 4.3)
Percent Change		1020.0	610.8	405.3	593.1	410.0	(41.5)
F	-	2.05^{a}	5.71	3.60	2.07	4.27	1.02
Df	-	7	7	7	7	7	7
P	-	0.25	0.06	0.12	0.25	0.10	0.47

Means within a column followed by the same letter are not significantly different using the Tukeys HSD mean separation test ($\alpha = 0.05$).

Table 4.4 (cont.) Least square mean and mean percent changes in *P. megacephala* non-target foraging ants before and after treatment application

	Days After Treatment (DAT)						
Treatment	DAT-0	DAT-120	DAT-240	DAT-270	DAT-300	DAT-330	DAT-360
Advion							
LS Mean (± SE)	122	0^{a} (± 0.0)	-0.4^{a} (± 4.7)	-0.5^{a} (± 5.8)	48.2^{a} (± 22.0)	13.3^{a} (± 17.3)	20.9^{a} (± 23.6)
Percent Change		(100)	(100)	(100)	(60.5)	(89.1)	(82.9)
Top Choice							
LS Mean (± SE)	58.7	0^{a} (± 0.0)	0.1^{a} (± 4.3)	0.1^{a} (± 5.3)	52.4^{a} (± 20.0)	61.9^{a} (± 15.7)	103.6^{a} (± 21.4)
Percent Change		(100)	(99.8)	(99.8)	(10.7)	5.5	76.5
Untreated Control							
LS Mean (± SE)	13.0	0^{a} (± 0.0)	11.0^{a} (± 5.7)	13.6^{a} (± 7.1)	82.6^{a} (± 26.5)	103.2^{a} (± 21.0)	130.3^{a} (± 28.5)
Percent Change)		(100)	(15.4)	4.6	535.4	693.8	902.3
F	-	-	1.02	1.02	1.81	5.02	5.00
Df	-	7	7	7	7	7	7
P	-	-	0.47	0.47	0.28	0.08	0.08

Means within a column followed by the same letter are not significantly different using the Tukeys HSD mean separation test (α = 0.05).

Figure 4.1 Advion (Adv), Top Choice (TC), and untreated control (Con) plots located at Hampton Roads Agricultural Research Extension Center, Virginia Beach,Va. (Google Earth 2010)

Figure 4.2 Additional Advion (Adv), Top Choice (TC), and untreated control (Con) plots located at Hampton Roads Agricultural Research Extension Center, Virginia Beach, Va. (Google Earth 2010)

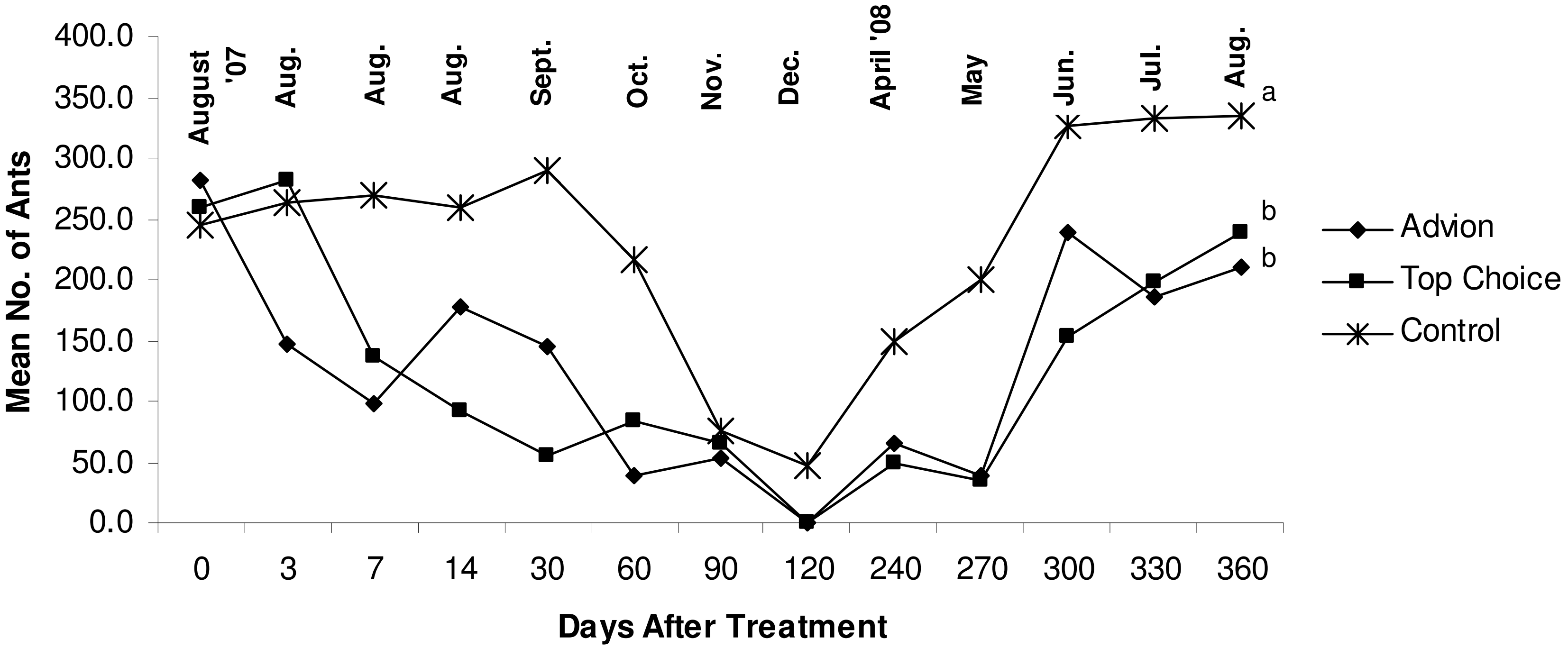

Fig. 4.3 Mean number of foraging ants collected in experimental plots before and after product applications. Trend lines followed by the same letter are not significantly different ($\alpha = 0.05$).

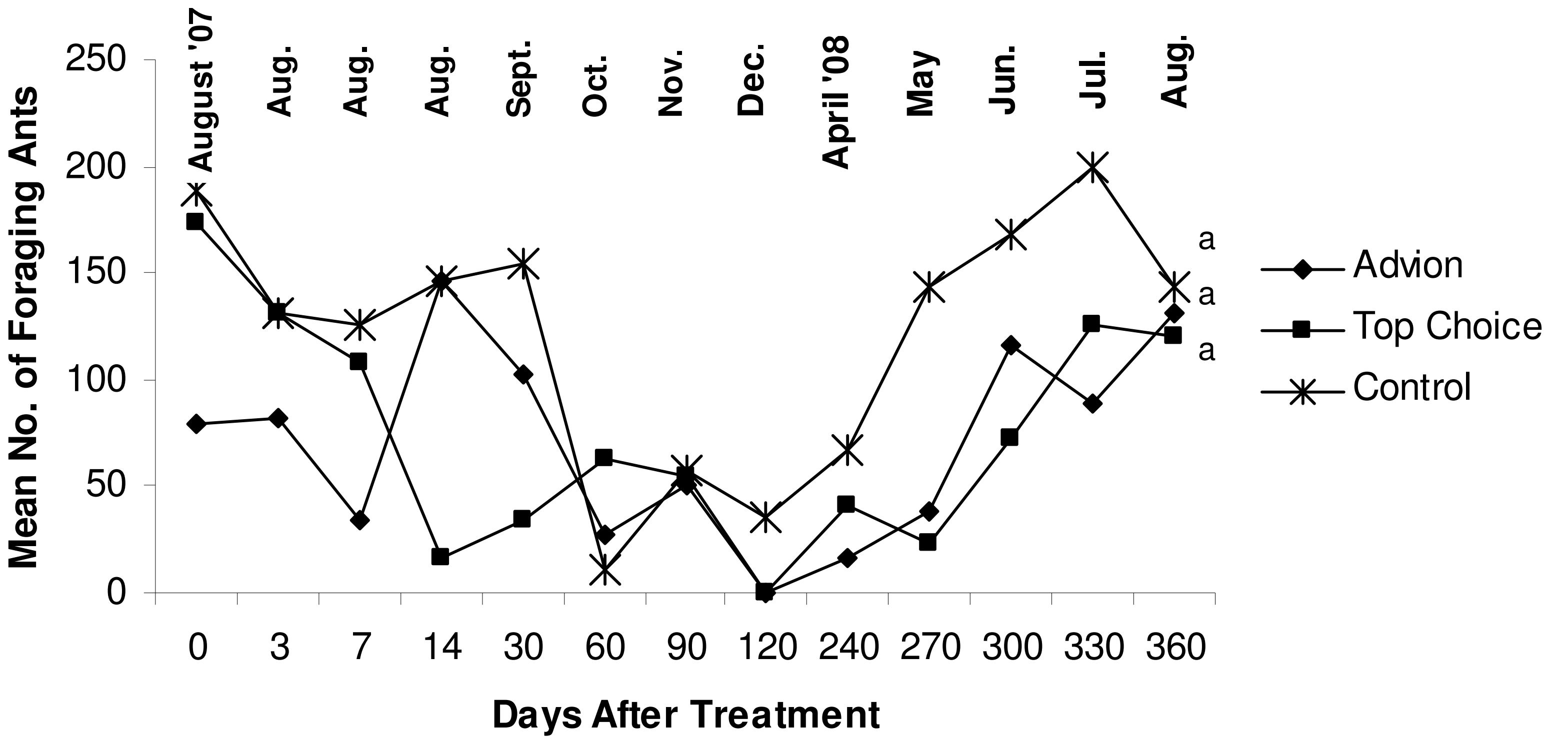

Figure 4.4. Mean number of foraging *L. neoniger* ants collected in experimental plots before and after product applications. Trend lines followed by the same letter are not significantly different (α = 0.05).

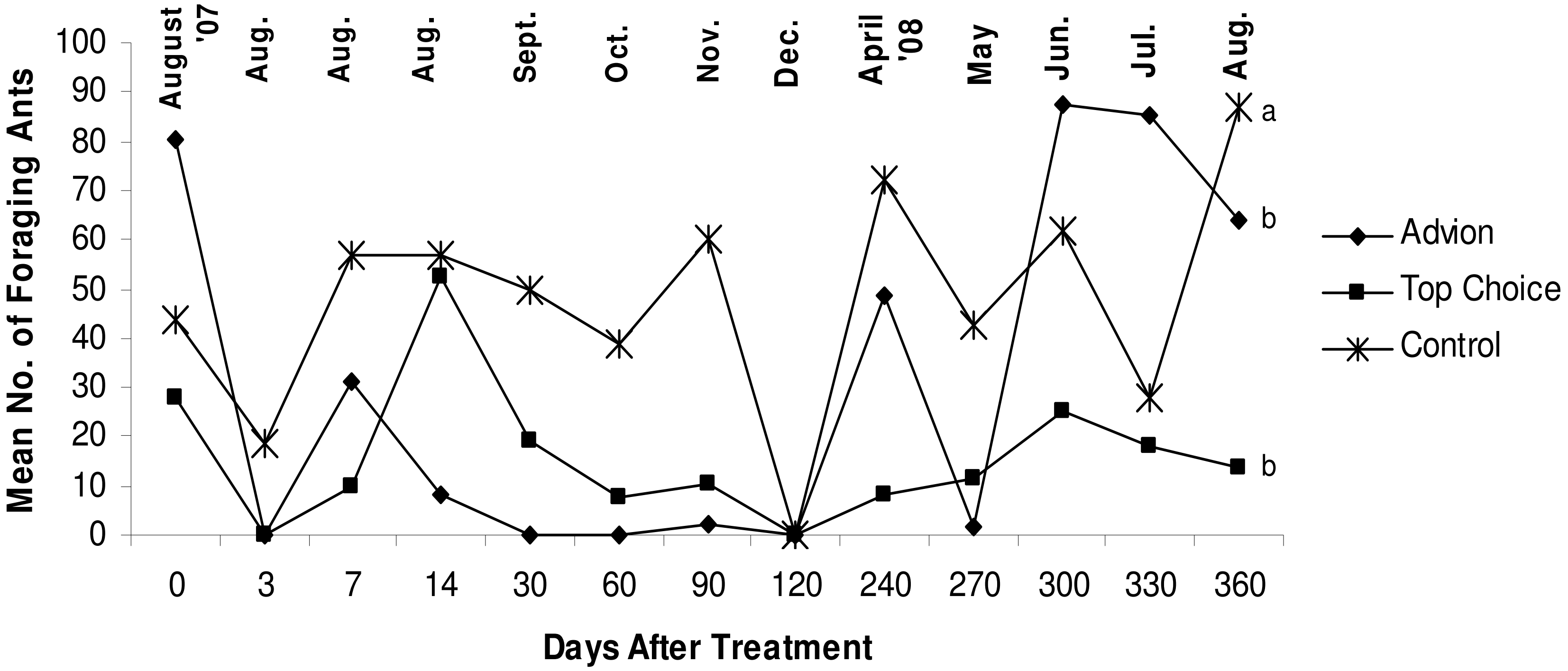

Figure 4.5 Mean number of foraging *M. minimum* ants collected in experimental plots before and after product applications. Trend lines followed by the same letter are not significantly different ($\alpha = 0.05$).

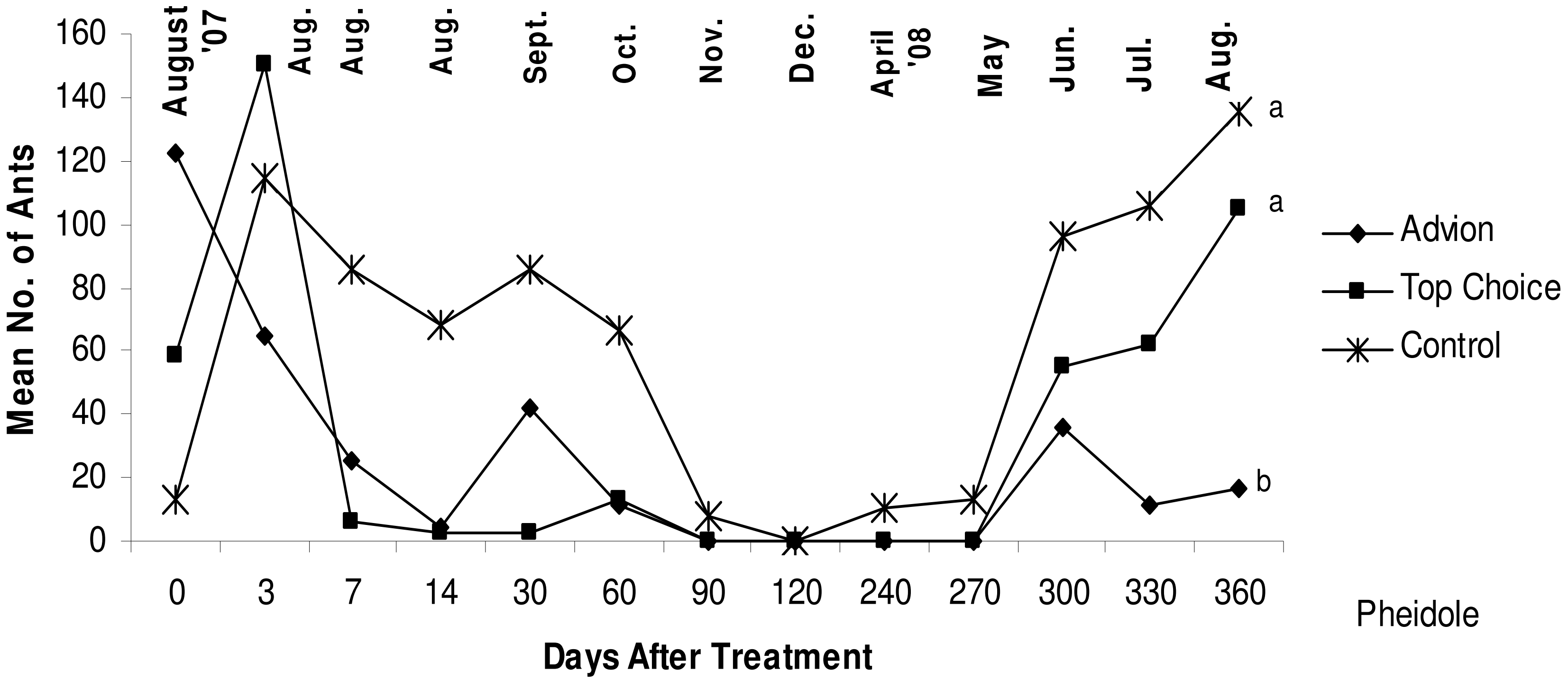

Figure 4.6 Mean number of foraging *P. megacephala* ants collected in experimental plots before and after product applications. Trend lines followed by the same letter are not significantly different (α = 0.05).

Chapter 5 - Characterization of *Solenopsis invicta* (Hymenoptera: Formicidae) populations in Virginia: Social form genotyping and pathogen/parasitoid detection

5.1 Introduction

Many social insect species have been inadvertently released by humans into non-natural habitats throughout the world. These invasive insects include different wasp, bee, and ant species (Moller 1996). Of these invasive insects, the red imported fire ant (RIFA) is one of the best studied (Vinson 1997, Williams et al. 2001, Tschinkel 2006). RIFA are native to South America but have been transported to such diverse locations as the United States, China, Taiwan, and Australia where they have been able to establish colonies (Vander Meer et al. 2007). In the U. S., fire ants have been extremely effective colonizers. Currently, RIFA colonies can be found in Alabama, Arkansas, Arizona, California, Florida, Georgia, Louisiana, Mississippi, New Mexico, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, and Puerto Rico (Kemp et al. 2000, Vander Meer et al. 2007). In fact, U.S. populations are known to have higher mound densities than fire ants living in their native habitat in South America (Porter et al. 1997).

The fact that RIFA mound density is greater in the United States than in their native habitat has been attributed to the absence of natural enemies and to the prevalence of polygyne fire ant colonies (Williams et al. 2003). Fire ant colonies exhibit one of two social forms. Colonies contain either one egg laying queen (monogyny) or multiple egg-laying queens (polygyny) (Glancey 1973). Monogyne and polygyne fire ant colonies differ in many aspects of their biology including their reproductive strategies, territoriality, and colony founding methods (Keller and Passera 1989, Keller and Ross 1999). Polygyne colonies are not intraspecifically territorial and usually establish new colonies that are close in proximity to one another (Tschinkel 2006). As a result, polygyne fire ant colonies have a higher mound density in a given area than monogyne colonies (Tschinkel 2006). These wide-spread, interconnected colonies have a greater influence on the local ecology and the multiple colonies have a greater influence on the local ecology than single queen colonies (King and Tschinkel 2009).

The spread of RIFA colonies in the U.S. have been facilitated by the lack of natural controls that occur in the RIFAs native South American habitat (Williams et al. 2003, Vander Meer et al. 2007). With no natural enemies or competitors, chemical control methods have been the only means available for *S. invicta* control (Williams et al. 2001). However, two biological control agents for RIFA have been released by the USDA in hopes of reducing the proliferation of this pest. These biological control agents in the U.S include the microsporidian parasite, *Kneallhazia solenopsae,* and fire ant decapitating flies found in the genus *Pseudacteon* (Pereira 2003).

K. solenopsae is a microsporidian parasite that is vertically transferred from queen fire ants to her offspring, infecting all castes and life stages (Sokolova and Fuxa 2008) of *S. invicta*. Fire ant queens infected with *K. solenopsae* have a reduced body weight, are less fecund, and have higher mortality than healthy queens. The life expectancy of worker ants also decreases with *K. solenopsae* infection (Valles et al. 2009). In addition to vertical transmission, *K. solenopsae* can be artificially transferred to RIFA colonies (Williams et al. 1999). Williams et al. (1999) inoculated laboratory RIFA colonies with *K. solenopsae* infected eggs, larvae, and pupae. Six months after inoculation the amount of live brood in infected colonies was on average 88% less than that of control colonies. Additionally, Williams et al. (1999) were also able to inoculate field colonies with *K. solenopsae* but post-treatment infection rates were not documented.

Phorid flies in the genus *Pseudacteon* were the first organisms released in U.S for fire ant control. Parasitization occurs when a female fly deposits a single egg inside the thoracic cavity of a worker ant. The developing larva then migrates to the ant's head consumes the contents inside the ants' head capsule, causing it to fall off. The larva completes its development inside the empty head capsule. In addition to causing fire ant death, laboratory studies have confirmed that *Pseudacteon spp.* flies have a negative impact on RIFA behavior, for example RIFA decrease their foraging activity in the presence of the *Pseudacteon* flies (Morrison 2000). However, field studies evaluating the effects of fire ant decapitating flies on foraging RIFA over large spatial and temporal scales have been less conclusive (Morrison and Porter 2005).

Since 1995, three phorid fly species including *Pseudacteon tricuspis*, *P. litoralis*, and *P. curvatus* have been successfully released in the U.S. These phorid fly species were released because each is specialized to parasitize specific size worker ants. For example, *P. tricuspis* parasitizes medium size worker ants while *P. curvatus* attacks small worker ants. *P. litoralis* parasitizes medium size-medium/large worker ants (Porter and Gilbert 2005).

In addition to the microsporidan parasite and phorid flies, three different *Solenopsis invicta* viruses (SINV-1, SINV-2, and SINV-3) have been discovered in U. S. fire ant populations (Valles et al. 2009). The pathogens are positive strand RNA viruses that can cause colony collapse and death. However, laboratory test results documenting the effect of each virus on fire ant colonies have differed between the three viruses (Valles et al. 2009). In laboratory tests the three viruses were responsible for colony mortality. However, before death worker ants collected from infected colonies did not display any identifiable disease symptoms.

Fire ant infestations have been observed throughout the southeastern U.S. since the 1930's, but these aggressive ants were not documented in the state of Virginia until 1989 (Gina

Goodwyn, Virginia Department of Agriculture and Consumer Services, personal communication). Since 1989, the number of fire ant-infested areas and mounds documented by VDACS has increased annually. From 1989 to 1999, VDACS identified 136 sites that were infested with *S. invicta*. From 2000-2006 the number of infested sites increased to 541. The largest number of mounds identified and treated by VDACS has been located in the expanding coastal cities of Chesapeake (3,000 mounds) Norfolk (1,700 mounds), and Virginia Beach (2,400 mounds) (Gina Goodwyn, Virginia Department of Agriculture and Consumer Services, personal communication), which are all cities located in the Hampton Roads area.

As of 2009, *S. invicta* populations in Virginia were not as widespread as infestations observed in other southern states such as Texas and Florida, however several predictive models have suggested that fire ants can certainly become established within the Virginia cities and counties immediately outside of the Hampton Roads area (Korzukhin et al. 2001, Morrison et. al 2004). In 2009, the cities of Chesapeake, Hampton, Newport News, Norfolk, Poquoson, Portsmouth, Suffolk, Virginia Beach, and Williamsburg and the counties of James City and York were placed temporarily under the Federal Red Imported Fire Ant Quarantine which aims to restrict the movement of fire ants from the quarantined areas to non-quarantined areas in the state. As a result of the quarantine's implementation, VDACS is no longer responsible for treating fire ant mounds in the quarantined cities and counties. The quarantine implementation leaves current infestations within the quarantine area solely in the hands of homeowners and pest management professionals who may not have any experience with fire ants. Because Virginians know so little about RIFA that there is an obvious need to determine the ants' potential to spread and if there are any biological control organisms in Virginia colonies that may limit their proliferation in Virginia.

The purpose of this study was twofold, I intended to assess the prevalence of monogyne and polygyne fire ant colonies in Virginia. My second goal was to determine if any biological control agents were present in Virginia *S. invicta* colonies.

5.2 Materials and Methods

Fire Ant Collection

Red imported fire ant workers (*Solenopsis invicta* Buren) were collected from 26 different colonies in seven different cities (Norfolk, Virginia Beach, Chesapeake, Portsmouth, Suffolk, Roanoke, and Richmond) within in the state of Virginia (2007-2008) (Table 5.1). Individual ant workers were collected by agitating the fire ant mound with a stick and removing the emerging workers using a hand-held aspirator (BioQuip, Rancho Dominguez, CA). All ant samples were transferred to glass vials (Acme Glass and Vial Co., Paso Robles, CA) containing 90-95% ethanol. Vials were labeled with the date of collection and location information.

DNA Preparation to Determine Social Form and the Potential Presence of *K. solenopsae* and *Pseudacteon spp*. Phorid Flies

Genomic DNA was extracted from the ants as described by Valles *et al*. (2002). Ten worker ants from each sample location were removed from the vial and blotted dry with a clean paper towel to remove ethanol. The dried ants (10 per sample) were placed into separate 1.5 ml microcentrifuge tubes (Eppendorf, Westbury, NY) containing 150 µl of lysis buffer (50 mM Tris-HCl, pH 8, 4% sodium dodecyl sulfate, and 5% 2-mercaptoethanol). Ants were homogenized by hand with a plastic pestle for 15-20 s. After homogenization, 200 µl of phenol:chloroform:isoamyl alcohol (Tris-HCl-saturated, pH 8) was added to the microcentrifuge tube. The microcentrifuge tube was inverted 4-5 times until the solution became milky white. The samples were then centrifuged for 5 min at 20,817*g* in a 5417C centrifuge (Eppendorf, Westbury, NY). Fifty microliters of the DNA-containing layer (supernatant) was removed from the microcentrifuge tube with a micropipette and transferred to a new 1.5 ml microcentrifuge tube (Eppendorf, Westbury, NY). DNA was precipitated by adding 900 µl of 100% isopropanol to the microcentrifuge tube. The mixture was inverted five times and centrifuged for 5 minutes at 21,000 *g*. The isopropanol was decanted from the microcentrifuge tube. The pellet was washed twice with 500 µl of 70% ethanol and centrifuged for 5 min at 20,817*g*. The ethanol was decanted and the centrifuge tubes were pulsed in a 5415D centrifuge for 4 seconds (Eppendorf, Westbury, NY). The remaining ethanol was removed by micropipette. Pellets were allowed to dry in an incubator set at 37°C for 5 minutes. Pellets were resuspended and dissolved in 50 µl of TE buffer (10mM Tris-HCl, 1 mM EDTA, pH 8.0). The purified DNA was analyzed spectrophotometrically to determine the concentration. All samples were diluted with TE buffer to achieve concentrations in the range of 50 to 100 ng DNA/µl.

RNA Preparation for SINV Detection

Total RNA was isolated from 10 fire ant workers from each sample. Fire ant workers were again blotted dry with a paper towel and placed in a 1.5 ml microcentrifuge tube. The ants were homogenized with a plastic pestle in 0.5 ml of Trizol reagent (Invitrogen, Carlsbad, CA). Chloroform (0.2 ml) was added to the homogenate which was vortexed briefly and centrifuged at 20,817 g for 5 min. at room temperature. The supernatant was transferred to a clean 1.5 ml microcentrifuge tube and 0.5 ml of isopropanol was added. The RNA pellet was rinsed once with 70% ethanol, dried, and suspended in 20 µl of DEPC-treated water. The RNA concentration was measured spectrophotometrically and diluted with DEPC-treated water to a concentration between 10 and 50 ng RNA/µl.

Polymerase Chain Reaction to Determine Colony Social Form

Multiplex PCR (polymerase chain reaction) was carried out in a PTC 100 thermal cycler (MJ Research, Waltham, MA) as described by Valles and Porter (2003). The *Gp-9*^{B} allele primers, 16BAS and 26BS were used to specifically identify samples from monogyne colonies. The *Gp-9*^{b} allele specific primers, 24bS and 25bAS were used to identify polygyne samples. The *Gp-9*^{B} alleles corresponded to positions 2167-2199 on the 16BAS primer and 1683-1703 on the 26BS primer. The *Gp-9*^{b} allele primers correspond to positions 1307-1334 on the 24bS primer and 1702-1729 on the 25bAS primer. The PCR process was conducted in a 50 µl volume containing 0.5 µl of prepared DNA (25 to 50 ng), 5 µl PCR buffer (10X), 2 µl 50 mM $MgCl_2$, 1 µl 10mM dNTP, 0.4 µl Platinum Taq polymerase (Invitrogen, Carlsbad, CA), 33.1 µl of H_20, and 2 µl of each primer (P16, P24, P25, and P26). The PCR process was conducted under the following temperature conditions: 1 cycle at 94°C for 2 min, then 35 cycles at 94°C for 15 sec, 55 °C for 15 sec, and 68 °C for 30 sec, and a single elongation step at 68 °C for 5 min. PCR products (17 µl) were separated on a 1% agarose gel and visualized by ethidium bromide staining. Positive and negative controls were run for all treatments.

K. solenopsae **and** ***Pseudacteon spp.*** **Phorid Fly Detection**

Oligonucleotide primers specific to the 16S rDNA gene (Moser *et al.,* 1998; Moser *et al.,* 2000; Valles et al. 2002; accession number: AF031538) of *K. solenopsae* (P1: 5'CGAAGCATGAAAGCGGAGC and P2: 5'CAGCATGTATATGCACTACTGGAGC) and the 18S rDNA gene of *Pseudacteon* flies (P800: 5'GTAGTACACCTATACATTGGGTTCGTACATTACTCTA and P801: 5'ATAAGTTTCAACGCTATAATCCTGAAAGCATC) were used in a multiplexed PCR to detect these parasites in the ant samples. Multiplex PCR was conducted by the hot start method in a PTC 100 thermal cycler (MJ Research, Waltham, MA) under the following optimized temperature regime: 1 cycle at 94° C for 2 min, then 35 cycles at 94° C for 15 sec, 55° C for 15 sec, and 68° C for 50 sec, followed by a final elongation step of 5 min at 68° C (Valles et al. 2009). The reactions were conducted in a 25 µl volume containing 2 mM $MgCl_2$, 200 µM dNTP mix, 0.5 units of Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, CA), 0.4 µM of primers P1 and P2, 0.05 µM of primers P800 and P801, and 25 to 50 ng of genomic DNA. PCR products were separated on a 1% agarose gel and visualized by ethidium bromide staining. For all experiments, positive and negative controls were conducted simultaneously.

Verification of *Pseudacteon* DNA Amplification

To verify that the corresponding *Pseudacteon* amplicon was produced in the PCR, the amplicon was cloned and sequenced. The agarose gel-purified amplicon was ligated into pCR4-TOPO vector, transformed into TOP10 competent cells (Invitrogen, Carlsbad, CA), and sequenced by the Interdisciplinary Center for Biotechnology Research (University of Florida). The sequences were subjected to BLASTn analysis (Altschul et al. 1997) then aligned with identified sequences with the Vector NTI software suite (Invitrogen, Carlsbad, CA).

Detection of *Solenopsis invicta* Viruses

One-step reverse transcriptase polymerase chain reaction (RT-PCR) was used to identify three different *Solenopsis invicta* viruses (SINV-1, SINV-2, SINV-3) in fire ant worker ants. cDNA was synthesized and subsequently amplified using the One-Step RT-PCR kit (Invitrogen, Carlsbad, CA) with oligonucleotide primers p517 (5'CAATAGGCACCAACGTATATAGTAGAGATTGGA) and p519 (5'GGAATGGGTCATCATATAGAAGAATTG) to detect SINV-1 (Hashimoto et al. 2007). Primers p64 (5'ATTTGTTTTGGCCACGGTCAACA) and p65

(5'GATGATACAAAGCATTAGCGTAGGTAAACG) were used to detect SINV-2 (Valles et al. 2007). Primers p812 (5’AATATCAGCATATTGATGATCCAAAATGCCAA) and p813 (5’AAGAGAACGTATGCCTACTCCATCAGAAGCAT) were used to detect SINV-3 (Valles et al. 2009). RT-PCR was conducted in a PTC 100 thermal cycler (MJ Research, Waltham, MA) under the following optimized temperature regime: 1 cycle at 45° C for 30 min, 1 cycle at 94° C for 2 min, 35 cycles of 94° C for 15 sec, 56° C for 15 sec, 68° C for 30 sec, followed by a final elongation step of 68° C for 5 min. DNA fragments were separated by gel electrophoresis on a 1.2% agarose gel and visualized by ethidium bromide staining.

5.3 Results

Colony Social Form

Both monogyne and polygyne fire ant colonies were positively identified from the Virginia ant samples (Table 5.2). Among the 26 fire ant colonies sampled, twenty (76.9%) were heterozygous at the *Gp-9* ($Gp\text{-}9^{Bb}$) locus indicating that the ants were of the polygyne form (Table 5.2). The remaining six colonies were homozygous ($Gp\text{-}9^{BB}$), indicating that they were monogyne.

Detection of *Kneallhazia solenopsae* in *S. invicta* colonies

Multiplexed PCR products for *K. solenopsae* are shown in Figure 5.1. PCR products for *K. solenopsae* infected ant colonies produced a 318 bp amplicon. Of the twenty-six ant colony samples colonies, eleven (42%) were infected with *K. solenopsae* (Table 5.3). Among the 11 colonies infected with *K. solenopsae*, eight (73%) were polygyne and three (27%) were monogyne.

Detection of Decapitating Phorid Flies in *S. invicta* Colonies

Multiplexed PCR products for *Pseudacteon spp. phorid flies* are shown in Figure 5.1. PCR products for worker ants parasitized by *Pseudacteon spp*. phorid flies produced a 800 bp amplicon (Figure 5.1). Two of the twenty-six fire ant colony samples were found to have worker ants parasitized by phorid flies. Phorid fly genetic material was found in ant samples collected from two different locations: Chesapeake and Virginia Beach (Table 5.3). One of the *Pseudacteon*-parasitized colonies was polygyne and one was monogyne. The *P. tricuspis* 18S gene was sequenced in an effort to determine which *Pseudacteon* species was discovered in the two Virginia colonies. Unfortunately, definitive species identification could not be determined (Table 5.4) because the sequences for *P. litoralis* and *P. obtusus* are identical to *P. tricuspis* in the sequenced region. However, sequence differences were sufficient to exclude *P. culatus* and *P. curvatus*. Thus, the Virginia fire ant samples were either *P. litoralis*, *P. obtusus*, or *P. tricuspis*. Each of these three species has been released in the United States as RIFA biological control agents.

Detection of *Solenopsis invicta* Viruses

SINV-1 was detected in five of the twenty-six (19%) fire ant colony samples and SINV-3 was detected in one of the twenty-six samples (3.8%) (Table 5.5). Multiplexed PCR products for SINV infected fire ant workers are shown in Figure 5.2. PCR products for SINV-1 infected workers produced a 481 bp amplicon and SINV-3 infected workers produced a 259 bp amplicon. SINV-2 was not detected in any of the colonies.

5.4 Discussion

Twenty-three of our twenty-six ant colony samples were collected from colonies located in the Hampton Roads area of Virginia (Table 5.2). According to data provided by Virginia's

Department of Agriculture and Consumer Services, population and mound densities in Hampton Roads are higher than those observed in other areas of the state. Hampton Roads is located on the southeastern coast of Virginia near the North Carolina border which contains several fire ant infested counties. Thus, the potential for repeated introductions of the ant through North Carolina into other areas of Virginia is high.

Both polygyne and monogyne fire ant colonies are present in Virginia and are established within the state. Although both social forms were collected, sample data suggest that the majority of *S. invicta* colonies in Virginia would most likely be polygyne. This finding is particularly relevant to control efforts because polygyne colonies tend to be associated with higher mound and population densities (Macom and Porter 1996). This higher mound density is typically the result of colony budding rather than annual mating flights which are typical of monogyne colonies. The process of budding is a more rapid means of colony expansion. During the budding process, workers from an established colony leave their original nest with one or more fecund queens: this group will found a new colony several meters away from the parent colony (Holldobler and Wilson 1990, Tschinkel 2006). Budding fire ant colonies are able to multiply quickly and one mound has the potential to split off into as many as eight new mounds in less than five months (Vargo and Porter 1989).

The discovery of polygyne colonies in Virginia has particular relevance to current ,as well as future, *S. invicta* management practices (King et al. 2008). At present, some of the more successful fire ant control products are not yet labeled for use in Virginia. Also, with the recent implementation of the Federal Fire Ant Quarantine non-chemical control measures will be needed including biological control agents. Of the eleven Virginia colonies infected with *K. solenopsae*, three were monogyne. This finding was of particular interest because *K. solenopsae* infections of monogyne colonies are considered rare among North American colonies (Oi et al. 2004). However, Milks et al. (2007) sampled 1,318 Louisiana fire ant colonies for *K. solenopsae* and reported that 9.9% of the sampled colonies were infected with the parasite. Furthermore, 20% of the infected colonies in Louisiana were monogyne.

Pseudacteon flies were detected in two of the Virginia fire ant samples (Table 5.3). This discovery was interesting because the nearest release site for *Pseudacteon* flies was approximately 100 miles away from the sampled region on the east coast of North Carolina (S.D. Porter, USDA-CMAVE, Gainesville, Florida, personal communication). Identification of this fly in Virginia suggests that the parasitoid *Pseudacteon* flies may have been transported to Virginia from states in the southeast where these flies have been released or that the flies could be spreading naturally in the U.S. Several different fly species including *P. curvatus*, *P. litoralis*, *P. obtusus*, and *P. tricuspis* have been released in the U.S. Attempts were made to differentiate between the two positive finds in the Virginia ant colonies using genetic sequencing (Table 5.4). However, sequence data for the 18S rDNA gene could only exclude *P. cutelatus* (which has not been released as a biological control agent) and *P. curvatus*.

Results presented in this study suggest that biological control organisms (*Pseudacteon* flies, *K. solenopsae*, and SINV) are present in Virginia RIFA colonies. Because fire ant infestations in Virginia are not that widespread and chemical control options are limited within the state, the presence of these organisms may be an indicator of the need to design a RIFA integrated pest management program in Virginia that is similar to the USDA Areawide Fire Ant Suppression Program. The preliminary results of the USDA Areawide Fire Ant Suppression Program have demonstrated that *K. solenopsae* and fire ant decapitating *Pseudacteon spp.* phorid flies are established in all of their release locations (Florida, Mississippi, Oklahoma, South

Carolina, and Texas) and have reduced *S. invicta* populations by 85-99% (Vander Meer et al. 2007) in those areas.

Two RIFA colonies located in Chesapeake and three colonies located in Virginia Beach were infected with SINV. SINV-1 was detected in five of the colonies, and SINV-3 was detected in one Chesapeake colony. Although SINV was detected in several Virginia fire ant colonies, the impacts of these viruses on active colonies outside of the laboratory are unknown (Valles et al. 2009).

This study is the first to document the establishment, social form, and associated pathogens and parasites of *S. invicta* within the state of Virginia. Monogyne and polygyne RIFA colonies are present in Virginia with the greatest percentage being the polygyne form. Many colonies are also infected with *K. solenopsae*, *Pseudacteon* flies, or SINV. Most of the Virginia fire ant samples were collected from a single region, the Hampton Roads area. However, fire ant infestations may be more widespread within the state. Therefore, a larger scale sampling project would be beneficial to further characterize fire ant populations in Virginia.

Table 5.1. Specific locations of *S. invicta* colonies sampled in Virginia.

Sample No.	Location	UTM
1	Chesapeake	Zone 18: 384981E, 4064800N
2	Chesapeake	Zone 18: 391663E, 4067337N
3	Roanoke	Zone 17: 591947E, 4129615N
4	Richmond	Zone 18: 285178E, 4157227N
5	Chesapeake	Zone 18: 392529E, 4070161N
6	Suffolk	Zone 18: 343340E, 4052718N
7	Virginia Beach	Zone 18: 408276E, 4070310N
8	Portsmouth	Zone 18: 375384E, 4080830N
9	Chesapeake	Zone 18: 392069E, 4067492N
10	Chesapeake	Zone 18: 384197E, 4065296N
11	Chesapeake	Zone 18: 383905E, 4065331N
12	Virginia Beach	Zone 18: 405373E, 4066017N
13	Chesapeake	Zone 18: 392897E, 4070626N
14	Chesapeake	Zone 18: 387433E, 4069160N
15	Norfolk	Zone 18: 385682E, 4080082N
16	Suffolk	Zone 18: 359582E, 4055267N
17	Chesapeake	Zone 18: 387529E, 4069089N
18	Virginia Beach	Zone 18: 397194E, 4071136N
19	Suffolk	Zone 18: 359384E, 4055251N
20	Virginia Beach	Zone 18: 399733E, 4076552N
21	Virginia Beach	Zone 18: 393678E, 4073028N
22	Roanoke	Zone 18: 591947E, 4129615N
23	Chesapeake	Zone 18: 390683E, 4068443N
24	Virginia Beach	Zone 18: 397770E, 4064090N
25	Chesapeake	Zone 18: 393194E, 4066399N
26	Chesapeake	Zone 18: 393194E, 4066399N

Table 5.2. Location and percentage of monogyne and polygyne *S. invicta* colonies collected in Virginia cities.

Site	No. of Samples	No. of Monogyne of Colonies	% Monogyne	No. of Polygyne Colonies	% Polygyne
Chesapeake*	12	1	8.3	11	91.7
Norfolk*	1	0	0	1	100
Portsmouth*	1	0	0	1	100
Richmond	1	1	100	0	0
Roanoke	2	0	0	2	100
Suffolk*	3	2	66.7	1	33.3
Virginia Beach*	6	2	33.3	4	66.7
Total	26	6	23.1	20	76.9

* Indicates cities in Hampton Roads.

Table 5.3. Location and percentage of Virginia RIFA colonies parasitized by *Pseudacteon spp*. phorid flies and/or infected with *K. solenopsae*.

Site	No. of Samples	No. of *Pseudacteon spp*. fly parasitized colonies	Percentage of *Pseudacteon spp.* fly parastized colonies	No. of *K. solenopsae* parasitized colonies	Percentage of *K. solenopsae* infected colonies
Chesapeake	12	1	8.3	5	41.7
Norfolk	1	0	0	1	100
Portsmouth	1	0	0	0	0
Richmond	1	0	0	1	100
Roanoke	2	0	0	0	0
Suffolk	3	0	0	1	33.3
Virginia Beach	6	1	16.7	3	50
Total	26	2	7.7	11	42.3

Table 5.4. Nucleotide differences observed in the small ribosomal subunit (18S) sequences of *Pseudacteon* phorid flies from Virginia and GenBank database sequences.

	Nucleotide position			
Sequence origin	266	630	796	887
Pseudacteon cultelatus	C	T	A	C
Pseudacteon littoralis	A	T	A	C
Pseudacteon obtusus	A	T	A	C
Pseudacteon curvatus	A	C	T	T
Pseudacteon (Chesapeake)	A	T	A	C
Pseudacteon (Virginia Beach)	A	T	A	C

[A] GenBank accession numbers are provided in the text.
*Note that nucleotide sequences for *P. obtusus* and *P. curvatus* are the same.

Table 5.5. Location and percentage of Virginia RIFA colonies infected with SINV.

Site	No. of Samples	No. of Colonies Infected with SINV	Percentage of Colonies infected with SINV
Chesapeake	12	2	16.7
Norfolk	1	0	0
Portsmouth	1	0	0
Richmond	1	0	0
Roanoke	2	0	0
Suffolk	3	0	0
Virginia Beach	6	3	50
Total	26	5	19.2

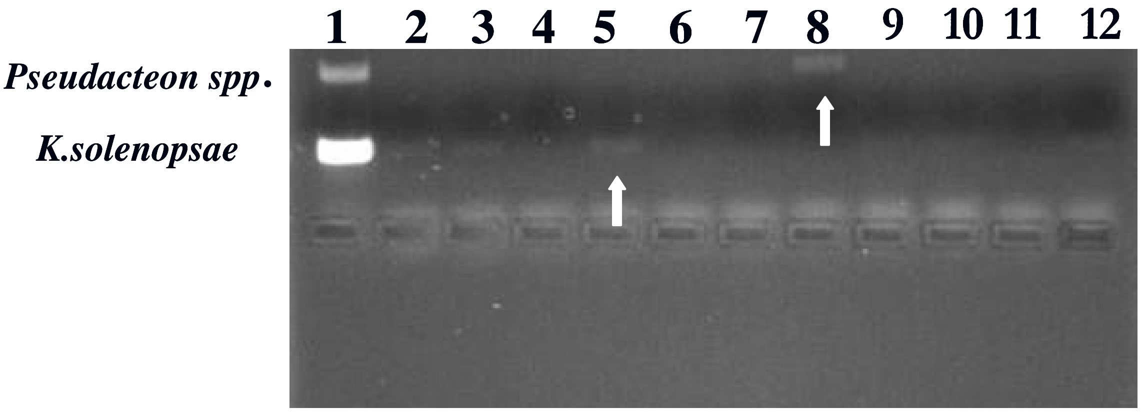

Fig. 5.1 Multiplex PCR banding patterns of *Pseudacteon spp*. phorid and *K. solenopsae* on a 1% agarose gel. Lane 1, top band positive *Pseudacteon spp*. control (800 bp amplicon), bottom band positive *K. solenopsae* control (318 bp amplicon); lane 5, *K. solenopsae* infected fire ant worker, lane 8 *Pseudacteon spp.* phorid fly parasitized fire ant worker

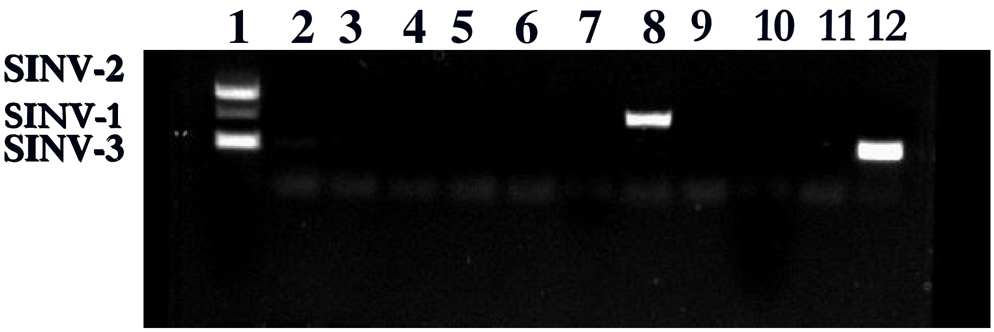

Fig. 5.2 Multiplex PCR banding patterns of SINV-1, SINV-2, and SINV-3. Lane 1, top band SINV-2 positive control (717 bp amplicon), middle band SINV-1 positive control (481 bp amplicon), bottom band SINV-3 positive control (259 bp amplicon); lane 8, SINV-1 infected fire ant worker, lane 12 SINV-3 infected fire ant worker

Chapter 6 - Predicting the Potential Range Expansion of the Red Imported Fire Ant, *Solenopsis invicta*, in Virginia

6.1 Introduction

The red imported fire ant, *Solenopsis invicta* (Buren), is a destructive pest that is native to South America but has been transported into several foreign countries including the United States (U.S.), China, Taiwan, and Australia (McCubbin and Weiner 2002, Zhang et. 2007). Although the ant has been transported to several different countries, colony propagation has been the most widespread and problematic in the U.S (Tschinkel 2006). The ant was first introduced to the U.S. in the 1930s by way of ship ballast and other materials transported from South America to seaports located in Mobile, AL. Because this pest ant is so easily transported, active colonies have been able to become established throughout the southeastern U.S. and as far west as California. In spite of the species' proficiency at coloniziation, the geographic spread in the northern U.S has ultimately been limited by temperature (Porter and Tschinkel 1993). Because temperature is such an influential range limiting factor for fire ants, several climate dependent predictive range models have been developed for the red imported fire ant (Pimm and Bartell 1980, Stoker et al. 1994, Killion and Grant 1995, Korzukhin et al. 2001, and Sutherst and Maywald 2005). These climate based models are important tools which can be used to predict where future control and quarantine efforts may be needed in recently invaded states.

The first statistical and mechanistic models created by Pimm and Bartell (1980) and Stoker et al. (1994) provided useful information regarding fire ant range expansion in the U.S. However model results either exaggerated or underrepresented range expansion of RIFA in the U.S. The models created by Pimm and Bartell (1980) and Stoker et al. (1994) models were only designed to be specific for the state of Texas.

Korzukhin et al. (2001) developed a model based on shortcomings of the two previous models. First, Korzukhin et al. (2001) used ambient temperature data from 4,357 meteorological stations in the U.S. located in 15 fire ant infested states and 9 states where fire ants were not established. Additionally, soil temperature data obtained from 137 of the 4,357 stations were incorporated into the model. Ultimately, ambient and soil temperature data were used to estimate the colony growth rate and female alate production for the life of the colony. The Korzukhin et al. (2001) model successfully identified all of the 2001 USDA fire ant quarantine area as "certainly" being able to support fire ant colony growth. The model also predicted that fire ants could "certainly" extend their "2001" range to establish future colonies in western Texas, southern New Mexico, southern Arizona, California, Oregon, and coastal areas of Virginia. Although Korzukhin et al. (2001) incorporated soil temperature and moisture into model parameters, the authors did not rely heavily on empirical biological data regarding fire ant colony growth.

One modeling system that incorporates the biology of the target species into model parameters is the CLIMEX model (Sutherst and Maywald 1985). The CLIMEX model can predict the establishment potential of an organism in any geographic location using climate data from the target species' current geographic distribution. The CLIMEX system has been used to assess the potential geographic range of several invasive plant and insect species within the United States, including the Chinese tallow tree, *Triadica sebifera* (Pattison and Mack 2008), *Cytisus scoparius* (Potter et al. 2009), red palm weevil, *Rhyncophorus ferrugineuse* (RuiTing et al. 2008), and the red imported fire ant (Sutherest and Maywald 2005).

Sutherest and Maywald's (2005) developed a fire ant model using CLIMEX that differed from previous models by including moisture data in addition to temperature data in model parameters. The CLIMEX model was used to estimate the potential range expansion of the fire ant in the United States. CLIMEX model results were validated by comparing the predicted range to current USDA fire ant quarantine maps. Interestingly, the results of the model indicated that the fire ant could extend its 2004 range further north within the state of California and to latitudes on the east coast beyond the state of Virginia. Although, the CLIMEX model predicted fire ants could potentially extend their range along the east coast of Virginia, climate data from Virginia used in the model were obtained from only three weather stations.

Currently (2010), active fire ant colonies can be found throughout the southeastern coastal region of Virginia. Model results from Korzukhin et al. (2001) and Sutherst and Maywald (2005) both indicated that the fire ant would advance their northern spread on the east coast beyond Virginia.

Since 1989, the number of fire ant reports recorded in Virginia by the Virginia Department of Agriculture and Consumer Services (VDACS), and the range of the insect has increased (Gina Goodwyn, personal communication) within the state. Thousands of mounds have been recorded over the last two decades in the southeast region of the state. In 2009, VDACS working in conjunction with the USDA implemented a quarantine for several counties and cities in Virginia (http://www.aphis.usda.gov/plant_health/plant_pest_info/fireants/downloads/fireant.pdf).

However, even with the quarantine in place, fire ants are still likely to be transported into non-quarantine areas of Virginia. While the ambient and soil temperatures in these non-quarantine counties may be too low to support RIFA colony growth, we cannot safely assume this will be true for all counties within the state. Therefore, a predictive model specific to the state of Virginia would help to determine where future control/quarantine efforts might be directed. In the present study, the CLIMEX model was used to determine the potential future geographic range of RIFA in Virginia counties based on their different climatic characteristics.

6.2 Materials and Methods

The CLIMEX model is a climate based species prediction model that utilizes the biology and geographic distribution of a target species in its native habitat to determine additional locations in the world where climates might be for species establishment.

Model Parameters

Sutherst and Maywald (2004) used CLIMEX to predict the potential spread of RIFA in the United States. The model parameters used in the 2004 study were based on the geographical distribution and quarantine map for the insect in the United States in 2003 (Table 6.1). The same model parameters used by Sutherst and Maywald (2004) were also used in the present study. These parameters include information on soil moisture, ambient temperature, dry stress, wet stress, cold stress, heat stress, degree-days to complete one fire ant generation, and degree-days to produce alates.

The CLIMEX modeling system contains a database composed of weather data (temperature, precipitation, and relative humidity) obtained from all 50 states. However, the CLIMEX weather station data for the state of Virginia is limited to three locations; Norfolk, Richmond, and Wytheville. To increase the resolution of the model for Virginia, weather data (daily maximum/minimum temperature, average monthly rainfall (mm), average daily and maximum relative humidity) were collected from 72 Virginia locations (Table 2). These data were obtained from the National Climate Data Center (http://www.ncdc.noaa.gov/oa/ncdc.html).

Model Output

Employing the “Compare Locations” function included in the CLIMEX model, fire ant colony growth parameters (Sutherst and Maywald 2004) and Virginia weather data were used to predict to potential spread of fire ants in Virginia. The “Compare Locations” function produces several different descriptive indices and maps to illustrate the potential geographic range of a target organism. The Temperature Index (TI), Growth Index (GI), and the Ecoclimatic Index (EI) were the indices of interest in the current study. The TI describes the potential for colony establishment based solely on temperature. The GI gives the probability of an organism to survive during the favorable season (when temperatures are suitable for fire ant colony growth). The GI and the potential for fire ant colonies to establish under four stress related factors (cold, hot, wet, and dry) are combined to produce the EI. The EI estimates the overall abundance and persistence of a species (Sutherst and Maywald 2004). Values produced in the GI, TI, and EI range from 0 to 100. A value of 0 signifies a low chance of survival, whereas a value of 100 indicates high survival probability.

6.3 Results

The potential RIFA range distribution maps produced by the CLIMEX model for Virginia are shown in Figures 6.1, 6.2, and 6.3. Each color coded map was produced to represent the potential for fire ant colony establishment according to the different indices (Temperature Index, Growth Index, and Ecoclimatic Index). Index values used to indicate high probability of RIFA establishment were based on values obtained from Virginia cities where fire ants are currently established.

Temperature Index (TI)

The temperature index was used to determine which areas in Virginia possess temperatures suitable for fire ant colony establishment (Fig. 6.1). TI values for Virginia greater > 20 indicate a high probability of fire ant colony establishment (Table 6.2). Model results indicated that 54 of the 75 (72 %) Virginia cities evaluated in the study have temperatures suitable for colony establishment. All of the desirable locations are located in eastern Virginia. The western cities of Abingdon (19), Big Meadows (6), Blacksburg (13), Bland (14), Covington (16), Dale (18), Floyd (13), Galax (12), Gathright Dam (17), Hot Springs (16), Lebanon (17), Louisa (14), Monterey (10), Mount Weather (14), Pulaski (15), Richland (17), Saltville (19), Staffordsville (15), Staunton (16), and Wytheville (16) did not produce TI values suitable for fire ant colony growth.

Growth Index (GI)

The GI measured the potential for fire ant colonies to survive during the favorable season. Again, GI values >20 for Virginia locations indicate a high probability of fire ant colony survival throughout the winter (Fig 6.3). Because the GI calculates the probability of fire ants to establish during the favorable season, cities with TI values >20 also produced GI values necessary for fire ant colony growth (Table 6.2). In addition, the cities of Covington and Louisa, which had TI values < 20 but both produced GI values of 22.

Ecoclimatic Index (EI)

The GI and four stress indices (hot, cold, wet, dry) were combined to produce the EI. The EI was used to measure the likely persistence and potential abundance of fire ant colonies in Virginia (Fig. 6.3). EI values ≥ 15ndicate a high probability of fire ant colony establishment and persistence (Table 6.2). Of the 75 Virginia cities evaluated by the model, 14 were determined by

CLIMEX to be suitable for fire ant colony growth. These cities include Arlington (17) Hopewell (22), Hampton (29), Norfolk (30, 31, and 32), Painter (21), Richmond (15), Suffolk (28) and Virginia Beach (30), Wakefield (21), Warsaw (18) West Point (20), and Williamsburg (18). Of the 14 cities suitable for fire ant colony growth, five are located in Hampton Roads, which currently has the highest prevalence of fire ants in Virginia.

6.4 Discussion

The purpose of the present study was to use the CLIMEX model to determine which locations in Virginia could support fire ant colony establishment and growth. The CLIMEX model produced three different climate based indices (TI, GI, and EI) which were used to quantify potential colony establishment.

The TI and GI both showed that fire ants could establish colonies beyond the Hampton Roads area. EI values indicate that fire ants will be able to establish in the following areas in Virginia including, Hampton Roads, the Greater Richmond area, and several cities in Northern Virginia (Table 6.2). The results of this study, however, should be viewed critically. Fire ants have been collected from Hampton Roads and Richmond consistently since 1989, but fire ant observations in Northern Virginia have so far been limited (Gina Goodwyn, personal communication). Winter temperatures in Northern Virginia are too low to support fire ant colony growth. However, because Northern Virginia is a highly urbanized area the presence of stream tunnels and other sources of heat in and around buildings may provide enough warmth to support fire ant colony survival.

The fire ant range distribution maps produced by this study suggest that the future range of the fire ant may be limited to several areas in Virginia. The CLIMEX model for potential RIFA distribution presented in this study adds to the information provided by Sutherst and

Maywald (2004) by examining Virginia cities in more specific detail. TI, GI, and EI results presented in the current study are similar to those presented by Sutherst and Maywald (2004). Results from both studies indicate that fire ants can overwinter in all of the cities located in eastern Virginia. However, while the Sutherst and Maywald (2004) model evaluated the entire states' potential to support RIFA colonies based on three weather stations, the addition of an additional 300 station provided much greater resolution regarding suitable temperatures that might exist in more urban locations. As a result, the model developed in this study suggests that indicated that fire ants could establish in areas outside of southeastern Virginia.

Currently, cities placed under the fire ant quarantine are all located in southeastern Virginia. However, results from this study may be used to direct future RIFA monitoring efforts in more urban locations. Other states facing possible fire ant invasion may also find the CLIMEX model useful for identifying those locations that are most suitable for fire ant colony establishment.

Table 6.1. CLIMEX parameter values obtained from Sutherst and Maywald (2005).

Paramater Description	Value
Moisture parameters (proportion of soil moisture holding capacity)	
Lower threshold of soil moisture (SM0)	0.15
Lower limit of optimal range of soil moisture (SM1)	0.8
Upper limit of optimal range of soil moisture (SM2)	2.0
Upper threshold of soil moisture (SM3)	3.0
Temperature parameters	
Lower threshold of temp for pop growth (DV0)	17.0°C
Lower optimal temp for pop growth (DV1)	26.0°C
Upper optimal temp for pop growth (DV2)	30.0°C
Upper threshold temp for pop growth (DV3)	35.0°C
Stress indices	
Dry stress threshold (proportion of soil capacity) (SMDS)	0.15
Weekly rate of accumulation of dry stress (HDS)	-0.008
Wet stress threshold (proportion of soil capacity) (SMWS)	4.0
Weekly rate of accumulation of wet stress (HWS)	0.002
Cold stress threshold (temp) (TTCS)	0°C
Heat stress threshold (TTHS)	38°C
Weekly rate of accumulation of heat stress (THHS)	0.0018
Constraints	
Degree-days to complete one generation (nanitics) (PDD)	330
Degree-days to produce alates (PDD)	510

Table 6.2 Temperature index (TI), growth index (GI), and ecoclimatic index (EI) values for selected cities in Virginia. Values followed by * indicates high probability of fire ant establishment.

Location	TI	GI	EI
Norfolk	31*	31*	31*
Richmond	20*	28*	15*
Wytheville	14	15	0
Abingdon 3s	19	19	0
Amelia 4SW	24*	24*	0
Appomattox	23*	23*	0
Ashland	25*	25*	0
Back Bay Wr	30*	30*	30*
Bedford	23*	23*	0
Big Meadows	6	6	0
Blacksburg	13	15	0
Bland	14	14	0
Bremo Bluff	23*	24*	0
Brookneal	23*	23*	0
Camp Pickett	25*	25*	0
Charlotte Court House	25*	25*	0
Charlottesville 2w	25*	25*	9
Chase City	22*	24*	0
Chatham	23*	23*	0
Colonial Beach	26*	26*	14
Corbin	25*	25*	0
Covington Filter Plant	16	22*	0
Crozier	24*	24*	0
Dale Enterprise	18	19	0
Danville	26*	26*	3
Dulles Airport/Sterling	22*	22*	0
Emporia 1WNW	26*	26*	7
Farmville 2N	25*	25*	2
Floyd 2NE	13	13	0
Galax Radio WBRF	12	12	0
Gathright Dam	17	18	0
Holland 1E	26*	26*	10
Hopewell	25*	27*	22*
Hot Springs	16	16	0
John H. Kerr Dam	27*	27*	13
Langley Air Force Base	29*	29*	29*
Lawrenceville 3E	25*	25*	0
Lebanon	17	17	0
Lexington	20*	22*	0
Lincoln-Loudoun Co	21*	21*	0
Louisa	14	22*	0

Table 6.2 (cont.) Ecoclimatic index, temperature index, and growth index values for selected cities in Virginia. Values followed by * indicates high probability of fire ant establishment.

Location	TI	GI	EI
Luray 5E	22*	20*	0
Martinsville Filter Plant	23*	23*	0
Matthews 4SE	25*	25*	0
Monterey	10	10	0
MountWeather	14	14	0
Norfolk International Airport	30*	30*	30*
Norfolk NAS	32*	32*	32*
Oceana NAS	30*	30*	30*
Painter 2W	28*	23*	21*
Pennington Gap	21*	21*	0
Piedmont Research Station	23*	23*	0
Pulaski	15	15	0
Quantico MCA	27*	27*	10
Reagan Airport/Arlington	27*	27*	17*
Richlands	17	17	0
Roanoke Woodrum AP	24*	24*	0
Rocky Mount	23*	23*	0
Saltville 1N	19	19	0
Staffordsville 3ENE	15	15	0
Staunton Sewage Plant	16	16	0
Sterling RCS	21*	19	0
Stuart	22*	22*	0
Suffolk Lake Kilby	29*	29*	28*
Tye River 1SE	23*	23*	0
Wakefield 1NW	27*	27*	21*
Walkerton 2NW	27*	27*	3
Wallops Island Flight	23*	20*	0
Warrenton 3SE	21*	21*	0
Warsaw 2NW	27*	27*	18*
West Point 2NW	27*	27*	20*
Williamsburg 2N	27*	27*	18*
Winchester 7SE	21*	21*	0
Woodstock 2NE	21*	18*	0
Wytheville 1S	16	16	0

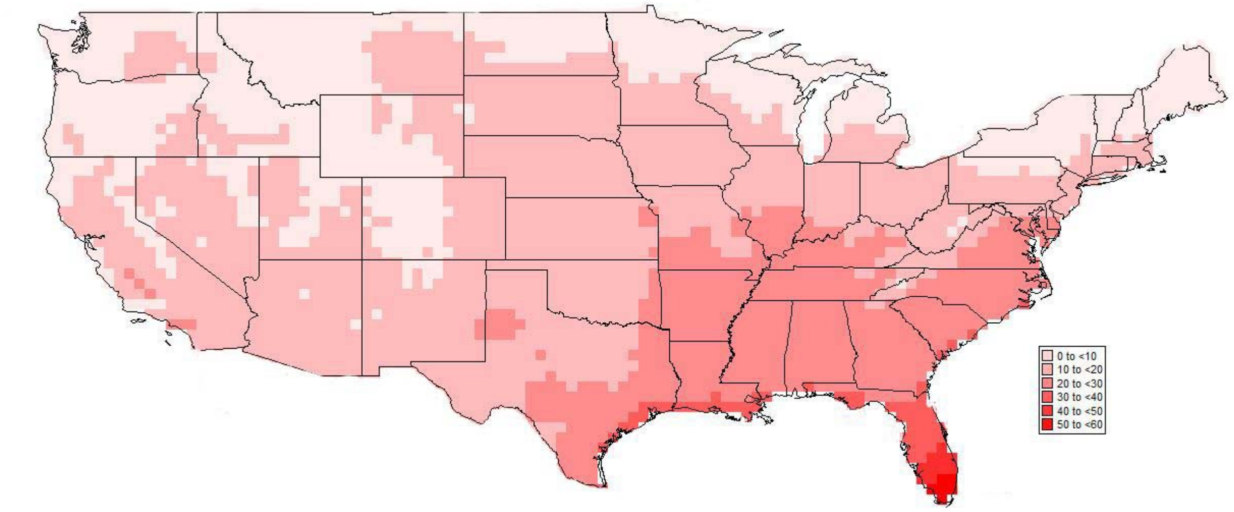

Fig. 6.1 Potential distribution of *S. invicta* in the United States as predicted by CLIMEX temperature index (TI).

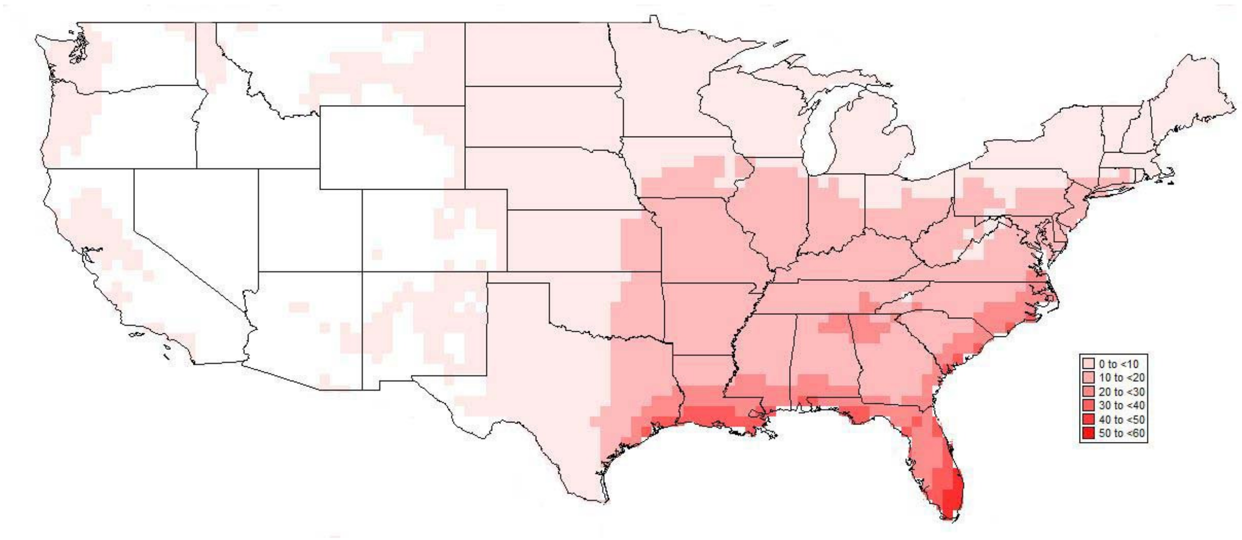

Fig. 6.2 Potential distribution of *S. invicta* in the United States as predicted by CLIMEX growth index (GI).

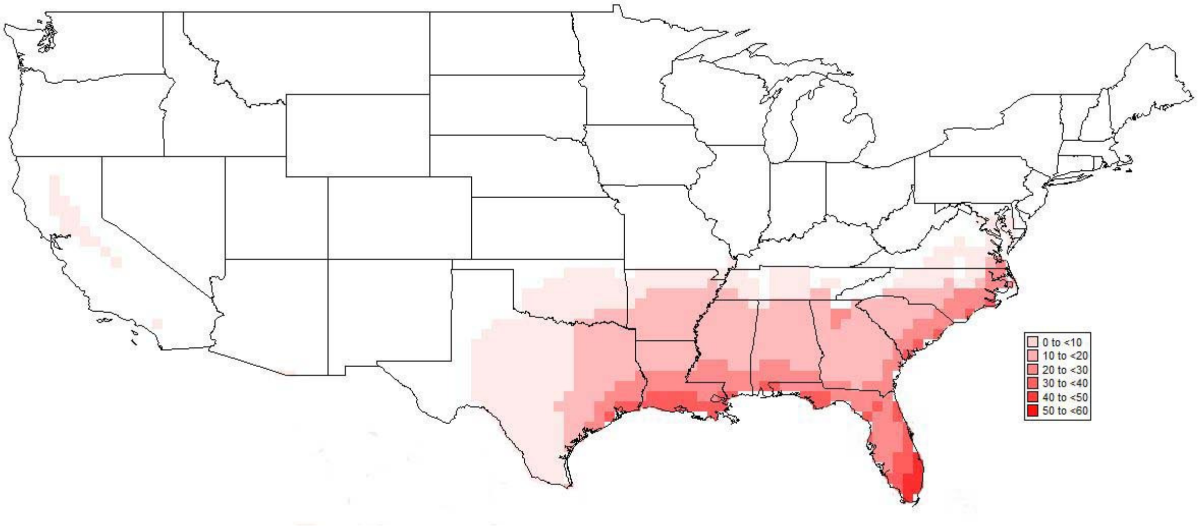

Fig. 6.3 Potential distribution of *S. invicta* in the United States as predicted by CLIMEX ecoclimatic index (EI).

Summary

The red imported fire ant, *Solenopsis invicta* Buren, is one of the most feared invasive insect species in the U.S. The impact of the fire ant in the U.S. has been well documented and the ants are associated with health risks and high control costs. In fact, the economic impact of the fire ant in the U.S, has been estimated at $6 billion annually. Fire ants have been found throughout the southeastern U.S. since their initial introduction in the 1930s. Since that time they have continued to extend their range further west and north. One of the more recently invaded U.S. states is Virginia (1989), which is one of the northernmost states in the U.S. with active fire ant colonies. The presence of fire ants in Virginia prompted the USDA and the Virginia Department of Agriculture and Consumer Services (VDACS) to place the cities of Chesapeake, Hampton, Newport News, Norfolk, Poquoson, Portsmouth, Suffolk, Virginia Beach, and Williamsburg and the counties of James City and York under the Federal Fire Ant Quarantine. As a result, pest management professionals and homeowners are responsible for fire ant control efforts. However, information regarding the status of this pest in Virginia is largely absent. Therefore, the biology, and potential geographic spread of fire ants in Virginia were studied, and potential control methods were evaluated.

VDACS currently treats each mound outside of the quarantine area individually using MaxForce® fire ant bait (1.0% hydramethylnon) (Bayer CropScience, Kansas City, MO) followed six weeks later by an acephate mound drench. Previous studies have proposed that broadcast products provide greater colony control (Barr and Best 1999, Barr 2002b). Therefore, my first objective was to compare the efficacy of three broadcast fire ant control products not currently used in Virginia (Indoxacarb 0.045%; DuPont, Wilmington, DE; Top Choice granular Fipronil 0.143%; Bayer Environmental Sciences, Cary, North Carolina; and an Advion/Top

Choice combination) to the individual mound treatment method currently used by VDACS to control fire ants. Studies were conducted at Fun Junktion Park in Elizabeth City, NC. Products were applied to plots measuring 30 x 30m. The plots were sampled for one year using hot dog baits to quantify any increases/decreases in the number of foraging fire ants. After one year, the products were reapplied and evaluated for 90 days to determine the speed of foraging knockdown.

The repeated-measures MANOVA and ANCOVA results indicated that, Advion provided the fastest knockdown (14 days) of fire ants foraging while Top Choice provided the greatest residual control (300 days after treatment). However, the Advion/Top Choice combination treatment outperformed all other treatments providing > 80% control three days after treatment. Suppression greater than 80% lasted until 300 days after treatment.

Because Advion and Top Choice have not been widely used in Virginia, their impacts on non-target ant species populations were not known. Previous studies have shown that fire ant control products applied over large areas may have negative impacts on other ant species (Edmonson 1981, Vogt et. al. 2005). Hence, I evaluated the impact of broadcast applications of Advion and Top Choice on non-target ant species in Virginia. Studies were conducted at the Hampton Roads Agricultural Research and Extension Center in Virginia Beach, VA. Both products were applied to research plots measuring 10 x 10 m. After treatment, the effect of both products on the foraging activity of non-target ants was measured for one year using sugar water baits. Over the course of the test period, 5 ant species were collected, however *Pheidole megacephala* (Fabricus), *Lasius neoniger* (Emery), and *Monomorium minimum* (Buckley) were the most abundant species collected. MANOVA results indicated that both broadcast products did cause a decrease in the mean number of foraging ants, but the ant populations were able to rebound one year after treatment. MANOVA results also indicated that there was not a significant treatment and species interaction ($P = 0.08$). However, ANCOVA results indicated that Advion caused rapid declines in the mean number of foraging *M. minimum*. Yet, by the end of the test populations had rebounded. On the other hand, *P. megacephala* treated with Advion were not able to rebound.

Top Choice provided faster and longer residual control of *L. neoniger* than *P. megacephala* or *M. minimum*. Fourteen days after treatment *L. neoniger* foraging was 92 percent lower than pre-treatment levels. At the conclusion of the test the number of foraging *L. neoniger* remained 32 percent lower than pre-treatment numbers.

VDACS has documented fire ants infestations in Virginia have been documented since 1989. However, VDACS did not record any specific biological data, only the colony location information. Since 1989, the number of fire ant occurrences in Virginia has continued to increase. Researchers have stated there are two major factors that contribute to high fire ant densities. These factors are polygyny and a lack of biological control organisms in the U.S. (Williams et. al. 2001). I conducted a study to determine the prevalence of polygyny in Virginia fire ant colonies and to determine if any colonies in Virginia were infected with known fire ant biological control organisms (*Pseudacteon* spp. phorid flies, and the microsporidian parasite *Kneallhazia solenopsae*, and *Solenopsis invicta* virus*, SINV*). Red imported fire ant workers were sampled from 26 colonies in Virginia during 2007 and 2008. Polymerase chain reaction (PCR) assays were used to determine colony social form (monogyny or polygyny) by genotyping ants at the *Gp-9* locus. Twenty of the Virginia colonies (77%) were found to be polygyne. Multiplex PCR was also used to detect the presence of *Pseudacteon* spp. phorid flies and *K. solenopsae* genetic material in worker ants. *K. solenopsae* was detected in 11 of 26 colonies

(42%) and worker ants from two colonies had been parasitized by phorid flies. Colonies were also examined by reverse transcription PCR (RT-PCR) for the presence of *S. invicta* viruses SINV-1, SINV-2, and SINV-3. Results indicated that 4 colonies were infected with SINV-1, none with SINV-2, and one with SINV-3.

Two population model studies have evaluated Virginia climate data and determined that fire ants have the potential to establish colonies beyond the current quarantine area (Korzukhin et. al. 2001, Sutherst and Maywald 2005) in Virginia. Although useful, models had shortcomings with regard to predicting fire ant spread in Virginia. Korzukhin et al. (2001) did not incorporate fire ant biological data into their model. Sutherst and Maywald (2005) did base their model on fire ant biological data, but the authors only used climate data from three weather stations in Virginia (Norfolk, Richmond, and Wytheville). Because Sutherst and Maywald (2005) used empirical data to create their model, I decided to use their methods as a conceptual framework. Sutherst and Maywald (2005) used the CLIMEX modeling system to predict the spread of fire ants in the U.S. The CLIMEX model is a climate based species prediction model that utilizes the biology and geographic distribution of a target species in its native habitat to identify other locations in the world where the climate is suitable for species establishment. To improve the resolution of Sutherst and Maywald's (2005) model in Virginia, I obtained weather data from 72 Virginia weather stations and incorporated these data into the CLIMEX model. However, I did not change model data specific to fire ant biology (ie. soil moisture parameters, temperature parameters, and stress indices). Model results indicated that fire ants would be able to establish in several of the urbanized centers of Virginia. These areas include the current quarantine zone, Richmond, and the cities of Arlington, Quantico, and Sterling located in Northern Virginia.

The findings presented in this study were the first to describe fire ant populations in Virginia and provides the foundation for future fire ant studies within the state. My study documented that fire ants are indeed established in Virginia and that polygyne fire ant colonies are more prevalent than monogyne colonies. Polygyne colonies are known to have a larger mound density, they establish new colonies faster than monogyne colonies, and are potentially harder to control than monogyne colonies.

CLIMEX model results indicated that fire ants could establish in the urban areas of Virginia. Combining the model results with the colony social form data suggest that there is a real potential for fire ants to rapidly colonize in the urban centers of Virginia. Therefore, fire ant control will be of major importance to Virginians. Results from the product efficacy tests concluded that broadcast fire ant control products perform better than individual mound treatments. Thus, broadcast control products should be used to control fire ant colonies in Virginia because fire ants are polygyne and have the ability to rapidly spread within areas outside of the quarantine.

References

1. Adams, C. T., W. A. Banks, and C. S. Lofgren. 1988. Red imported fire ant (Hymenoptera: Formicidae): correlation of ant density with damage to tow cultivars of potatoes (*Solanum tuberosum* L.). J. Econ. Entomol. 81:905-909.

2. Adams, C. T., J. K. Plumley, C. S. Lofgren, and W. A. Banks. 1976. Economic importance of the red imported fire ant, *Solenopsis invicta* Buren. Preliminary investigations of impact on soybean harvest. J. Ga. Entomol. Soc. 11:165-169.

3. Adams, C. T., W. A. Banks, C. S. Lofgren, B. J. Smittle, and D. P. Harlan. 1983. Impact of the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), on the growth and yield of soybeans. J. Econ. Entomol. 76:1129-1132.

4. Adams, E. S. 2003. Experimental analysis of territory size in a population of the fire ant, *Solenopsis invicta*. Behavioral Ecology. 14:48-53.

5. Adams, E. S., and J. F. A. Traniello. 1981. Chemical interference competition by Monomorium minimum. Oecologia. 51:265-270.

6. Adams, E. S., and W. R. Tschinkel. 1995. Effects of foundress number on brood raids and queen survival in the fire ant, *Solenopsis invicta*. J. Anim. Ecol. 64:315-324.

7. Adams, E. S. and W. R. Tschinkel. 2001. Mechanisms of population regulation in the fire ant, *Solenopsis invicta*: an experimental study. J. Anim. Ecol. 64:473-480.

8. Agnew, C. W., and W. L. Sterling. 1981. Predation of boll weevils in partially-open cotton bolls by the red imported fire ant. Southwest. Entomol. 6:215-219.

9. Allen, C. R., S. Demaris, and R. S. Lutz. 1994. Red imported fire ant impact on wildlife: an overview. Tex. J. Sci. 46:51-59.

10. Allen, C. R., S. Demaris, and R. S. Lutz. 1997. Effects of red imported fire ants on recruitment of white-tailed deer fawns. J. Wildl. Manage. 61:911-916.

11. Allen, C. R., R. S. Lutz., and S. Demarais. 1995. Red imported fire ant impacts on northern bobwhite populations. Ecol. Appl. 5:632-638.

12. Allen, C. R., K. G. Rice, D. P. Wojcik, and H. F. Percival. 1997. Effect of red imported fire ant envenomization on neonatal American alligators. J. Herpetol. 31:318-321.

13. Allen, C. R., D. M. Epperson., and A. S. Garmestani. 2004. Red imported fire ant impacts on wildlife: a decade of research. Am. Midl. Nat. 152:88-103.

14. Allen, C. R., R. D. Willey, P. E. Myers, P. M. Horton, and J. Buffa. 2000. Impact of red imported fire ant infestation on northern bobwhite quail abundance trends in southeastern United States. J. Agric. Entomol. 17:43-51.

15. Allen, G. E., and W. F. Buren. 1974. Microsporidian and fungal diseases of *Solenopsis invicta* Buren in Brazil. J. NY. Entomol. Soc. 82:125-130.

16. Allen, G. E., W. F. Buren, R. N. Williams, M. deMenezes, and W. H. Whitcomb. 1974. The red imported fire ant, *Solenopsis invicta*: distribution and habitat in Mato Grosso, Brazil. Ann. Entomol. Soc. Am. 67:43-46.

17. Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402.

18. Aubuchon, M. D., and R. K. Vander Meer. 2008. Areawide suppression of fire ants. pp. 261-270. In O. Koul, G. Cuperus, and N. Elliot (eds.) Areawide pest management: theory and implementation. CABI, Wallingford, UK.

19. Aubuchon, M. D., G. R. Mullen, and M. D. Eubanks. 2006. Efficacy of broadcast and perimeter applications of S-methoprene bait on the red imported fire ant in grazed pastures. J. Econ. Entomol. 99:621-625.

20. Banks, W. A., B. M. Glancey, C. E. Stringer, D. P. Jouvenaz, C. S. Lofgren, and D. E. Weidhaas. 1973. Imported fire ants: Eradication trials with Mirex bait. J. Econ. Entomol. 66:785-789.

21. Banks, W. A., C. S. Lofgren, and J. K. Plumley. 1978. Red imported fire ants: effects of insect growth regulators on caste formulation and colony growth and survival. J. Econ. Entomol. 71:75-78.

22. Banks, W. A., D. F. Williams, and C. S. Lofgren. 1988. Effectiveness of fenoxycarb for control of red imported fire ants (Hymenoptera: Formicidae) J. Econ. Entomol. 81:83-87.

23. Banks, W. A., C. T. Adams, and C. S. Lofgren. 1991. Damage to young citrus trees by the red imported fire ant (Hymenoptera: Formicidae). J. Econ. Entomol. 84:241-246.

24. Barr, C. L. 2002a. The active ingredient indoxacarb as a broadcast bait for the control of red imported fire ants. Texas Coop. Ext., Texas A&M Univ. System.

25. Barr, C. L. 2002b. Indoxacarb bait effects on mound activity and foraging of red imported fire ants. Texas Coop. Ext., Texas A&M Univ. System.

26. Barr, C. L. 2004. How fast is fast?: Indoxacarb broadcast bait. P. 46-50. In: Proceeding of the Annual Red Imported Fire Ant Conference, Baton Rouge, LA. 201 pp.

27. Barr, C. L., and R. L. Best. 1999. Comparison of Amdro®, Spectracide® fire ant bait and Diazinon using broadcast and individual mound treatment applications. Results and Demonstration Handbook. 1997-1999. Texas Agricultural Extension Service. Bryan, TX.

28. Barr, C. L., and B. M. Drees. 1994. Diagnosing and treating animals for Red imported fire ant injury. Texas Agricultural Extension Service, Texas A&M University, College Station, Texas. 4 pgs.

29. Bessin, R. T., and T. E. Reagan. 1993. Cultivar resistance and arthropod predation of sugarcane borer (Lepidoptera: Pyralidae) affects incidence of deadhearts in Louisiana sugarcane. J. Econ. Entomol. 86:929-932.

30. Bloomquist, J. R. 1996. Ion channels as targets for insecticides. Annu. Rev. Entomol. 41:163-190.

31. Borgmeir, T. 1963. Revision of the North American phorid flies. Part 1. The Phorinae, Aenigmatiinae and Metopiniae, except Megaselia (Diptera, Phoridae). Studia Entomoligica (Petropolis). 6:1-256.

32. Briano, J. A., R. S. Cordo. 1995. Long-term studies of the black imported fire ant (Hymenoptera: Formicidae) infected with a microsporidium. Environ. Entomol. 24:1328-1332.

33. Broome, J. R., P. P. Sikorowski, and B. R. Norment. 1976. A mechanism of pathogenicity of *Beauveria bassiana* on larvae of the imported fire ant, *Solenopsis richteri*. J. Invertebr. Pathol. 28:87-91.

34. Buren, W. F. 1972. Revisionary studies on the taxonomy of the imported fire ants. J. Ga. Entomol. Soc. 7:1-27.

35. Buren, W. F. 1982. Red imported fire ant now in Puerto Rico. 1982. Red imported fire ant now in Puerto Rico. Fla. Entomol. 65:188-189

36. Buren, W. F. 1982. Red imported fire ant now in Puerto Rico. Fla. Entomol. 65:188-189.

37. Burns, E. C., and D. G. Melancon. 1977. Effect of imported fire ant (Hymenoptera: Formicidae) invasion on lone star tick (Acarina: Ixodidae) populations. J. Med. Entomol. 14:247-249.

38. Byron, D. W. and S. B. Hays. 1986. Occurrence and significance of multiple mound utilization by colonies of the red imported fire ant (Hymenoptera: Formicidae). J. Econ. Entomol. 79:637-640.

39. Callcott, A. M. and H. L. Collins. 1996. Invasion and range expansion of imported fire ants (Hymenoptera: Formicidae) in North America from 1918-1995. Fla. Entomol. 79:240-251.

40. Callcott, A. M., H. Collins, and S. Jaronski. 1988. Evaluation of *Beauveria bassiana* (Abbot Laboratories strain). Annual report, USDA-APHIS, Imported Fire Ant Station, Gulfport, MS.

41. Cancienne, E. A. 1959. Fire ant control: effects on the sugar cane borer. Sugar J. 21:11-17.

42. Canter, L. W. 1981. Final programmatic environmental impact statement for the cooperative imported fire ant progam. APHIS-ADM-81-01-F. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Hyattsville, Maryland. 240 pp.

43. Chen, J. S. C., Shen ChinHui, and Lee HowJing. 2006. Monogynous and polygynous red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), in Taiwan. Environ. Entomol 35:167-172.

44. Clemmer, D. I. and R. E. Sterling 1975. The imported fire ant dimensions of the urban problem. South. Med. J. 68:1133-1138.

45. Creighton, W. S. 1930. The New World species of the genus *Solenopsis* (Hymenoptera:Formicidae). Proc. Nat. Acad. Sci. USA. 66:39-151.

46. Culpepper, G. H. 1953. Status of the imported fire ant in the southern states in July 1953. U.S.D.A., Bur. Entomol. & Plant Quar. Special Rpt. E-867. Washington D.C.

47. Cupp, E. W., and J. O'Neal. 1973. The morphogenetic effects of two juvenile hormone analogues on larvae of imported fire ants. Environ. Entomol. 2:191-194.

48. DeHeer, C. J. 2002. A comparison of the colony-founding potential of queens from single- and multiple-queen colonies of the fire ant *Solenopsis invicta*. Anim. Behav. 64:655-661.

49. DeShazo, R. D., D. F. Williams, and E. S. Moak. 1999. Fire ant attacks on residents in health care facilities: a report of two cases. Ann. Intern. Med. 131:424-429.

50. Diaz, R., A. Knutson, and J. S. Bernal. 2004. Effect of the red imported fire ant on cotton aphid population density and predation of bollworm and beet armyworm eggs. J. Econ. Entomol. 97:222-229.

51. Dickinson, V. M. 1995. Red imported fire ant predation on Crested Caracara nestlings in south Texas. Wilson Bull. 107:761-762.

52. Drees, B. M. 1994. Red imported fire ant predation on nestlings of colonial waterbirds. Southwest. Ento. 19: 355-359.

53. Drees, B. M., S. B. Vinson, R. E. Gold, M. E. Merchant, E. Brown, M. Keck, P. Nester, D. Kostroun, K. Flanders, F. Graham, K. Loftin, J. Hopkins, K. Vail, R. Wright, W. Smith, D. C. Thompson, J. Kabashima, B. Layton, P. G. Koehler, D. Oi, and A. M.

Callcott. 2006. Managing imported fire ants in urban areas. Texas Coop. Ext., Texas A&M Univ. System. MP426 23 p.

54. Edmonson, M. B. 1981. The effect of Amdro on non-target ant species associated with *Solenopsis invicta* Buren in Florida. M. S. Thesis. University of Florida, Gainesville.

55. Eubanks, M. D. 2001. Estimates of the direct and indirect effects of red imported fire ants on biological control in field crops. Biol. Control. 21:35-43.

56. Eubanks, M. D., S. A. Blackwell, C. J. Parrish, Z. D. Delamar, and H. Hull-Sanders. 2002. Intraguild predation of beneficial arthropods by red imported fire ants in cotton. Environ. Entomol. 31:1168-1174.

57. Fillman, D. A., and W. L. Sterling. 1983. Killing power of the red imported fire ant (Hymenoptera: Formicidae): a key predator of the boll weevil (Coleoptera: Curclionidae). Entomophaga. 28:339-344.

58. Fletcher, D. J. C., and M. S. Blum. 1981. Pheromonal control of dealation and oogenesis in virgin queen fire ants. Science. 12:73-75.

59. Folgarait, P. J., R.J.W. Patrock, and G. Albioni-Montenegro. 2008. *Solenopsis invicta*: Evidence for recen internal immigration across provinces in Argentina. Fla. Entomol. 91:131-132.

60. Fritz, G. N., and R. K. Vander Meer. 2003. Sympaty of polygyne and monogyne colonies of the fire ant, Solenopsis invicta (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 96:86-92.

61. Furman, B., and R. E. Gold. 2006. Determination of the most effective concentration and quantity of Advion™, as well as the most effective placement of the bait for individual Solenopsis invicta mound treatments (Hymenoptera: Formicidae). Sociobiology. 48:101-116.

62. Furman, B., and R. E. Gold. 2006. The effectiveness of label-rate broadcast treatment with Advion at controlling multiple ant species (Hymentoptera: Formicidae). Sociobiology. 48:559-570.

63. Gardner, W. A., S. Diffie, R. K. VanderMeer, and M. A. Brinkman. 2008. Distribution of the fire ant (Hymenoptera: Formicidae) hybrid in Georgia. J. Entomol. Sci. 43:133-137.

64. George, J. L. 1958. The program to eradicate the Imported Fire Ant. Report to the Conservation Foundation and the New York Zoological Society. 39 pages.

65. Gibbons, J. W., D. E. Scott, T. Ryan, K. Buhlmann, T. Tuberville, J. Greene, T. Mills, Y. Leiden, S. Poppy, C. Winne, and B. Metts. 2000. The global decline of reptiles, Déjà vu Amphibians. BioScience. 50:653-666.

66. Glancey, B. M. and C. S. Lofgren. 1988. Adoption of newly-mated queens: a mechanism for proliferation and perpetuation of polygynous red imported fire ants, *Solenopsis invicta* Buren. Fla. Entomol. 4:581-587.

67. Glancey, B. M., C. H. Craig, C. E. Stringer, and P. M. Bishop. 1973. Multiple fertile queens in colonies of the imported fire ant, *Solenopsis invicta*. J. Ga. Entomol. Soc. 8:327-328.

68. Glancey, B. M., J. D. Coley, and F. Killibrew. 1979. Damage to corn by the red imported fire ants. J. Ga. Entomol. Soc. . 14:198-201.

69. Glancey, B. M., C. S. Lofgren, and D. F. Williams. 1982. Avermectin B1a: effects on the ovaries of red imported fire ant queens (Hymenoptera: Formicidae). J. Med. Entomol. 19:743-747.

70. Greenberg, L., D. Reierson, and M. K. Rust. 2003. Fipronil trials in California against the red imported fire ant, *Solenopsis invicta* Buren, using sugar water consumption and mound counts as measures of ant abundance. J. Agr. Urban Entomol. 20:221-233.

71. Goodisman, M. A. D., and K. G. Ross. 1996. Relationship of queen number and worker size in polygyne colonies of the fire ant *Solenopsis invicta*. Insectes Soc. 43:303-307.

72. Goodisman, M. A. D., P. D. Mack, D. E. Pearse, and K. G. Ross. 1999. Effects of a single gene on worker and male body mass in the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae).

73. Haight, K. L., and W. R. Tschinkel. 2003. Patterns of venom synthesis and use in the fire ant, *Solenopsis invicta*. Toxicon. 42:673-682.

74. Harvey, C. T., and M. D. Eubanks. 2005. Intraguild predation of parasitoids by Solenopsis invicta: a non-disruptive interaction. Entomologia Experimentalis et Applicata. 114:127-135.

75. Hashimoto, Y, and S. M. Valles. 2008. Infection characteristics of *Solenopsis invicta* virus 2 in the red imported fire ant, Solenopsis invicta. J. Invertebr. Pathol. 99:136-140.

76. Hölldobler, B., and E. O. Wilson. 1990. The Ants. Belknap Press of Harvard University Press. 752 pp.

77. Holtcamp, W. N., W. E. Grant, and S. B. Vinson. 1997. Patch use under predation hazard: effect of the red imported fire ant on deer mouse foraging behavior. Ecology. 78:308-317.

78. Hood, M. W., P. M. Horton, and J. W. McCreadie. 2003. Field evaluation of the red imported fire ant (Hymenoptera: Formicidae) for the control of wax moths (Lepidoptera: Pyralidae) in stored honey bee comb. J. Agr. Urban Entomol. 20:93-103.

79. Hu, X, and D. Song. 2007. Field evaluation of label-rate broadcast treatment with baits for controlling the red imported fire ant, Solenopsis invicta (Hymenoptera: Formicidae). Sociobiology. 50:1107-116.

80. Hung, A. C. E., S. B. Vinson, and J. W. Summerlin. 1974. Male sterility in the red imported fire ant, Solenopsis invicta. Ann. Entomol. Soc. Am. 67:909-912.

81. Jacobson, A. L., D. C. Thompson, L. Murray, and S. F. Hanson. 2006. Establishing guidelines to improve identification of fire ants *Solenopsis xyloni* and *Solenopsis invicta*. J. Econ. Entomol. 99:313-322.

82. Jetter, K. M., J. Hamilton, and J. H. Klotz. 2002. Eradication costs calculated. Red imported fire ants threaten agriculture, wildlife, and homes. Calif. Agric. 56:26-34.

83. Jones, D. B., L. C. Thompson, and K. W. Davis. 1997. Use of fenoxycarb followed by acephate for spot eradication of imported fire ants (Hymenoptera: Formicidae). J. Kans. Entomol. Soc. 70:169-174.

84. Jouvenaz, D. P. 1983. Natural enemies of fire ants. Fla. Entomol. 66:11-121.

85. Kaiser, K. L. E. 1978. The rise and fall of mirex. Environ. Sci. Technol. 12:520-528.

86. Keller, L., and L. Passera. 1989. Regulation of queen number in the Argentine ant, *Iridomyrmex humilis* (Hymenoptera: Formicidae). Actes des Colloques Insectes Soc. 6:287-293.

87. Keller, L., and K. G. Ross. 1995. Gene by environment interaction: effects of a single gene and social environment on reproductive phenotypes of fire ant queens. Funct. Ecol. 9:667-676.

88. Keller, L., and K. G. Ross. 1999. Major gene effects on phenotype and fitness: the relative roles of Pgm-3 and Gp-9 in introduced populations of the fire ant *Solenopsis invicta*. J. Evol. Biol. 12: 672-680.

89. Kemp, S. F., R. D. DeShazo, J. E. Moffitt, D. F. Williams, and W. A. Buhner II. 2000. Expanding habitat of the imported fire ant (*Solenopsis invicta*): a public health concern. J. Allergy Clin. Immunol. 105:683-691.

90. King, J. R., and W. R. Tschinkel. 2008. Experimental evidence that human impacts drive fire ant invasions and ecological change. Proc. Nat. Acad. Sci. USA. 105:20339-20343.

91. King, J. R., Tschinkel, W. R., and Ross, K. G. 2009. A case study of human exacerbation of the invasive species problem: transport and establishment of polygyne fire ants in Tallahassee, Florida, USA. Biol. Invasions 11:373-377.

92. Kolaczninski and C. Curtis. 2001. Laboratory evaluation of fipronil, a phenylpyrazole insecticide, against adult *Anopholes* (Diptera: Culicidae) and investigation of its possible cross-resistance with dieldrin in *Anopheles stephensi*. Pest Management Science 57:41-45.

93. Kopachena, J. G., A. J. Buckley, and G. A. Potts. 2000. Effects of the red imported fire ant (*Solenopsis invicta*) on reproductive successs of barn swallows (*Hirundo rustica*) in Northeast Texas. Southwest. Nat. 45:477-482.

94. Killion, M. J., and W. E. Grant. 1995. A colony-growth model for the imported fire ant: potential geographic range of an invading species. Ecological Modell. 77:73-84.

95. Korzukhin, M. D., S. D. Porter, L. C. Thompson, and S. Wiley. 2001. Modelling temperature-dependent range limits for the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae) in the United States. Environ. Entomol. 30:645-655.

96. Lechner, K. A., and D. O. Ribble. 1996. Behavioral interactions between red imported fire ants (*Solenopsis invicta*) and three rodent species of south Texas. Southwest. Nat. 42:123-128.

97. Lee, J. S., J. Johnson, and V. L. Wright. 1990. Quantitative survivorship analysis of the velvetbean caterpillar (Lepidoptera: Noctuidae) pupae in soybean fields in Louisiana. Environ. Entomol. 19:978-986.

98. Lockley, T. C. and H. L. Collins. 1990. Imported fire ant quarantine in the United States of America: Past, Present, and Future. J. Mississippi Acad. Sci. 35: 23-26.

99. Lofgren, C. S., and D. F. Williams. 1984. Avermectin B1a: highly potent inhibitor of reproduction by queens of the red imported fire ant (Hymenoptera: Formicidae). J. Econ. Entomol. 75:798-803.

100. Lofgren, C. S. and R. K. Vander Meer. 1986. Fire ants and leaf-cutting ants. Westview Press, Boulder and London. 435 pgs.

101. Lofgren, C. S., F. J. Bartlett, and C. E. Stringer. 1963. Imported fire ant toxic bait studies: Evaluation of carriers for oil baits. J. Econ. Entomol. 56:62-66.

102. Lofgren, C. S., F. J. Bartlett, C. E. Stringer, and W. A. Banks. 1964. Imported fire ant toxic bait studies: further tests with granulated mirex-soybean bait. J. Econ. Entomol. 57:695-698.

103. MacKay, W. P., B. Vinson, J. Irving, S. Majdi, and C. Messer. 1992. Effect of electrical fields on the red imported fire ant (Hymenoptera: Formicidae). Environ. Entomol. 21:866-870.

104. Macom, T. E. and S. D. Porter. 1996. Comparison of polygyne and monogyne red imported fire ant (Hymenoptera: Formicidae) population densities. Ann. Entomol. Soc. Am. 89:535-543.

105. Mallipudi, N. M., S. J. Stout, A. H. Lee, and E. J. Orloski. 1986. Photolysis of AMDRO fire ant insecticide active ingredient hydramethylnon (AC 217,300) in distilled water. J. Agric. Food Chem. 34:1050-1057.

106. Markin, G. P. and J. H. Dillier. 1971. The seasonal life cycle of the imported fire ant, *Solenopsis saevissima richteri*, on the gulf coast of Mississippi. Ann. Entomol. Soc. Am. 64:562-565.

107. Markin, G. P., H. L. Collins, and J. H. Dillier. 1972. Colony founding by queens of the red imported fire ant, *Solenopsis invicta*. Ann. Entomol. Soc. Am. 65: 1053-1058.

108. Markin, G. P., J. H. Dillier, and H. L. Collins. 1973. Growth and development of colonies of the red imported fire ant, *Solenopsis invicta*. Ann. Entomol. Soc. Am. 66: 803-808.

109. Markin, G. P. H. L. Collins, and J. Davis 1974. Residues of the insecticide mirex in terrestrial and aquatic invertebrates following a single aerial application of mirex bait, Louisiana – 1971-72. Pesticides Monitoring Journal. 8:131-134.

110. Markin, G. P., J. H. Dillier, S. O. Hill, M. S. Blum, and H. R. Hermann. 1971. Nuptial flight and flight ranges of the imported fire ant, *Solenopsis saevissima richteri* (Hymenoptera: Formicidae). J. Ga. Entomol. Soc. 6:145-156.

111. McCubbin, K. I., and J. M. Weiner. 2002. Fire ants in Australia: a new medical and ecological hazard. Med. J. Aust. 176:518-519.

112. Mescher, M. C., K. G. Ross, D. D. Shoemaker, L. Keller, and M. J. B. Krieger. 2003. Distribution of the two social forms of the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae) in the native South American range. Ann. Entomol. Soc. Am. 96:810-817.

113. Miller, S. E., M. S. Henry, B. J. V. Mey, and P. M. Horton. 2000. Averting cost measures of the benefits to South Carolina households of red imported fire ant control. J. Agric. Urban. Entomol. 17:113-123.

114. Milks, M. L., Fuxa, J. R., and Richter, A. R. 2007. Prevalence and impact of the microsporidium *Thelohania solenopsae* (Microsporidia) on wild populations of red imported fire ants, *Solenopsis invicta*, in Louisiana. J. Invertebr. Pathol. 97:91-102.

115. Moller, H. 1996. Lessons for invasion theory from social insects. pp. 125-142. *In* J. R. Carey, P. Moyle, M. Rejanek, and G. Vermeij. (eds.), Proceedings, Symposium: Special issue: Invasion Biology, May 1994. University of California, Davis, USA.

116. Morrison, L. W. 2000. Biology of *Pseudacteon* (Diptera: Phoridae) ant parasitoids and their potential to control imported Solenopsis fire ants (Hymenoptera: Formicidae). Recent Res. Dev. Entomol. 3:1-13.

117. Morrison, L. W. 2002. Long-term impacts of an arthropod community invasion by the imported fire ant, *Solenopsis invicta*. Ecology. 83:2337-2345.

118. Morrison, L. W. 2003. Positive association between densities of the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), and generalized ant and arthropod diversity. Environ. Entomol. 2003. 32:548-554.

119. Morrison, L. W., and S. D. Porter. 2005. Testing for population-level impacts of introduced *Pseudaceteon tricuspis* flies, phorid parasitoids of *Solenopsis invicta* fire ants. Biol. Control. 33:9-19.

120. Morrison, L. W., M. D. Korzukhin, and S. D. Porter. 2005. Predicted range expansion of the invasive fire ant, *Solenopsis invicta*, in the eastern United States based on the VEMAP global warming scenario. Diversity Distrib. 11:199-204.

121. Morrison, L. W., S. D. Porter, E. Daniels, and M. D. Korzukhin. 2004. Potential global range expansion of the invasive fire ant, *Solenopsis invicta*. Biol. Control. 29:179-188.

122. Moser, B. A., Becnel, J. J., Maruniak, J., and Patterson, R. S. 1998. Analysis of the ribosomal DNA sequences of the microsporida *Thelohania* and *Vairimorpha* of fire ants. J. Invertebr. Pathol. 72: 154-159.

123. Moser, B. A., Becnel, J. J., and Williams, D. F. 2000. Morphological and molecular characterization of the *Thelohania solenopsae* complex (Microsporidia: Thelohaniidae). J. Invertebr. Pathol. 75:174-177.

124. Mount, R. H. 1981. The red imported fire ant, *Solenopsis invicta*, as a possible serious predator on southeastern vertebrae: Direct observation and subjective impressions. J. Ala. Acad. Sci. 52: 66-70.

125. Mueller, J. M., C. B. Dabbert, and A. R. Forbes. 2001. Negative effects of imported fire ants on deer: the "increased movement" hypothesis. Tex. J. Sci. 53:87-90.

126. Oi, D., and F. M. Oi. 2006. Speed of efficacy and delayed toxicity characteristics of fast-acting fire ant (Hymenoptera: Formicidae) baits. J. Econ. Entomol. 99:1739-1748.

127. Oi, D., R. M. Pereira, J. L. Stimac, and L. A. Wood. 1994. Field applications of *Beauveria bassiana* for control of the red imported fire ant (Hymenoptera: Formicidae). J. Econ. Entomol. 87:623-630.

128. Oi, D. H., S. M. Valles, and R. M. Pereira. 2004. Prevalence of Thelohania solenopsae (Microsporidia: Thelohaniidae) infection in monogyne and polygyne red imported fire ants (Hymenoptera: Formicidae). Environ. Entomol. 33:340-345.

129. Orrock, J. L., and B. J. Danielson. 2004. Rodents balancing a variety of risks: invasive fire ants and indirect and direct indicators of predation risk. Oecologia. 140:662-667.

130. Pedersen, E. K., T. L. Bedford, W. E. Grant, S. B. Vinson, J. B. Martin, M. T. Longnecker, C. L. Barr, and B. M. Drees. 2003. Southwest. Nat. 48:419-426.

131. Pereira, R. M. 2003. Areawide suppression of fire ant populations in pastures: project update. J. Agr. Urban Entomol. 20:123-130.

132. Pimm, S. L., and D. P. Bartell. 1980. Statistical model for predicting range expansion of the red imported fire ant, *Solenopsis invicta*, in Texas. Environ. Entomol. 9:653-658.

133. Porter, S. D. 1988a. Impact of temperature on colony growth and development rates of the ant, *Solenopsis invicta*. J. Insect Physiol. 34:1127-1133.

134. Porter, S. D. 1998b. Invasion of red imported fire ants (Hymenoptera: Formicidae): microgeography of competitive replacement. Ann. Entomol. Soc. Am.. 81:913-918.

135. Porter, S. D. 1991. Origins of new queens in polygyne red imported fire ant colonies (Hymenoptera: Formicidae). J. Entomol. Sci. 26:474-478.

136. Porter, S. D., and L. E. Gilbert. 2005. Parasitoid case history: an evaluation of methods used to assess host ranges of fire ant decapitating flies. Second International Symposium on Biological Control of Arthropods, Davos, Switzerland. Pp. 634-650.

137. Porter, S. D., and D. A. Savignano. 1990. Invasion of polygyne fire ants decimates native ants and disrupts arthropod community. Ecology. 71:2095-2106.

138. Porter, S. D. and W. R. Tschinkel. 1986. Adaptive value of nanitic workers in newly founded red imported fire ant colonies (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 79:723-726.

139. Porter, S. D. and W. R. Tschinkel. 1987**.** Foraging in *Solenopsis invicta* (Hymenoptera: Formicidae) effects of weather and season. Environ. Entomol. 16: 802-808.

140. Porter, S. D., and W. R. Tschinkel. 1993. Fire ant thermal preferences: behavioral control of growth and metabolism. Behav. Ecol. Sociobiol. 32: 321-329.

141. Porter, S. D., M. A. Pesquero, S. Campiolo, and H. G. Fowler. 1995. Growth and development of *Pseudacteo*n phorid fly maggots (Diptera: Phoridae) in the heads of *Solenopsis* fire ant workers (Hymenoptera: Formicidae). Environ. Entomol. 24:475-479.

142. Porter, S. D., D. F. Williams, R. S. Patterson, and H. G. Fowler. 1997. Intercontinental differences in the abundance of *Solenopsis* fire ants (Hymenoptera: Formicidae): escape from natural enemies. Environ. Entomol. 26:373-384.

143. Porter, S. D., L. A. N. Sa, and L. W. Morrison. 2004. Establishment and dispersal of the fire ant decapitating fly *Pseudacteon tricuspis* in North Florida. Biol. Control 29:179-188.

144. Reagan, S. R., J. M. Ertel, and V. L. Wright. 2000. David and Goliath retold: fire ants and alligators. J. Herpetol. 34:475-478.

145. Reinert, J. A., and S. J. Maranz. 2001. Controlling the red imported fire ant, *Solenopsis invicta*, in urban landscapes. Int. Turfgrass Soc. Res. J. 9:787-792.

146. Rhoades, R. B., C. T. Stafford, F. K. James. 1989. Survey of fatal anaphylactic reactions to imported fire ant stings. J. Allergy Clin. Immunol. 84:159-162.

147. Ross, K. G. 1992. Strong selection on a gene that influences reproductive competition in a social insect. Nature. 355:347-349.

148. Ross, K. G., and L. Keller. 1995. Ecology and evolution of social organization: insights from fire ants and other highly eusocial insects. Annu. Rev. Ecol. Syst. 26: 631-656.

149. Ross, K. G. and L. Keller. 1998. Genetic control of social organization in an ant. Proc. Natl. Acad. Sci. 95:14232-14237.

150. Ross, K. G. and L. Keller. 2002. Experimental conversion of colony social organization by manipulation of worker genotype composition in fire ants (*Solenopsis invicta*). Behav. Ecol. Sociobiol. 51:287-295.

151. Ross, K. G., and D. D. Shoemaker. 1993**.** An unusual pattern of gene flow between the two social forms of the fire ant, *Solenopsis invicta*. Evolution 47: 11595-1605.

152. Ross, K. G., and D. D. Shoemaker. 2005. Species delimitation in native South American fire ants. Mol. Ecol. 14:3419-3438.

153. Ross, K. G., E. L. Vargo, and D. J. C. Fletcher. 1987. Comparative biochemical genetics of three fire ant species in North America, with special reference to the two social forms of *Solenopsis invicta* (Hymenoptera:Formicidae). Evolution. 41:979-990.

154. Sikes, J., and K. A. Arnold. 1986. Red imported fire ant (*Solenopsis invicta*) predation on cliff swallow (*Hirundo pyrrhonota*) nestlings in east-central Texas. Southwest. Nat. 31:105-106.

155. Slowik, T. J., H. G. Thonvilson, and B. L. Green. 1997. Response of red imported fire ants to magnetic fields in the environment. Southwest. Entomol. 22:301-306.

156. Sokolova, Y. Y., and J. R. Fuxa. 2008. Biology and life-cycle of the microsporidium *Kneallhazia solenopsae* Knell Allen Hazard 1977 gen. n., comb. n., from the fire ant *Solenopsis invicta*. Parisitology. 135:903-929.

157. Stoker, R. L., D. K. Ferris, W. E. Grant, and L. J. Folse. 1994. Simulating colonization by exotic species: a model of the red imported fire ant (*Solenopsis invicta*) in North America. Ecol. Modell. 73:281-292.

158. Streett, D. A., T. B. Freeland Jr., and R. K. VanderMeer. 2006. Survey of imported fire ant (Hymenoptera:Formicidae) populations in Mississippi. Fla. Entomol. 89:91-92.

159. Summerlin, J. W., and S. E. Kunz. 1978. Predation of the red imported fire ant on stable flies. Southwest. Entomol. 3:260-262.

160. Sutherst, R. W., and G. Maywald. 2005. A climate model of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae): implications for invasion of new regions, particularly Oceania. Environ. Entomol. 34:317-335.

161. Tracy, J. M., J. G. Demain, J. M. Quinn, D. R. Hoffman, D. W. Goetz, and T. M. Freeman. 1995. The natural history of exposure to the imported fire ant (*Solenopsis invicta*). J. Allergy Clin. Immunol. 95:824-828.

162. Trager, J. C. 1991. A revision of the fire ants, *Solenopsis geminata* group (Hymenoptera: Formicidae). J. NY. Entomol. Soc. 99:141-198.

163. Tschinkel, W. R. 1988a. Colony growth and the ontogeny of worker polymorphism in the fire ant, *Solenopsis invicta.* Behav. Ecol. Sociobiol. 22: 103-115.

164. Tschinkel, W. R. 1988b. Distribution of the fire ants *Solenopsis invica* and *S. geminata* (Hymenoptera: Formicidae) in northern Florida in relation to habitat and disturbance. Ann. Entomol. Soc. Am. 81:76-81.

165. Tschinkel, W. R. 1992a. Brood raiding and the population dynamics of founding and incipient colonies of the fire ant, *Solenopsis invicta*. Ecol. Entomol. 17:179-188.

166. Tschinkel, W. R. 1992b. Brood raiding in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae): laboratory and field observations. Ann. Entomol. Soc. Am. 85:638-646.

167. Tschinkel, W. R. 1993. Resource, allocation, brood production, and cannibalism during colony founding in the fire ant, Solenopis invicta. Behav. Ecol. Sociobiol. 33:209-223.

168. Tschinkel, W. R. 1995. Stimulation of fire ant queen fecundity by a highly specific brood stage. Ann. Entomol. Soc. Am. 88:876-882.

169. Tschinkel, W. R. 1998. An experimental study of pleometrotic colony founding in the fire ant, *Solenopsis invicta*: what is the basis for association? Behav. Ecol. Sociobiol. 43:247-257.

170. Tschinkel, W. R. 2006. The Fire Ants. Cambridge, Mass: The Belknap Press of Harvard University Press; 723 pp.

171. Tschinkel, W. R., and D. F. Howard. 1983. Colony founding by pleometrosis in the fire ant, *Solenopsis invicta*. Behav. Ecol. Sociobiol. 12:103-113.

172. Tschinkel, W. R., E. S. Adams, and T. Macom. 1995. Territory area and colony size in the fire ant, *Solenopsis invicta.* J. Anim. Ecol. 64: 473-480.

173. Tsutsui, N. D., A. V. Suarez, and F. W. Allendorf. 2003. The colony structure and population biology of invasive ants. Conserv. Biol. 17:48-58.

174. Tuberville, T. D., J. R. Bodie, J. B. Jensen, L. Laclaire, and G. J. Whitfield. 2000. Apparent decline of the southern hog-nosed snake, *Heterodon simus*. J. Elisha Mitchell Soc. 116:19-40.

175. Valles, S. M., Oi, D. H., Pereira, O. P., and Williams, D. F. 2002. Detection of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) in *Solenopsis invicta* (Hymenoptera: Formicidae) by multiplex PCR. J. Invert. Pathol. 81:196-201.

176. Valles, S. M., C. A. Strong, P. M. Dang, W. B. Hunter, R. M. Pereira, D. H. Oi, A. M. Shapiro, and D. F. Williams. 2004. A picorna-like virus from the red imported fire ant, *Solenopsis invicta*: initial discover, genome sequence, and characterization. Virology. 328:151-157.

177. Valles, S. M., D. H. Oi, and Porter S. D. 2009. *Kneallhazia* (=*Thelohania*) solenopsae infection rate of *Pseudacteon curvatus* flies determined by multiplex PCR. Fla. Entomol. 92:344-349.

178. Vander Meer, R. K., and L. E. Alonso. 2002. Queen primer pheromone affects conspecific fire ant (*Solenopsis invicta*) aggression. Behav. Ecol. Sociobiol. 51:122-130.

179. Vander Meer, R. K., and G. N. Fritz. 2003. Sympatry of polygyne and monogyne colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 96:86-92.

180. Vander Meer, R. K., C. S. Lofgren, and F. M. Alvarez. 1985. Biochemical evidence for hybridization in fire ants. Fla. Entomol. 68:501-506.

181. Vander Meer, R. K., L. Morel, and C. S. Lofgren. 1992. A comparison of queen oviposition rates from monogyne and polygyne fire ant, *Solenopsis invicta*, colonies. Physiol. Entomol. 17:384-390.

182. Vander Meer, R. K., R. M. Pereira, S. D. Porter, S. M. Valles, and D. H. Oi. 2007. Area-wide suppression of invasive fire ant *Solenopsis* spp. populations. pp. 487-496. In M. J. B. Vreysen, A. S. Robinson, and J. Hendrichs (eds.), Area-wide control of insect pests: from research to field implementation. Springer SBM, Dordrecht, Netherlands.

183. Vargo, E. L. and D. J. C. Fletcher. 1987. Effect of queen number on the production of sexuals in natural populations of the fire ant, *Solenopsis invicta*. Physiol. Entomol. 12:109-116.

184. Vargo, E. L. and D. J. C. Fletcher. 1989. On the relationship between queen number and fecundity in polygyne colonies of the fire ant, *Solenopsis invicta*. Physiol. Entomol. 14:223-232.

185. Vargo, E. L., and S. D. Porter. 1989. Colony reproduction by budding in the polygyne form of *Solenopsis invicta* (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 82:307-313.

186. Varlamoff, S., W. J. Florkowski, J. L. Jordan, J. Latimer, and K. Braman. 2001. Georgia homeowner survey of landscape management practices. HortTechnology. 11:326-331.

187. Vasquez, R. J., S. D. Porter, and J. A. Briano. 2006. Field release and establishment of the decapitating fly Pseudacteon curvatus on red imported fire ants in Florida. BioControl 51:207-216

188. Vickers, A. J. 2001. The use of percentage change from baseline as an outcome in a controlled trial is statistically inefficient: a simulation study. BMC Medical Research Methodology 6: 1-4.

189. Vinson, S. B. 1997. Invasion of the red imported fire ant (Hymenoptera: Formicidae): spread, biology, and impact. Am. Entomol. 43:23-39.

190. Vogt, J. T., J. T. Reed, and R. L. Brown. 2005. Timing bait applications for control of imported fire ants (Hymenoptera: Formicidae) in Mississippi: efficacy and effects on non-target ants. Int. J. Pest Manag. 51:121-130.

191. Walin, L., P. Seppa, and L. Sundstrom. 2001. Reproductive allocation within a polygyne, polydomous colony of the ant Myrmica rubra. Ecol.26:537-546.

192. Waters, E. M., J. E. Huff, and H. B. Gerstner. 1977. Mirex. An Overview. Environ. Res. 14:212-222.

193. Way, M. J., and K. C. Khoo. 1992. Role of ants in pest management. Annu. Rev. Entomol. 37:479-503.

194. Wendel, L. E., and S. B. Vinson. 1978. Distribution and metabolism of a juvenile hormone analogue within colonies of the red imported fire ant. J. Econ. Entomol. 71:561-565.

195. Wetterer, J. K., and J. A. Moore. 2005. Red imported fire ants (Hymenoptera: Formicidae) at gopher tortoise (Testudines: Testudinidae) burrows. Fla. Entomol. 88:349-354.

196. Wetterer, J. K., and R. R. Snelling. 2006. The red imported fire ant, *Solenopsis invicta*, in the Virgin Islands (Hymenoptera: Formicidae). Fla. Entomol. 89:431-434.

197. Wheeler, D. E. 1991. The developmental basis of worker caste polymorphism in ants. Am. Nat. 138:1218-1238.

198. Williams, D. F. 1983. The development of toxic baits for the control of the imported fire ant. Fla. Entomol. 66:162-172.

199. Williams, D. F., G. J. Knue, and J. J. Becnel. 1998. Discovery of *Thelohania solenopsae* from the red imported fire ant, *Solenopsis invicta*, in the United States. J. Invertebr. Pathol. 71:175-176.

200. Williams, D. F., D. Oi, and G. J. Knue. 1999. Infection of red imported fire ant (Hymenoptera: Formicidae) colonies with the entomopathogen *Thelohania solenlopsae* (Microsporidia: Thelohaniidae). J. Econ. Entomol. 92:830-836.

201. Williams, D. F, H. L. Collins, and D. H. Oi. 2001. The Red Imported Fire Ant (Hymenoptera: Formicidae): An Historical Perspective of Treatment Programs and the Development of Chemical Baits for Control. Am. Entomol. 47:146-149.

202. Williams, D. F., D. Oi, S. D. Porter, R. M. Pereira, and J. A. Briano. 2003. Biological control of imported fire ants (Hymenoptera: Formicidae) Am. Entomol. 49:150-163.

203. Williams, R. N., J. R. Panaia, D. Gallo, and W. H. Whitcomb. 1973. Fire ants attacked by Phorid flies. Fla. Entomol. 56:259-262.

204. Wilson, E. O. 1952. A report on the imported fire ant *Solenopsis saevissima* var. Forel in the gulf states. J. Ala. Acad. Sci. 52:21-22.

205. Wilson, E. O., and J. H. Eads. 1949. A report on the imported fire ant *Solenopsis saevissima* var. richteri Forel in Alabama. Special mimeographed report to the Director of the Alabama State Department of Conservation.

206. Wilson, N. L., J. H. Dillier, and G. P. Markin. 1971. Foraging territories of imported fire ants. Ann. Entomol. Soc. Am. 64:660-665.

207. Wojcik, D. P., C. R. Allen, R. J. Brenner, E. A. Forys, E. A. Jouvenaz, and R. S. Lutz. 2001. Red imported fire ants: impact on biodiversity. Am. Entomol. 47:16-23.

208. Wood, L. A., and W. R. Tschinkel. 1981. Quantification and modification of worker size variation in the fire ant, *Solenopsis invicta.* Insectes Soc. 28: 117-128.

209. Zar, J. H. 1999. Data Transformations pp. 275-277 IN T. Ryu ed. Biostatistical Analysis. Prentice Hall. 663 pp.

210. Zhang RunZhi, Li YingChao, Liu Ning, and S. D. Porter. 2007. An overview of the red imported fire ant (Hymenoptera: Formicidae) in mainland China. Fla. Entomol. 90:723-731.