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Integrated management of the red imported fire ant in pastures

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INTEGRATED MANAGEMENT
OF THE RED IMPORTED FIRE ANT
IN PASTURES

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ABSTRACT

The effects and interactions of chemical, cultural, and biological control of red imported fire ant (RIFA) populations were evaluated in two replicated studies in pastures. The first study was conducted from 2005 through 2007 in Washington parish, Louisiana (WP) where the biological control *Psuedacteon tricupsis* phorid flies were present and at the LSU AgCenter St. Gabriel Research Station in St. Gabriel, Louisiana (SG) where *Psuedacteon tricuspis* flies were absent. The second study was conducted from May 2009 to May 2010 at St. Gabriel in the presence of phorid flies. In both studies, RIFA population indices in sites treated with methoprene in the presence of phorid flies were significantly less than at pretreatment after one year compared to six months in the absence of phorid flies. In study one, methoprene treatments did not significantly reduce the abundance of phorid flies or *Kneallhazia solenopsae* infections. In study two, the effects of plowing and methoprene treatments did not interact, and the height of RIFA mounds in plowed pastures was significantly reduced for nine months while methoprene treatment had no effect on mound height. The results of these two studies suggest that the effects of methoprene treatments on RIFA populations are temporally extended in areas where phorid flies are present and that methoprene treatments do not negatively impact the abundance of phorid flies. Since plowing significantly reduces the height of RIFA mounds for nine months and methoprene does not reduce the height of RIFA mounds, plowing is a more effective tool that producers can use rather than methoprene treatments to negate RIFA related damage to hay equipment. However, treatment of areas to reduce RIFA population density to diminish direct impact of RIFA's on animal and human health can be achieved with methoprene applications, and the temporal and economic benefits of these treatments should be extended in areas where phorid flies are present.

INTRODUCTION

The red imported fire ant (RIFA), *Solenopsis invicta* Buren, is an invasive pest that was inadvertently introduced into the United States in the early 1900's (Creighton 1930). The most recent estimate of economic losses to the US economy was six billion dollars per year due to costs of control and losses in urban and agricultural settings (Lard et al. 2001). In a survey that was done in Texas to characterize and estimate RIFA related agricultural losses, respondents reported RIFA related damage and control costs which collectively totaled greater than 90 million dollars per year (Lard et al. 2006), and 55% of the production losses was in cattle operations. The RIFA causes problems in pastures where large mounds can damage hay equipment and result in losses in forage production (Barr and Drees 1996).

Unfortunately, chemical treatments of pastures are too expensive for producers to justify the costs when weighing the benefits of treating pastures with chemicals to minimize damages caused by the RIFA. Therefore, integrated strategies involving chemical, cultural, and biological control agents to the RIFA need to be developed in order to help producers reduce RIFA related damages and control costs. The recent introductions of *Psuedacteon* phorid flies have established populations that are currently acting as biological control agents to the RIFA (Callcott et al. 2011), and the microsporidian pathogen *Kneallhazia solenopsae* has been found to be present in wild populations of the RIFA in the U.S. (Sokolova et al. 2004). However, there have been no replicated studies that have presented data on the effects or interactions of phorid flies and *K. solenopsae* infections in integrated strategies to manage RIFA populations. Furthermore, the effects and interactions of common cultural practices, such as plowing, have not been evaluated as parts of integrated strategies for RIFA management in pastures. The first objective of this study was to evaluate the temporal effects of methoprene treatments on RIFA populations in the presence and absence of phorid flies, and to evaluate the effects of methoprene treatments on the incidence of *K. solenopsae* infections and the abundance of *P. tricuspis* flies in methoprene treated pastures. The second objective of this study was to perform a longitudinal evaluation of the temporal effects of methoprene in the presence and absence of phorid flies as well as to compare effects of and possible interactions of plowing and methopene treatments.

CHAPTER 1. LITERATURE REVIEW

1.1 Biology of Red Imported Fire Ants

Imported fire ants were first reported in the United States in the Mobile Bay, Alabama, area in 1930 (Creighton 1930). At that time, the imported fire ants were classified as *Solenopsis saevissima richteri*. Later studies described *S. saevissima richteri* as having a light and dark color phase (Wilson and Eads 1949; Wilson 1951; Wilson and Brown 1958). Subsequently, Buren (1972) separated *S. saevissima richteri* into two species. The light color phase was named *Solenopsis invicta* Buren and the dark color phase was named *Solenopsis richteri* Buren. The species *S. invicta* is now called the red imported fire ant (RIFA). The RIFA is considered highly invasive (Porter and Savignanno 1990) and the area of its distribution in the United States is estimated to be at least 130 million ha spread over 12 Southeastern states (Pereira 2003) and is currently expanding throughout the Southwest and northward along the West Coast (Korzukhin et al. 2001).

The RIFA, like all other ants, is in the family Formicidae in the order Hymenoptera (Johnson and Triplehorn 2005). The RIFA is a predator of other arthropods, seeds, and small mammals, birds and reptiles (Seaman and Marino 2003, Parys and Johnson 2012, Wetterer et al. 2007, Allen et al. 2000, Orrock 2004). However, the RIFA also have been shown to mutually exist with honeydew producing hemipterans, such as aphids and scale insects (Kaplan and Eubanks 2005). Members of RIFA colonies excavate soil to build mounds as nests. The RIFA is classified as eusocial, which means that an individual RIFA colony operates using a caste system that is comprised of at least two generations of siblings that are either sterile workers, which tend brood or forage, or reproductives (Gullen and Cranston 2005).

The RIFA exhibits two social form structures, the monogyne social form and the polygyne social form (Glancey et al. 1975). The term "gyne" means "queen" in RIFA social form definition. Monogyne and polygyne RIFA differ behaviorally; monogyne ants are aggressive toward conspecifics from other colonies while polygyne ants are not (Ross and Keller 1995). Monogyne colonies occupy a single mound and do not share resources or workers with conspecifics from other colonies (Glancey et al. 1975). On the other hand, polygyne colonies can have multiple queens in a single mound and/or multiple queens in multiple mounds that all share food and workers (Glancey et al. 1975).

Ross and Keller (1998) reported that a single genomic element marked by the protein encoding gene Gp-9 is responsible for the existence of the two RIFA social forms. The gene Gp-9 encodes for a pheromone binding protein commonly thought to be involved in chemoreception in insects (Kreiger and Ross 2002). Kreiger and Ross (2002) stated that monogye RIFA queens have homozygous *BB* alleles and that workers with *B* are always associated with monogyne queens. Polygyne queens have heterozygous *Bb* alleles and workers with *b* are always associated with polygyne queens. Polygyne queens do produce a portion of worker and female sexual offspring that have the *BB* alleles; but the social behavior of those ants is likely regulated by a *b*-like allele (Kreiger and Ross 2002). The workers in polygyne colonies execute queens that do not carry the recessive *b* allele or a *b*-like allele. Therefore, the polygyne social form is preserved through natural selection by the workers (Keller and Ross 1998). Hallar et al. (2007) stated that the *b* allele is a deleterious double recessive allele in the RIFA. Homozygous *bb* RIFA queens do not mature normally and gain very little weight after pupation (Keller and Ross 1993, 1999). Homozygous *bb* workers also are smaller than workers with the *B* allele (Goodisman et al. 1999).

In addition to the difference in aggressiveness toward conspecifics, monogyne and polygyne RIFA colonies also have different reproductive strategies. The monogyne RIFA exclusively participate in mating flights to establish new colonies. The virgin queens leave the colony mound and take flight to mate with male reproductive RIFA while in the air (Morrill 1974). Male and female RIFA reproductives have been captured as high as 140 m above ground (Fritz 2011). Once mated, RIFA queens descend to the ground carrying a lifetime supply of sperm in the spermathaecae (Tschinkel 2006). The queen will then lose her wings, and find a suitable place to start a new colony. After her wings are shed, histolysis of the flight muscles begins, and the proteins are reabsorbed to use in egg production while fat reserves provide the energy for the newly mated queen (Burns et al. 2007). Although polygyne RIFA reproductives can participate in mating flights, polygyne RIFA also establish new colonies through a process called budding in which workers migrate with queens from a parent colony to a new location.

1.2 Impact of Red Imported Fire Ants as Invasive Species

The latest estimate of RIFA related damage and control costs in the United States economy was $ 6 billion USD/ year (Pereira 2003). The RIFA is aggressive and has a potent sting which can cause a wheal at the site of the sting with a pustule forming after 24 h. Allergic reactions to RIFA envenomation, which rarely result in death, also have been reported (deShazo et al. 1990). Furthermore, RIFA predation has been shown to significantly reduce populations of native insects and reptiles, birds, and mammals (Pereira 2003). For example, the RIFA competitively reduces the species richness and total abundance of native ant populations (Porter and Savignano 1990). Even as early as 1949, reports of the negative effects of RIFA predation on squirrel and rabbit young had surfaced (Bruce et al. 1949). Allen et al. (2000) regressed changes in the abundance of the northern bobwhite quail against the number of years post RIFA invasion and reported that the presence of the RIFA has a negative effect on the abundance of the northern bobwhite quail and proposed that the RIFA negatively impacted northern bobwhite quail abundance directly by preying on pipping quail chicks or indirectly by competing with the northern bobwhite quail for food. Subsequently, Seymour (2007) reported the first anecdotal evidence that the RIFA is capable of breaching eggs of the northern bobwhite quail. Landers (1980) also reported that RIFA are predators of gopher tortoise hatchlings.

The effects of the RIFA on native wild fauna are great, but the damages that the RIFA causes in production agriculture are perhaps the most noted by humans. Eubanks (2002) reported that the RIFA negatively affected the abundances of approximately 40 species of natural enemies of arthropod pests in cotton and soybean. In a survey that was done in Texas to characterize and estimate RIFA related agricultural losses, 938 respondents across a variety of agricultural production systems reported RIFA related damage and control costs which collectively totaled greater than 90 million dollars per year (Lard et al. 2006). The largest portion of production losses (55 percent) was in cattle operations, followed by companion animal operations at 14 percent, and field crops and hay at 12 percent. Large RIFA mounds in pastures can damage equipment and reduce forage production.

Although the RIFA has been associated with many types of damage to production agriculture, the presence of the RIFA in production agriculture can be beneficial because the RIFA preys on many arthropod agricultural pests. Among the most notable pests reduced by RIFA predation in row crops is the sugar cane borer. Reagan et al. (1972) reported that sugar cane borer infestation and damage increased by an average of 69 % in sugar cane plots that were treated with mirex to control RIFA populations. Burns and Melancon (1977) attributed a dramatic reduction in the number of lone star ticks in areas infested with the RIFA. The RIFA is also a predator of the horn fly. Hu and Frank (1996) seeded dung pats in the field with laboratory reared horn fly eggs and reported that the presence of the RIFA contributed to a 79 % average increase horn fly mortality.

1.3 Chemical Control of Red Imported Fire Ants

Despite the benefit that the RIFA provides to agriculture as a predator of other arthropod agricultural pests, the USDA- APHIS developed three programs to attempt eradication of the RIFA using large scale chemical treatments (Tschinkel 2006). The chemical chlordane was used in the first program that was initiated in 1948 because of rising concerns over losses in the hay industry caused by the RIFA. Chlordane was made available to farmers as a dust that was to be applied to individual RIFA mounds. When subsequent surveys in Mississippi showed that the RIFA was still spreading into Mississippi, it was apparent that the area wide control program with chlordane would not result in eradication of the RIFA, and the program was terminated in 1951 (Tschinkel 2006). The second program to reduce RIFA populations involved spraying eight to ten million hectares of land across ten southeastern states with clay granules containing heptachlor and dieldrin, but the program ended in 1962 after many deaths of wildlife, pets and livestock were linked to the toxicities of heptachlor and dieldrin (Tschinkel 2006). The third RIFA eradication program distributed bait containing the chemical mirex over 45 million hectares of land between 1967 and 1975 using aerial and ground applications. However, the dangers of large scale use of mirex baits became more apparent after toxicity studies indicated that kepone, a chemical byproduct released during mirex degradation, caused cancer in mice and bioaccumulated and biomagnified in the environment. These health concerns prompted the cancellation of the mirex label in 1973, and mirex use was subsequently banned in 1978.

Clearly, eradicating the RIFA by using chemicals alone was never feasible, but chemical treatments are considered useful to attain temporary control of RIFA populations.

Presently, only baits containing hydramethylnon, pyriproxifen and (s)-methoprene, or a mixture of hydramethylnon and (s)-methoprene are registered for treating pastures to control RIFA (eXtension.org). Pyroproxifen and (S)-methoprene are classified as insect growth regulators (IGRs). Feeding IGR's to RIFA laboratory colonies can result in the death of colonies due to a decrease or complete stoppage of the production of eggs, larvae, and pupae (Vinson and Robeau 1974). Treatment of RIFA colonies with an IGR also can cause shifts in social caste differentiation. For example, Robeau and Vinson (1976) reported that RIFA colonies that were only producing minor workers began producing major workers, intercastes and sexuals following exposure to an IGR. Field applications of IGR's can greatly reduce RIFA populations in treated sites 2-6 mo. post treatment, but RIFA populations often equal or exceed pretreatment levels after 12 mo. post treatment (Banks 1986, Calcott and Collins 1992, Aubuchon et al. 2006).

1.4 Cultural Control of Red Imported Fire Ants in Pastures

Dragging and plowing are routine pasture management techniques practiced in livestock and hay production operations. Dragging pastures fertilizes soil and kills internal parasites of livestock by scattering dung pats (Chamblis et al. 2006). Plowing is often done in the fall to prepare soil for the planting of supplemental winter forage. For example, rye grass is commonly planted in pastures throughout the southeastern U.S. in the fall, grazed by the cattle during the winter and harvested for hay in the warmer months. Dragging and plowing both reduce the size of RIFA mounds in pastures. However, the dragging of pastures has not produced consistent results as a cultural control of RIFA populations, and only one study exists on the effects of plowing pastures on RIFA populations (Colby et al. 2008). There have been two studies that evaluated the temporal effects of dragging to reduce RIFA populations in pastures. Wilson (1981) reported that the effects of dragging pastures two to five times during the warmer months on RIFA populations were temporally insignificant, but that the height of RIFA mounds in pastures dragged once or twice during the winter was reduced by 50 % for 7 months and 25 % for 11 months. On the other hand, a study in southern Oklahoma found no significant differences in the height, width, or number of RIFA mounds measured in pastures dragged in the winter compared to control pastures at 104 days after dragging (Vogt et al. 2001). Colby et al. (2008) investigated the effects of plowing pastures on RIFA populations and reported that most RIFA colonies persisted in pastures plowed in the fall, but that the mean height of the mounds in the plowed pastures was significantly smaller than those of mounds in unplowed pastures five months after plowing. Since plowing and dragging are routine farm practices that reduce the size of RIFA mounds, perhaps plowing and/ or dragging could be beneficial for integrated strategies to suppress RIFA populations in pastures.

1.5 Biological Control of Red Imported Fire Ants

Since the two major economically and environmentally hazardous government attempts to eradicate the RIFA by indiscriminately treating millions of hectares of land throughout the southeast with toxic chemical baits (Heptachlor, Dieldren, and Mirex) multiple times had failed, efforts to find other means of managing RIFA populations began in the United States. Jouvanez (1983) conducted a survey to identify natural enemies of the RIFA in the native range in South America. Jouvanez (1983) reported that the RIFA hosted a variety of pathogens and parasitic arthropods that could not be found in the U.S. Currently, parasitoids and pathogens have been imported and released by the USDA-ARS in cooperation with several universities in the southeastern United States (Williams et al. 2003). The first two natural enemies imported to the

U.S. were a phorid fly parasitoid, *Psuedacteon tricuspis* Borgmeier, and a microsporidian pathogen, *Kneallhazia solenopsae* Knell.

Female phorid flies attack foraging RIFA workers to oviposit an egg into the thorax of the RIFA worker. Consoli et al. (2001) reported that that the egg and first instar larvae of the phorid fly develop in the thorax of the RIFA worker but that the second and third instar larvae develop in the head capsule before the fly pupates out of the head capsule. Henne and Johnson (2007) conducted a laboratory study on RIFA colonies containing workers that were parasitized by phorid flies and reported that parasitized workers remained inside of the RIFA mound until about eight to ten hours before pupation. Henne and Johnson (2007) also stated that the parasitized workers leaving the mound were highly mobile, and would travel to a grass thatch area and hide before the fly would pupate.

Even though phorid flies do kill RIFA workers, the concept of introducing phorid flies for biological control was that phorid flies could shift interspecific competitive interactions in favor of native ant species (Freener and Brown 1992). Mehdiabadi and Gilbert (2002) conducted a laboratory study on the effects of *P. tricuspis* phorid flies on the foraging abilities of the RIFA in the presence of another native ant competitor, *Forelius mccooki.*, and reported that the presence *P. tricuspis* allowed *F. mccooki* to better compete exploitatively.

Sequential releases to establish populations of six species of decapitating phorid flies in the genus *Psuedacteon* have been conducted in the southeast since 1998 (Calcott et al. 2011, Plowes et al. 2011, Porter et al. 2011). Currently, populations of two species, *P. tricuspis* and *P. curvatus*, are established in Louisiana. (Callcott et al. 2011). However, the impact that phorid flies are potentially having as biological control agents of the RIFA and the interactions between phorid flies and chemical control treatments in integrated strategies to manage the RIFA in the U.S. have not been evaluated in a replicated field study.

Prior to being re-assigned to the genus *Knellhazia* by Sokolova and Fuxa in 2008, the microsporidian *Knellhazia solenopsae* was named *Thelohania solenopsae* Knell.. Sokolova and Fuxa stated that molecular, morphological, ultrastructural, and life cycle data indicated the need to re-classify *T. solenopsae* to a different genus than *Thelohania* and proposed that a new genus named *Knellhazia* be created since SSUrDNA-sequence data indicated that the microsporidian belonged to a separate clade on the phylogenetic tree. The microsporidian *Kneallhazia solenopsae*, was the most commonly found microbial pathogen of the RIFA in Argentina (Briano et al. 1995). Briano and Williams (1997) suggested that *K. solenopsae* might be a potential candidate to serve as a biological control agent of the RIFA in the U.S. after observing significant reductions in the production of brood in RIFA colonies that were infected with *K. solenopsae*. Shortly afterwards, *K. solenopsae* was discovered in wild populations of the RIFA in Florida (William et al. 1998), and later in Louisiana (Sokolova et al. 2004).

The pathogen *K. solenopsae* primarily infects polygyne RIFA; however, reduced brood production in monogyne and polygyne laboratory colonies infected with *K. solenopsae* has been reported (Oi and Williams 2002.). Infection causes progressive destruction of the fat body of RIFA leading to reduced longevity of workers and reduced fecundity of queens (Briano and Williams 1997). *K. solenopsae* infections can be spread in RIFA populations artificially by feeding live brood infected with *K. solenopsae* to uninfected RIFA colonies. Williams (2002) reported that *K. solenopsae* infected RIFA populations in the field were reduced to a maximum of 63 %, and Valles and Pereira (2003) reported that RIFA colonies that were infected with *K. solenopsae* and treated with the chemical hydramethylnon experienced a two-fold cumulative mortality rate compared to uninfected colonies at 21 days post treatment. Therefore, *K. solenopsae* could be a useful element in integrated strategies to manage RIFA populations.

1.6 Integrated Control of Red Imported Fire Ants

The USDA-ARS conducted two unreplicated projects to demonstrate the efficacy of integrating chemical treatments with the two biological control agents *Knellhazia solenopsae* and *Pseudacteon tricuspis* to control RIFA populations. The first project was conducted from 2000 to 2003. The project compared the temporal effectiveness of a single treatment with 0.1 % granular fipronil on RIFA populations in the presence and absence of the fly *P. tricuspis* and the pathogen *K. solenopsae*. The project had a single site for each treatment, and was conducted in South Carolina on the fringe of the geographic range of the RIFA at that time. The RIFA population of the site treated with fipronil in the absence of the two biological control agents was approximately 85% of the pretreatment population for 1.4 years after treatment, while the RIFA population of a site treated with fipronil in the presence of the two biological control agents was approximately 95% of the pretreatment population for 3 years after treatment. The control site had an average 32% increase in population estimates (Oi et al. 2008). The second demonstration project which integrated a chemical treatment consisting of a 1:1 mixture of hydramethylnon and methoprene with *P. tricuspis* and *K. solenopsae* began in 2002. The project was conducted in Florida, Mississippi, Oklahoma, South Carolina, and Texas. Florida, Mississippi, and Texas were within the well-established range of the RIFA where a higher annual invasion rate than in South Carolina would likely occur. In 2003, Pereira issued an update on the progress of the project. Pereira reported that RIFA populations in sites that received chemical treatments in the presence of the two biological controls had been reduced by 85 – 95 % but that whether or not the reduction of the RIFA populations would be sustained was indeterminable at that time. In

2007, another report on this project surfaced (Vander Meer et al. 2007). However, no additional results were reported Oi et al. (2008) indicated that the fast dispersal of phorid flies and the increasing prevalence of *Kneallhazia solenopsae* would make it difficult to find areas that could be used as replicated control sites for study of interactions between chemical control and biological control. The purpose of this study was to analyze and evaluate data collected in a replicated RIFA integrated control study with sites both with and without *Psuedacteon tricuspis*.

CHAPTER 2. EVALUATING THE TEMPORAL EFFECTS OF METHOPRENE TREATMENTS ON RIFA POPULATIONS IN THE PRESENCE AND ABSENCE OF *PSEUDACTEON TRICUSPIS* FLIES AND THE INTERACTION OF METHOPRENE TREATMENTS WITH *PSUEDACTEON TRICUSPIS* POPULATIONS AND *KNEALLHAZIA SOLENOPSAE* INFECTIONS

2.1 Introduction

The red imported fire ant (RIFA), *Solenopsis invicta* Buren, was inadvertently brought into the United States in the early 1900's (Creighton 1930). Currently, the presence of RIFA populations causes estimated economic losses of six billion dollars per year in the U.S. (Pereira 2003). The distribution of the RIFA in the U.S. covers the Southeast and is expanding throughout the Southwest and northward along the West Coast (Korzukhin et al. 2001). Red imported fire ants aggressively attack humans and animals with painful stings, which can rarely lead to lethal allergic reactions (DeShazo 1990).

Apart from its painful and potentially dangerous sting, the RIFA creates problems in production agriculture. In a survey that was done in Texas to characterize and estimate RIFA related agricultural losses, 938 respondents across a variety of agricultural production systems reported RIFA related damage and control costs which collectively totaled greater than 90 million dollars per year (Lard et al. 2006). The largest portion of production losses (55 %) was in cattle operations, followed by companion animal operations at 14 %, and field crops and hay at 12 %. Large RIFA mounds in pastures can damage equipment and reduce forage production.

In the past, the U.S.D.A-APHIS developed three programs to attempt eradication of the RIFA using large scale chemical treatments (Tschinkel 2006). The chemical chlordane was used in the first program that was initiated in 1948 because of rising concerns over losses in the hay industry caused by the RIFA. When subsequent surveys in Mississippi showed that the RIFA was still spreading into Mississippi, it was apparent that the area wide control program with chlordane would not result in eradication of the RIFA, and the program was terminated in 1951 (Tschinkel 2006). The second program to reduce RIFA populations used the chemicals heptachlor and dieldrin, but ended in 1962 after many deaths of wildlife, pets and livestock were linked to the toxicities of heptachlor and dieldrin (Tschinkel 2006). The third program employed distribution of baits containing the chemical mirex in 1967. However, the dangers of large scale use of mirex baits became more apparent after toxicity studies indicated that kepones, a chemical byproduct released during mirex degradation, caused cancer in mice and bioaccumulated and biomagnified in the environment. These health concerns prompted the cancellation of the mirex label in 1973, and mirex use was subsequently banned in 1978. Clearly, eradicating the RIFA by using chemicals alone was never feasible, but chemicals treatments are considered useful to attain temporary control of RIFA populations.

The chemicals that are available in broadcast granular baits labeled for control of RIFA populations in pastures and rangelands grazed by cattle and companion animals are hydramethylnon, pyriproxifen, fenoxycarb, and methoprene. Hydramethylnon is an aminohydrazone compound that provides RIFA control from one to twelve months depending on the seasonal timing of the treatment (Williams et al. 1980). Pyriproxifen, fenoxycarb, and methoprene are insect growth regulators (IGR's) and depending on the time of treatment can provide control of RIFA populations for 4-12 months (extension.org). The insect growth regulators used for RIFA control mimic insect juvenile hormone and reduce or stop egg production in RIFA queens and kill or cause brood to differentiate into sexuals rather than workers which disrupts the replacement of workers in the colony (Cupp and O'Neal 1973). These effects of methoprene on treated colonies can be transient; in lab studies, queens have been shown to recover from the effects of IGR's and resume normal egg production (Bigley and Vinson 1979). Adult RIFA workers are unaffected by IGR's and eventually die of other causes. Death and lack of replacement of RIFA workers can be fatal to RIFA colonies. In cattle production operations, reducing RIFA populations, rather than eliminating them, is beneficial because RIFA populations prey on arthropod pests such as ticks and horn flies (Howard and Oliver 1978, Hu and Frank 1996, Melancon 1977). The cost of using chemicals to control RIFA populations is $23–60 per hectare for product per year, plus the price of labor, fuel and equipment required to make the treatments (Lard et al. 2006). In most cases, the expenses outweigh the benefits of treating pastures with chemicals to control RIFA populations.

Recently, biological control agents of the RIFA have been introduced into the U.S. The first introduced natural enemy of the RIFA was the parasitic phorid fly, *Psuedacteon tricuspis* Borgmeier. Releases to establish phorid fly populations in the southeastern states, including Louisiana, began in the late 1990's (Porter et al. 1999, Henne and Johnson 2007). The phorid fly *Pseudacteon tricuspis* is one of approximately twenty known species of *Psuedacteon* flies found parasitizing fire ants in South America (Jouvanez 1983). The flies attack foraging RIFA workers of a specific size regardless of social form (Morrison and Gilbert 1998). Attacks of phorid flies on foraging RIFA workers are thought to disrupt RIFA feeding behaviors which shifts inter and intra specific interactions in favor of native ants (Freener and Brown 1992).

An introduced pathogen of the RIFA, the microsporidian pathogen *Kneallhazia solenopsae*, was found to be the most common microbial pathogen found in RIFA populations in Argentina (Briano et al. 1995) *Knellhazia solenopsae* infections can be spread artificially by introducing infected RIFA pupae to uninfected RIFA colonies (Williams et al. 1999). Although *K. solenopsae* was imported and has been spread artificially, *K. solenopsae* infections do occur outside of areas where the pathogen has been artificially introduced into U.S. RIFA populations (Williams et al. 1998, Sokolova et al. 2004). Red imported fire ants have two social forms, monogyne (single queen) and polygyne (multiple queen) (Glancey et al. 1973a). *Kneallhazia solenopsae* infections are primarily found in polygyne RIFA colonies (Oi et al. 2004, Oi 2006, Milks et al. 2007). Infections of *K. solenopsae* reduce egg production of RIFA queens over a period of months causing a gradual decline in the number of RIFA workers, which is potentially deadly to RIFA colonies (Williams and Oi 1998). However, polygyne colonies of the RIFA infected with *K. solenopsae* can survive infections because some queens in polygyne RIFA colonies are likely to remain uninfected (Oi and Williams 2002).

The need for affordable ways to reduce RIFA populations in agriculture is particularly great in pastures. It is possible that the presence of biological control agents of the RIFA in the U.S. might temporally extend the suppression of RIFA populations in pastures achieved by chemical treatments. On the other hand, chemical treatments could reduce populations of biological control agents in treated areas. Therefore, there is a need to evaluate the effects and interaction of chemicals and biological controls in integrated strategies to control RIFA populations in replicated studies. One objective of this study was to evaluate the temporal efficacy of treating pastures with the chemical methoprene to control RIFA populations in pastures in the presence and absence of the phorid fly, *P. tricuspis*. A second objective of this study was to evaluate the effects of methoprene treatments on the incidence of both phorid flies and *K. solenopsae*.

2.2 Materials and Methods

- Study sites

The study was conducted in fourteen pastures, each approximately 4ha, from April 2005 to June 2007. Pasture grasses were a combination of crabgrass (*Digitaria spp*.), Bahaiagrass (*Paspalum notatum Flugge*), and common bermudagrass (*Cynodon spp.*) and were grazed by cattle. Eight pastures, which had phorid flies present, were in Washington parish, Louisiana (WP) where fine sandy loamy soils form rolling hills. Six pastures, which were devoid of phorid flies, were located on the St. Gabriel Research Station, St. Gabriel, Louisiana (SG) where soils are poorly drained and clayey and the land is flat. Climatic data from April 2005 through June 2007 were downloaded for one weather station at the LSU AgCenter Southeast Research Station in Franklinton, La. and one weather station at the LSU AgCenter St. Gabriel Research Station in St. Gabriel, La. by visiting the LSU AgCenter Agroclimatic Data System database (http://weather.lsuagcenter.com/reports.aspx?r=0). The climatic variables minimum, mean, and maximum ambient temperature and relative humidity as well as mean rainfall were compared using paired t-tests to evaluate differences between the climatic conditions at WP and in SG during the study. Methoprene treatments were replicated within pairs of pastures, four pair in WP and three pair in SG. The pastures were paired based on the least difference in the incidence of polygyny, which had been determined by PCR analysis (Valles and Porter 2003) of the RIFA samples from colonies in the pastures in the spring of 2004. In May 2005, on days when no rain was expected, Extinguish® granular fire ant bait (active ingredient [S]-methoprene) was broadcast over each treated pasture at a rate of 1.7 kg/ha using a Herd® spreader.

- Sampling and Data Analysis

Study data were collected from each pasture in WP and SG four times during the study. The first collection was made in April 2005 before treating the pastures with methoprene. Subsequent sampling was done every six months until October 2006. All data were collected inside a 0.3 ha circle painted in the center of each pasture. Centers were marked with a GPS to ensure that the centers could be found throughout the study (Table 2.1). Circles were made by two people. One person held one end of a 30.9 m rope, which represented the radius of a 0.3 ha circle, at the center. The other person held the opposite end of the rope, pulled the rope taunt, and walked the circumference of the circle while painting the circle boundary on the ground using a hand pump sprayer filled with a 3:1 mixture of latex paint to water. After the sampling area in a pasture was established, each RIFA mound was found and marked with a 35.5 cm tall flag.

Table 2.1. GPS coordiantes to the center of the eight sites in Washington parish (WP) and the six sites at Saint Gabriel (SG).

GPS Coordinates (hddd.ddddd Position Format; WGS 84 Map Datum)					
WP Sites	North (Lat.)	West (Lon.)	SG Sites	North (Lat.)	West (Lon.)
1	30.74288	89.9975	4SG	30.28314	91.0956
9	30.74771	89.8902	1CSG	30.27644	91.1035
5	30.76878	90.0297	WT4	30.26656	91.0933
4	30.7359	90.1577	WT6	30.26335	91.0924
3	30.72853	90.2253	E6-7	30.27157	91.095
2	30.59061	89.912	E2-3	30.27326	91.0952
7	30.76849	90.0362	.	.	.
8	30.74409	89.9635	.	.	.

- Red imported fire ant population index (RIFA PI)

Each RIFA mound in the circle was rated according to the USDA-Animal and Plant Health Inspection Service (APHIS) RIFA population index (RIFA PI) which was developed by Harlan et al. in 1981 and modified by Lofgren and Williams in 1982. Immediately after removing the top of each mound with a shovel, mounds were rated by first confirming the presence or absence of brood in the mound and then by visually estimating the number of RIFA workers in the mound. Based on the number of workers in a mound, a colony class rating six through ten was given to mounds with brood and one through five was given to mounds without brood (Table 2.2). Each colony class corresponds to a weighting factor (Table 2.2). The index is calculated as: $PI = \sum^{25} K(N_K)$, where K is the weighting factor and N is the number of mounds in the pasture that have corresponding values of K. More simply, the formula states that the value K is multiplied by the number of mounds with that K value (N_K), and the products of (N_k) for each value of K are summed to yield the RIFA population index (PI) for the pasture.

The PI value of each site was log transformed to normalize the data distribution and compared using a two-way ANOVA (PROC MIXED, SAS Institute, 2009) with repeated measures. The main effects in the model were: flies (± phorid flies), methoprene, (treated / untreated) and months (0, 6, 12, 18). The model also included all possible interactions between the main effects and compared the mean log RIFA PI of treated and untreated pastures at WP and SG within pairs of pastures to account for possible variation in treatment effects due to differences in the prevalence of polygyne mounds at the study sites, and a Tukey-Kramer adjustment was used to separate means ($\alpha = 0.05$).

- Polygyny and *Kneallhazia solenopsae* Infection

Twenty RIFA mounds were sampled to determine the incidence of polygyny and *Thelohania* infection in the RIFA population. Mounds were sampled at a calculated frequency. For example, if we found 60 mounds in a circle, the 20 samples were divided among the 60 mounds and every third mound would have been sampled. Samples consisted of at least 40 RIFA workers and were collected in 20 ml glass scintillation vials that were coated with Fluon®, which prevented the workers from escaping the vials. A vial was pushed into the top of each mound to collect RIFA workers that were responding to the disturbance of the RIFA mound. After at least forty RIFA workers were captured in each vial from each mound, and the vial was filled with 95 percent ethanol to preserve the workers. Multiplex polymerase chain reaction (PCR) methods (Valles and Porter 2003) were used to determine the social form of the workers in each sample. The presence of *Kneallhazia solenopsae* infection also was determined with multiplex PCR (Valles et al. 2002).

Table 2.2. Colony classification system developed by Harlan et al. (1981) and modified by Lofgren and Williams (1982) used to evaluate the effects of insecticides and insect growth regulators on RIFA populations.

	Brood Absent		Brood Present	
Number of Workers	Colony Class	Weighting Factor (K)	Colony Class	Weighting Factor (K)
<100	1	1	6	5
100-1,000	2	2	7	10
1,000-10,000	3	3	8	15
10,000-50,000	4	4	9	20
>50,000	5	5	10	25

Separate stepwise logistic regression analyses were used to analyze data on the prevalence of both polygyny and *Thelohania* infection in WP and at SG collectively at 0 (pretreatment) and 12 months post treatment (PROC LOGISTIC, SAS Institute, 2009). In the analysis of the data on the prevalence of polygyny, the main effects were methoprene (treated/ untreated), flies (± phorid flies), and time (pretreatment, 12 months). In the analysis of the data on the prevalence of *K. solenopsae* infection, the main effects were methoprene (treated/ untreated), flies (± flies) time (pretreatment and 12 months) and polygyne (polygyne/ monogyne). The polygyne effect was added to the model for of the prevalence of *K. solenopsae* infections because *K. solenopsae* infections have been shown to be strongly associated with the polygyne RIFA social form. In both models all possible interactions were considered, and correlations were significant when $P \leq 0.05$.

- Phorid Fly Counts

Phorid flies were counted at ten disturbed RIFA mounds in each circle after RIFA workers were alarmed by disturbing the upper portion of mounds with a shovel. Phorid flies were collected at each mound using an aspirator for five minutes or until no more flies arrived at the mound. A white paper plate was skewered by a 35.5 cm tall flag and erected adjacent to disturbed mounds to shelter the attacking phorid flies from wind and direct sunlight. Captured phorid flies were preserved in 95 % ethanol, transported to the laboratory, and counted.

Phorid fly count data from treated and control pastures in WP were log transformed and compared using a two –way ANOVA (PROC MIXED, SAS Institute, 2009) with repeated measures. The main effects in the model were treatment (treated/control) and time (pretreatment, 6, 12, 18). The interaction between the two main effects was the focus of this analysis, and a Tukey-Kramer adjustment was used to separate means ($\alpha = 0.05$).

2.3 Results

- Climatic Variables

The paired t-test comparisons of the mean minimum, maximum, and mean ambient temperature, relative humidity, and rainfall at SG and in WP indicated a significant difference between the minimum, maximum, and mean ambient temperature and minimum relative humidity (Table 2.3). The maximum and mean relative humidity and daily rainfall in WP were not different than at SG (Table 2.3).

Table 2.3. Paired t-test comparisons of mean +/- standard deviation climatic variables where weather data points (n) were collected on the same days from both weather stations, one in Washington parish (WP) and one in Saint Gabriel (SG), from April 2005 through October 2007.

Ambient Temperature (°C)	n	Mean ± Stdev. WP	Mean ± Stdev. SG	P (α = 0.05)
Minimum	519	15.92 ± 7.08	15.23 ± 7.00	<0.0001
Mean	519	22.62 ± 6.50	21.98 ± 6.31	<0.0001
Maximum	520	29.33 ± 6.35	28.73 ± 6.04	<0.0001
% Relative Humidity				
Minimum	461	39.41 ± 13.53	46.11 ± 13.45	<0.0001
Mean	460	65.56 ± 8.81	66.66 ± 17.31	0.16
Maximum	501	92.12 ± 6.49	92.83 ± 19.19	0.39
Rainfall (cm)				
Mean	518	0.10 ± 0.31	0.13 ± 0.44	0.05

- Red Imported Fire Ant Population Index (RIFA PI)

There was a statistically significant ($F = 4.10$, $df = 1, 10$, $P = 0.04$) three-way interaction between the main effects flies (± phorid flies), methoprene, (treated / untreated) and months (0, 6, 12, 18) in the statistical model (Table 2.4) For all paired pastures at both SG and WP, the pretreatment mean log RIFA PI was not significantly different, and for control pastures at both SG and WP, the mean log RIFA PI never differed significantly from pretreatment (Table 2.5). The mean log RIFA PI in treated pastures at six months post treatment was reduced by 44 percent and 77 percent at SG and WP, respectively (Table 2.5). The mean log RIFA PI in treated pastures at SG at 12 months had increased to 98 percent of the pretreatment PI, while the mean log PI in treated pastures in WP had increased to only 63 percent of the pretreatment PI (Table 2.5). Although the mean log RIFA PI in WP at 18 months post treatment did not differ statistically, the mean log RIFA PI was still only 81 percent of the pretreatment value (Table 2.5).

- Prevalence of Polygyny

The stepwise logistic regression analysis of the prevalence of polygyny indicated that the prevalence of polygyny was independent of the effects of methoprene because neither the methoprene main effect nor any methoprene interactions were retained in the model. The interaction between WP and SG through time was the only significant term in the model ($\chi^2 = 53.12$, $df = 1$, $P < 0.0001$). The incidence of polygyne colonies in pastures in WP at 12 months was 84 % less than at pretreatment, but the incidence of polygyne colonies in pastures at SG at 12 months was 68 % higher than at pretreatment (Figure 2.1).

Table 2.4. ANOVA table generated by PROC MIXED (SAS Institute 2009) for the analysis used to compare through time the mean log RIFA PI of methoprene treated and untreated sites in Washington parish and at Saint Gabriel.

Effect	Numerator Degrees of Freedom	Denominator Degrees of Freedom	F Value	Pr > F
Flies	1	10	4.71	0.06
Methopene	1	10	7.04	0.02
Methoprene*Flies	1	10	0.64	0.44
Months	3	10	47.59	<.0001
Months*Flies	3	10	12.13	0.001
Methoprene*Months	3	10	26.80	<.0001
Methoprene*Months*Flies	3	10	4.10	0.04

Table 2.5. Mean log RIFA PI ± SE in treated and control pastures at Washington parish (WP) and St. Gabriel (SG) from April 2005 to October 2007.

		Months Post Treatment		
WP	Pretreatment	6	12	18
Treated	6.86 ± 0.36 ab	1.61 ± 0.68 d	4.31 ± 0.38 ce	5.53 ± 0.33 ab
Control	6.58 ± 0.36 ab	5.20 ± 0.68 abcd	6.10 ± 0.61 abc	6.10 ± 0.61 abc
SG				
Treated	6.57 ± 0.42 abc	3.68 ± 0.78 bde	6.45 ± 0.44 abc	6.42 ± 0.38 ac
Control	6.81 ± 0.42 ab	6.30 ± 0.78 abc	6.68 ± 0.44 abc	6.31 ± 0.38 abc

Within rows means followed by the same letter were not significantly different (alpha = 0.05).

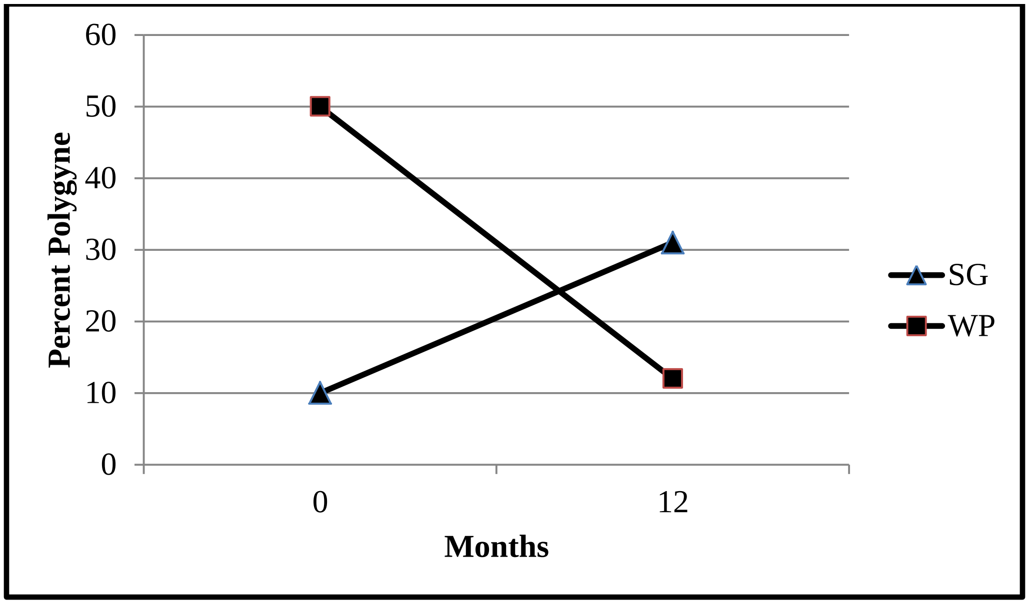

Figure 2.1. Change in percent polygyny from spring 2005 (0 months) to spring 2006 (12 months) in the RIFA populations in Washington parish (WP) and at Saint Gabriel (SG).

- Prevalence of *Knellhazia solenopsae* Infection

The stepwise logistic regression analysis of the prevalence of *K. solenopsae* infections in the RIFA populations in treated versus control pastures indicated that the effects of methoprene treatments on the prevalence of *K. solenopsae* infection were not statistically significant. The methoprene main effect (χ^2 = 6.52, df = 1, P = 0.01) and the polygyne main effect (χ^2 = 135.69, df = 1, P < 0.0001) were both significant in the model. The incidence of *K. solenopsae* infection in control sites was 38 % compare to 28 % in treated sites. The incidence of *K. solenopsae* infection in monogyne RIFA colonies was 4 % compared to 62 % in polygyne RIFA colonies.

- Phorid Fly Counts

The mean log phorid fly counts never differed statistically during the study in treated versus control sites (Table 2.6); however, phorids tended to be more abundant during fall sampling periods at 6 and 18 months compared to the spring sampling periods at 0 and 12 months (Table 2.6).

Table 2.6. Mean ± SE of *P. tricuspis* flies counted at treated and control pastures in Washington parish (WP) and at St. Gabriel (SG) at pretreatment and at six, twelve, and eighteen months post treatment.

		Months Post Treatment		
WP	Pretreatment	6	12	18
Treated	16.5 ± 8.2	40.5 ± 19.6	13.2 ± 4.8	115.7 ± 30.8
Control	22.5 ± 16.8	15.2 ± 6.6	3.2 ± 1.3	89.7 ± 31.4
SG				
Treated	0	0	0	0
Control	0	0	0	0

2.4 Discussion

At six months post treatment, the RIFA population estimates (PI) were 77 and 44 percent lower than pretreatment levels at WP and SG, respectively (Table 2.5). Aubuchon et al. (2006) reported that broadcast and bait station applications of methoprene reduced RIFA PI in grazed pastures at 8 and 16 weeks post treatment, but did not report any surveys past 16 weeks. At 12 months post treatment, the RIFA PI for treated pastures at SG was 98 percent of the pretreatment level while the RIFA PI for treated pastures in WP was 67 percent of the pretreatment level, which was statistically different from the pretreatment RIFA PI value (Table 2.5). There have been no other long-term studies on the effects of methoprene treatments on RIFA populations that have used the RIFA PI as a measure of population size. In a study that measured the effects of a chemical treatment on RIFA until the effects of the treatment were diminished, Callcott and

Collins (1992) reported that RIFA PI in fenoxycarb treated pastures was 57 % lower than the pretreatment PI at thirteen months, but the RIFA PI was 26 % higher than the pretreatment PI at 16 months post treatment. In this study, the RIFA PI in treated pastures at SG at 12 months was not different than at pretreatment. However, the RIFA PI in treated pastures in WP at 12 months was only 67 % of the pretreatment value, which was statistically significant. At 18 months, the RIFA PI in treated pastures in WP was still 20 % lower (not statistically significant) than the pretreatment RIFA PI (Table 2.5). The difference between the temporal suppression of the RIFA PI of treated pastures in WP and at SG could be attributed to differences in abiotic factors, such as soil type and minimum relative humidity, as well as biotic factors, such as the social form composition of the RIFA populations and the prevalence of *K. solenopsae*, or the presence and absence phorid flies.

The abiotic factors that were not different at WP and SG were average rainfall,maximum, average, and minimum ambient temperature, and maximum and average ambient relative humidity (Table 2.3). The abiotic factors that differed at WP and SG were minimum relative humidity and soil type. The minimum relative humidity was approximately 6.7 % lower in WP than SG during the study (Table 2.3). Pranschke and Bui (2003) measured mounds chosen randomly from plots at two locations in Louisiana and reported that increased relative humidity was correlated with increased RIFA PI. Milks et al. (2008) correlated soils high in phosphorus and organic matter with increased RIFA abundance in a study that evaluated RIFA populations randomly at 165 sites in Louisiana. Soils in WP were characterized as sandy loamy soils; soils at SG were silty, clayey soils called Sharkey. Sharkey soils are found in the flood plains of the Mississippi River and characteristically exhibit a higher content of organic matter compared to the soils of WP. The two previously mentioned studies evaluated abiotic factors on established

RIFA populations, while this study measured the growth of incipient or recovering RIFA colonies in chemically treated areas. The temporal changes in RIFA PI were evaluated by comparing RIFA PI of sites within WP and SG before and after chemical treatments. Therefore, differences between relative humidity or soil characteristics at WP or SG that might have contributed to different RIFA PI values at the two areas were accounted for in the statistical model.

The higher incidence of polygyny measured in WP compared to SG at pretreatment was another variable of concern when the experiment was being designed; hence, pastures were paired according to corresponding rates of polygyny to account for possible differences between the effects of methoprene treatments on the monogyne versus the polygyne RIFA social forms. However, the results showed that the effects of methoprene treatments were the same on monogyne and polygyne RIFA colonies, and that recolonization was not extremely social form biased

At pretreatment, 5 % of monogyne and 49 % of polygyne colonies at WP and SG combined were infected with the microsporidian, *K. solenopsae*. The observed higher incidence of *K. solenopsae* infection in the polygyne social form supports previous reports (Milks et al. 2008, Oi 2006). In this study, no significant decrease of the incidence of *K. solenopsae* was observed in treated sites at 12 months compared to pretreatment. Valles and Pereira (2003) found that polygynous *K. solenopsae* infected RIFA colonies exhibited significantly greater cumulative mortality than uninfected colonies exposed to hydramethylnon in laboratory and field studies. However, hydramethylnon treatments would eliminate colonies rapidly, while methoprene treatments would not kill all of the infected ants within colonies. There has only been one other study that reported the possible effects of methoprene treatments on the prevalence of *K. solenopsae* infection in RIFA populations. Sokolova et al. (2004) observed that the incidence of *K. solenopsae* infection in RIFA colonies in pastures that had been treated with methoprene one year prior to sampling was 1.6 percent compared to 15.4 – 73.3 percent in untreated pastures. Unfortunately, Sokolova et al. (2004) did not provide pretreatment data on *K. solenopsae* infection rates.

Another variable that was different between the study sites was the presence of the phorid fly, *P. tricuspis*. In this study, RIFA populations in treated pastures were suppressed significantly for 12 months in the presence of phorids compared to 6 months in the absence of phorid flies (Table 2.5). Similarly, in chapter three, a study on the temporal efficacy of methoprene treatments on RIFA populations in the presence of two species of phorid flies, *P. tricuspis* and *P. curvatus* conducted at SG is described, and the methoprene treatment was effective for one year in the presence of phorids compared to six months in the absence of phorids (Table 3.6).

There is only one other project that provided data on the temporal effects of chemical control of RIFA populations in the presence of biological control agents. Oi et al. (2008) conducted a non-replicated project to demonstrate the temporal efficacy of the chemical fipronil in the presence of *P. tricuspis* flies and *K. solenopsae* microsporidia and reported that RIFA populations were reduced by approximately 95 % for three years at sites where fipronil and biological control agents were integrated compared to 85 % for 1.4 years at sites that were treated with fipronil alone. These results suggest that the presence of biological control agents, particularly *P. tricuspis* and *K. solenopsae*, slows the growth of incipient and/or recovering RIFA colonies in areas where biological control agents are present and RIFA populations have been treated with chemicals. Unfortunately, the results of a subsequent study that was conducted in five states have not been reported.

The initial concept of introducing phorid flies for biological control into the U.S. was that the flies would inhibit the foraging behavior of the RIFA and shift interspecific competition in favor of native ants (Freener 2000). However, the effects of phorid flies on RIFA foraging should also directly affect growth of small RIFA colonies that are incipient or recovering in an area that has been treated with chemicals by decreasing foraging efficiency. Although direct proof of sustained effects of chemicals on RIFA populations being related to the effects of phorid flies is currently not available, the results of Oi et al. (2008), this study, and the study reported in chapter three each support the idea of sustained chemical treatment effects being attributable to the presence of phorid flies.

Tschinkel (1988) evaluated growth and ontogeny of RIFA worker polymorphism and reported that colony growth is logistic and that RIFA queens in incipient colonies began producing major workers, which are needed for foraging, as early as one month. *Psuedacteon tricuspis* flies preferably attack RIFA workers with a head width size of 0.92 ± 0.16 regardless of social form (Morrison and Gilbert 1998). The incipient monogyne colonies in Tschinkel's study produced workers large enough to be parasitized by *P. tricuspis* flies after five months. Since *P. tricuspis* flies were attacking RIFA workers at all of the treated sites in WP 12 months after pretreatment, incipient RIFA colonies were at a stage of growth suitable for producing workers of the size commensurate to host parasitism by *P. tricuspis* flies, which likely limited foraging behavior and the collection of required resources for rapid reproduction of workers (Tschinkel 1988).

Farnum and Loftin (2010) tested the impact of methoprene on *P. tricuspis* flies by exposing RIFA workers, which had been parasitized by the flies six and ten days prior, to exposure to methoprene. The authors reported that the proportion of emerged *P. tricuspis* flies from methoprene treated RIFA was significantly reduced regardless of post parasitism exposure time. In this study, the abundance of *P. tricuspis* flies at pretreatment and 12 months post treatment were comparable (Table 2.6); therefore, the impact of methoprene on local *P. tricuspis* populations was undetectable in the field.

Although the results of this study suggest that the presence of phorid flies could affect the recovery of RIFA populations after chemical treatments, coordinating a controlled field experiment to validate interaction of phorid flies and chemical treatments would be problematic. Establishing a phorid fly population takes time; and once established, a fly population disperses relatively quickly (Henne and Johnson 2007). Evaluations of RIFA population suppression through the use of chemical treatments requires at least two years because of natural seasonal changes in RIFA populations in conjunction with the temporal effects of chemical treatments, which can be detectable over one year post treatment. Therefore, future studies on the interactions of phorid flies and chemical treatments will likely be limited to conducting replicated studies in geographic areas with and without phorid flies, similar to what was done in this study.

It is common in studies comparing the effects of treatments between geographic areas with or devoid of a major variable like a parasite or agent of disease to use block designs to replicate treatments within a single experimental unit (Chaves 2010). In this study, the experimental units were WP, which had phorid flies and SG which did not have phorid flies and the blocks were the pairs of pastures within WP and SG. Hurlbert (1984) reviewed 167 experimental field ecology studies published in several ecological journals and found that 48 % of those studies that employed inferential statistics were psuedoreplicated. Hurlbert reported that the most common form of psudoreplication was simple psuedoreplication, which was the case in this study.   Hurlbert (1984) stated that simple psuedoreplication prevailed in those studies in which gross treatment effects were anticipated, only a rough estimate of the treatment effects were required, or the cost of replication was great. The author also stated that in many instances pseudoreplication is the best or only option available for undertaking such studies and that interspersing the experimental units with the research goal in mind can add validity to the statistical tests for significant differences. Pairing pastures in this study by percent polygyny adjusted the statistical model for differences in social form composition and rate of *K. solenopsae* infections; abiotic differences could not be accounted for by interspersing blocks but were accounted for in statistical model by comparing pretreatment RIFA population levels of treated and control pastures to post treatment population levels within WP and SG.

In summary, RIFA population estimates at sites treated with methoprene in the absence of phorid flies recovered to pretreatment levels within twelve months after treatment, but RIFA population estimates at sites treated with methoprene in the presence of phorid flies were still significantly lower than pretreatment estimates at twelve months post treatment. The incidence of the polygyne social form was significantly higher in WP than at SG and was adjusted for in the experimental design. Analysis of the methoprene treatment effects indicated that methoprene equally affects RIFA colonies regardless of social form, and that RIFA recolonization of chemically treated areas are not significantly social form biased. The incidence of *K. solenopsae* infection in the RIFA populations of WP and SG was much greater in the polygyne social form and was not reduced in treated pastures at 12 months post treatment. No long-term effects of methoprene treatments on phorid fly populations were detected. Collectively, these results suggest that the effects of IGR’s for controlling RIFA populations of either social form are temporally extended in the presence of phorid flies, which do appear to be viable biological elements in integrated strategies, but the value of *K. solenopsae* as an agent in integrated strategies when the chemical control agent is an IGR may be limited.

CHAPTER 3. COMPARING THE TEMPORAL EFFICACY OF PLOWING IN THE FALL AND TREATING WITH METHOPRENE IN THE SPRING AND EVALUATING POTENTIAL INTERACTIONS OF THETWO TREATMENTS IN THE PRESENCE OF *PSEUDACTEON* FLIES TO REDUCE RIFA POPULATIONS IN PASTURES

3.1 Introduction

The red imported fire ant (RIFA), *Solenopsis invicta* Buren, is a ubiquitous invasive ant in the southeastern United States (Calcott and Collins 1996). Red imported fire ant colonies excavate soil to form mounds that serve as nests. In many cases, RIFA mounds are large and numerous enough to damage farm equipment and reduce forage production (Barr and Drees 1996). A Texas survey revealed that agricultural producers most commonly suffer RIFA related production losses and damages in pastures (Lard et al. 2006). In the past, chemical control agents have been the champion of RIFA management strategies; however, in large areas such as pastures, chemical treatments are too expensive for producers to balance the benefits with the costs of using chemicals.

There are four chemicals (hydramethylnon, methoprene, pyroproxifen, and fenoxycarb) that are labeled for controlling RIFA populations in pastures (www.extension.org/pages/.../how-do-we-control-fire-ants-in-pastures). Hydramethylnon is an aminohydrazone toxicant, but methoprene, pyyroproxifen, and fenoxycarb are classified as insect growth regulators (IGR's). Insect growth regulators reduce or stop egg production of RIFA queens and kill or cause deformities or changes in cast differentiation in RIFA brood (Cupp and O'neal 1973, Robeau and Vinson 1974), but adult RIFA workers remain unaffected by IGR's (Banks et al. 1983). The greatest effect that IGR's have on RIFA populations occurs 4-6 months after treatment and is considered to be due to reduced forager replacement (Callcott and Collins 1992). Red imported fire ant population reduction rather than eradication is more favorable to producers because

RIFA populations significantly reduce populations of both horn flies and ticks in pastures (Howard and Oliver 1978, Hu 1996, Melancon 1977). The cost for product alone to treat pastures to manage RIFA populations is $23- 60 per hectare. After the cost of the equipment, fuel, and labor needed to apply the treatments also is considered, farmers often find treating pastures with chemicals to control RIFA populations financially infeasible. Therefore, chemical control must be integrated with other methods of control for RIFA population management to be cost effective.

Dragging and plowing are routine pasture management techniques practiced in livestock and hay production operations. Dragging pastures fertilizes soil and kills internal parasites of livestock by scattering dung pats (Chamblis et al. 2006). Plowing is often done in the fall to prepare soil for the planting of supplemental winter forage. For example, rye grass is commonly planted in pastures throughout the southeastern U.S. in the fall, grazed by the cattle during the winter and harvested for hay in the warmer months. Dragging and plowing both reduce the size of RIFA mounds in pastures. However, the dragging of pastures has not produced consistent results as a cultural control of RIFA populations, and only one study exists on the effects of plowing pastures on RIFA populations (Colby et al. 2008). There have been two studies that evaluated the temporal effects of dragging to reduce RIFA populations in pastures. Wilson (1981) reported that the effects of dragging pastures two to five times during the warmer months on RIFA populations were temporally insignificant, but that the height of RIFA mounds in pastures dragged once or twice during the winter was reduced by 50 % for 7 months and 25 % for 11 months. On the other hand, a study in southern Oklahoma found no significant differences in the height, width, or number of RIFA mounds measured in pastures dragged in the winter compared to control pastures at 104 days after dragging (Vogt et al. 2001). Colby et al.

(2008) investigated the effects of plowing pastures on RIFA populations and reported that most RIFA colonies persisted in pastures plowed in the fall, but that the mean height of the mounds in the plowed pastures was significantly smaller than those of mounds in unplowed pastures five months after plowing. Since plowing and dragging are routine farm practices that reduce the size of RIFA mounds, perhaps plowing and/ or dragging could be beneficial for integrated strategies to suppress RIFA populations in pastures.

The only study on the long-term effects of methoprene treatments in the presence of phorid flies was chapter two, and there are no studies on the interactions between the effects of plowing and methoprene treatments on RIFA populations. Furthermore, the temporal effects of plowing on RIFA populations have not been evaluated in the presence of *Psuedacteon* phorid flies. *Psuedacteon* flies have been imported into the U.S. and released as biological control agents of RIFA populations. The phorids are parasitoids of RIFA workers and disrupt RIFA foraging behavior which temporally limits the resources that drive colony growth.   The results of chapter two, suggested that extended temporal effects of methoprene on RIFA populations could have been attributed to the presence of the phorid fly *P. tricuspis*. Phorid flies were not present at the Saint Gabriel (SG) study site during study one described in Chapter two, but were detected prior to the initiation of this study. The objective of this study was to conduct a longitudinal evaluation of the temporal effects of methoprene on RIFA populations in the presence of phorid flies by comparing the temporal response of the RIFA population to chemical treatments at SG before and after phorid flies were present. However, since certain pastures had been plowed in the fall at SG prior to the start of the study, the opportunity was presented to evaluate the temporal effects and interactions of plowing and methoprene in the presence of phorid flies.

3.2 Materials and Methods

The study was done from May 2009 through May 2010 at St. Gabriel Research Station in St. Gabriel, Louisiana. The pastures at St. Gabriel were being rotationally grazed by cattle and horses, and rye grass was planted in certain pastures (Figure 3.1). Pastures with rye grass were plowed 15 to 20 cm deep in October 2008 with a 3.35 m John Deere Disk. In May 2009, twelve study sites were chosen. Sites were 100 m^2 and were at least 100 m apart. Six sites were in plowed pastures and six sites were in unplowed pastures. The six plowed and unplowed sites were paired based on proximity (Figure 3.1). One site from each of the three pairs of plowed and unplowed sites was randomly chosen to be treated with methoprene (Figure 3.1). On 11 May 2009 morning, methoprene treatments were made in treated sites at the label rate of 1.7 kg/ha using a Herd spreader mounted to a Honda Rancher four-wheeler.

Red imported fire ant population index (Harlen et al. 1981, Lofgren and Williams 1982) and mound measurement (length x width x height) data were collected in May 2009 (pretreatment), October 2009 and May 2010. A 0.1 ha circular sampling area was created in the center of each 100 m^2 site. All data collection was done inside of the sampling area. Sampling area centers were established and marked with a 30.48 cm diameter concrete stepping stone. The longitude and latitude of each center were recorded using a Magellan GPS (www.magellangps.com). Sampling area perimeters were measured using a 17.8 m rope. One end of the rope was held stationary at the center of the circle while the rope was pulled taunt and someone walked the perimeter, keeping the rope taunt, and placed flags in the ground.

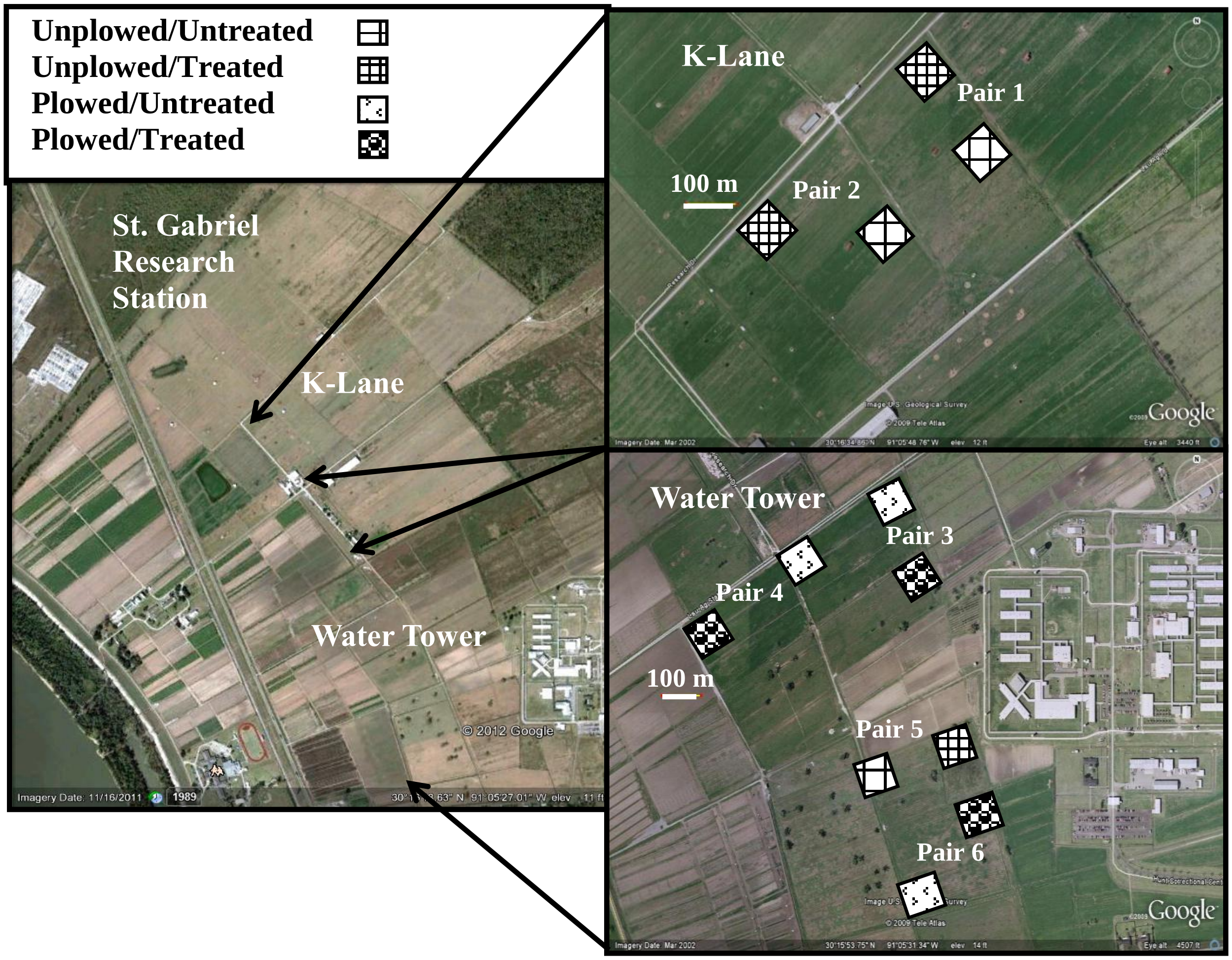

Figure 3.1. Map of the sites and arrangement of treatments (plowing and methoprene treatments) at the St. Gabriel Research Station in St. Gabriel, Louisiana.

Data on the dependent variables RIFA mound height (m), area (m^2), volume (m^3), and population index (PI) were analyzed using PROC MIXED (SAS Institute. 2008). Data were log transformed to normalize the distribution. Separate analyses were used to analyze each variable of interest using the same model. The main effects in the model were plowing, methoprene treatments, and time. The model was designed as a two by two factorial with repeated measures, and a Tukey – Kramer adjustment ($P < 0.05$) was used to separate means.

Red imported fire ant PI data collected from treated and control pastures at SG from April 2005 – October 2007 (study 1) in the absence of phorid flies was compared longitudinally to the RIFA PI data collected from treated and control pastures at SG in this study (study 2) in the presence of phorid flies from May 2009 to May 2010. The longitudinal comparisons were made using a two by two factorial with repeated measures (PROC MIXED, SAS Institute 2009), and a Tukey – Kramer adjustment ($P < 0.05$) was used to separate means. The changes in the prevalence of mature RIFA colonies in treated and control pastures at SG in study 1 and study 2 also were evaluated using a stepwise logistic regression analysis.

3.3 Results

- Mound Height (m)

Significant main effects in the analysis of mean log mound height data were plowing ($F = 20.81$, d.f. = 1, 27.6, $P < 0.0001$) and methoprene treatments ($F = 12.39$, d.f. = 1, 27.6, $P = 0.0015$). The main effect time was not significant ($F = 0.37$, d.f. = 2, 25.5, $P = 0.6944$). There was a significant temporal plowing effect on mean log mound height ($F = 3.62$, d.f. = 2, 22.5, $P = 0.0413$), but the temporal methoprene effect on mean log mound height was not significant ($F = 2.37$, d.f. = 2, 22.5, $P = 0.1138$). Since there was no significant interaction between plowing and methoprene ($F = 0.03$, d.f. = 2, 22.5, $P = 0.9734$), the results of the analysis on the effects of plowing and methoprene treatments on RIFA mound height are presented separately below.

In May 2009, six months after plowing, the mean log mound height was 35 percent less in plowed than in unplowed sites (Table 3.1). In October 2009, nine months after plowing, the mean log mound height was 57 percent less in plowed than in unplowed sites (Table 3.1). In

May 2010, 18 months after plowing, there was no difference between the mean log mound heights of plowed and unplowed sites (Table 3.1).

Table 3.1. Mean (± SE) log RIFA mound height (m) in plowed and unplowed sites 6, 9, and 18 months after the sites were plowed.

Sampling Date	Unplowed	Plowed
9-May	0.15 ± 0.01ab	0.10 ± 0.01c
9-Oct	0.18 ± 0.02a	0.10 ± 0.02bc
10-May	0.14 ± 0.01abc	0.12 ± 0.14abc

Means followed by the same letter are not statistically different ($P \leq 0.05$).

At pretreatment in May 2009, the mean log RIFA mound height was not different in methoprene treated versus control sites (Table 3.2). In October 2009, three months after treating the pastures with methoprene, the mean log mound height in methoprene treated pastures was 44 percent less than in control pastures (Table 3.2). However in May 2010, 12 months after treating the pastures with methoprene, there was no difference between the mean log mound heights of methoprene treated and untreated sites (Table 3.2).

Table 3.2. Mean (±SE) log RIFA mound height (m) in treated and untreated sites 0, 6, and 12 months after treating the sites with methoprene.

Sampling Date	Untreated	Treated
9-May	0.13 ± 0.01ab	0.12 ± 0.01ab
9-Oct	0.18 ± 0.03a	0.10 ± 0.02b
10-May	0.15 ± 0.01ab	0.11 ± 0.02b

Means followed by the same letter are not statistically significant ($P \leq 0.05$).

- Mound Area (m^2)

Plowing was the only significant main effect in the analysis of mean log mound area (F = 19.26, d.f. = 1, 45.5, P < 0.0001). The mean log mound area for plowed sites was 47 percent less than for unplowed sites when data from May 2009, October 2009, and May 2010 were pooled (Table 3.3). The analysis indicated no significant interactions.

- Mound Volume (m^3)

Plowing was the only significant (F = 24.32, d.f. = 1, 57.5, P < 0.0001) main effect in the analysis of mean log mound volume. The mean log mound volume of RIFA mounds was 58 percent less for plowed sites than for unplowed sites when data from May 2009, October 2009, and May 2010 were pooled. No interactions in the model were statistically significant.

Table 3.3. Mean (±SE) log mound area (m^2) and volume (m^3) of unplowed and plowed sites when data from May 2009, October 2009, and May 2010 were pooled within the statistical model.

	No Plow	Plow
Area	0.84 ± 0.06a	0.45 ± 0.07b
Volume	0.09 ± 0.01a	0.04 ± 0.01b

Within rows means followed by the same letter are not significantly different (P < 0.05).

- Red Imported Fire Ant Population Index (RIFA PI) Plowing and Methoprene

The main effects in the statistical model, plowing (F= 6.85, d.f. = 1, 24, P = 0.0151), methoprene treatments (F = 338.77, d.f. = 1, 24, P <0.0001) and time (F = 245.51, d.f. = 2, 25, P < 0.0001), were significant. The temporal effects, plowing and methoprene treatments, did not interact (F = 1.68, d.f. = 1, 24, P = 0.2078). However, plowing (F = 5.39, d.f. = 2, 24, P =

0.0116), and methoprene (F = 108.76, d.f. = 2, 24, P < 0.0001) treatments each had separate significant temporal interactions; therefore, the results of the temporal effects of plowing and of methoprene treatments on RIFA PI are presented separately in the following two paragraphs.

The comparison of the RIFA PI data from plowed and unplowed sites in May 2009, six months after plowing, indicated no significant difference in the mean log RIFA PI of plowed versus unplowed sites (Table 3.4). In October 2009, nine months after plowing, the mean log RIFA PI for plowed sites was 22 percent less than for unplowed sites (Table 3.4). However, in May 2010, 18 months after plowing, the mean log PI of plowed sites no longer differed from unplowed sites (Table 3.4).

Table 3.4. Mean log RIFA PI (± SE) of plowed and unplowed sites in May 2009, October 2009, and May 2010.

Sampling Date	No Plow	Plow
9-May	6.01 ± 0.14a	5.65 ± 0.14a
9-Oct	3.18 ± 0.14c	2.48 ± 0.14d
10-May	4.53 ± 0.14b	4.72 ± 0.14b

Means followed by like letters were not statistically different (P ≤ 0.05).

At pretreatment in May 2009 the mean log RIFA PI for methoprene treated sites was not different than for control sites (Table 3.5). At three months post treatment in October 2009, the mean log RIFA PI was 85 percent less for methoprene treated sites than for untreated sites (Table 3.5). At 12 months post treatment in May 2010, the mean log RIFA PI for treated sites was still significantly less and 32 % lower than in untreated sites (Table 3.5).

Table 3.5. Mean log RIFA PI (± SE) of methoprene treated and untreated sites in May 2009, October 2009, and May 2010.

Sampling Date	Untreated	Treated
9-May	5.93 ± 0.14a	5.72 ± 0.14a
9-Oct	4.93 ± 0.14b	0.74± 0.14d
10-May	5.49 ± 0.14ab	3.76 ± 0.14c

Means followed by like letters were not statistically different ($\alpha = 0.05$).

- Longitudinal Comparisons of RIFA PI

The temporal change in the mean log RIFA PI in treated versus control sites at SG from April 2005 to October 2007 (study 1) compared to May 2009 – May 2010 (study 2) was statistically significant ($F = 16.06$, df = 7, 16, $P \leq 0.0001$). The mean log RIFA PI of treated and control sites at SG in study 1 and study 2 were not different at pretreatment. The mean log RIFA PI of methoprene treated sites in study 1 at 12 months post treatment were not different than the RIFA PI at pretreatment or for control sites (Table 3.6); however, methoprene treated sites in study 2 had a mean log RIFA PI value that was 37 % lower than the pretreatment PI and 30 % lower than the control sites at 12 months post treatment (Table 3.6).

The stepwise logistic regression analysis indicated that the effect of methoprene on the prevalence of mature RIFA colonies was statistically significant. ($\chi^2 = 25.27$, df = 1, $P \leq 0.0001$), and that there was a statistically significant difference between the prevalence of mature RIFA colonies in study 1 compared to study 2 ($\chi^2 = 36.98$, df = 1, $P \leq 0.0001$). However, there was no statistically significant temporal effect of methoprene on the prevalence of mature RIFA mounds in treated and control pastures in study 1 compared to study 2 ($\chi^2 = 0.01$, df = 1, P = 0.90). The difference in the incidence of mature colonies measured in treated versus control pastures at 12 months post treatment in study 1 was only 5 % greater than in study 2 (Figure 3.2).

Table 3.6. Mean log RIFA PI (± SE) of methoprene treated and control sites in study 1 (phorids absent) and study 2 (phorids present).

Study 1 (-)	12 months
Treated	6.63 ± 0.22 a
Control	6.44 ± 0.31 ab
Study 2 (+)	
Treated	3.72 ± 0.25 c
Control	5.34 ± 0.25 b

+ Phorid flies present at SG
- Phorid flies absent at SG (also shown in Table 2.5)
  Mean log RIFA PI values followed by the same letter are not statistically different ($\alpha = 0.05$).

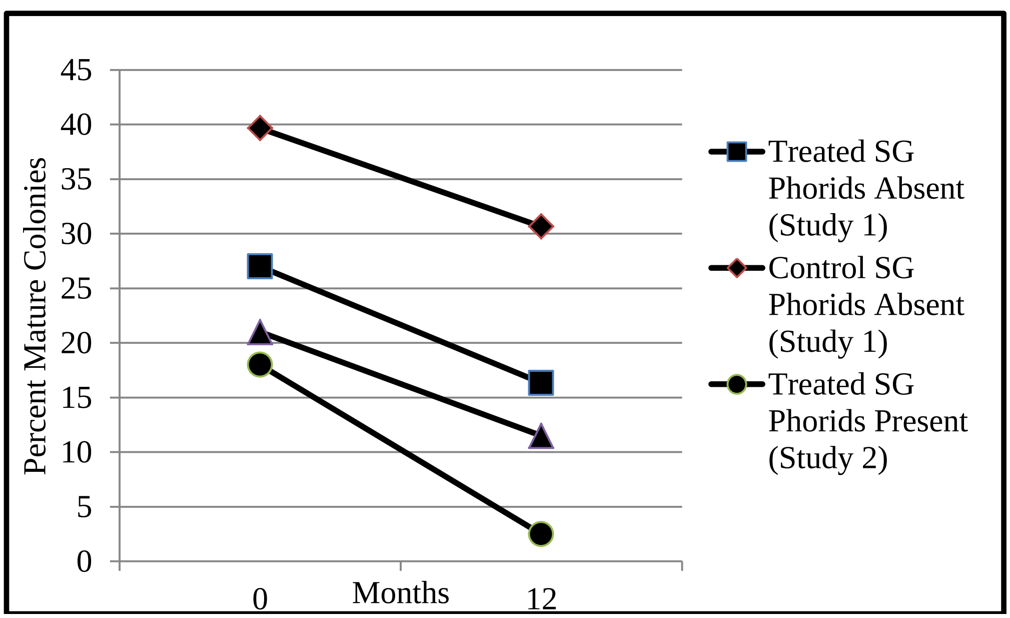

Figure 3.2. General change in prevalence of mature RIFA colonies in treated and control pastures at SG in the presence (Study 1) and absence (Study 2) of phorid flies.

3.4 Discussion

The RIFA mounds in plowed pastures were 44 % shorter than those in unplowed pastures at both six and nine months post plowing (Table 3.1). Colby et al. (2008) showed that RIFA mounds were significantly shorter in plowed sites than in unplowed sites at five months post plowing, but the longevity of the reduction in mound height was not monitored past five months. We did not have the opportunity to collect pretreatment data in the pastures, but given 1) the clear impact of plowing in the fall on RIFA mound height in the spring shown by Colby et al. (2008), 2) the fact that both studies were done at the same location, and 3) the statistical differences in the mound heights in plowed versus non-plowed pastures in May 2009 (Table 3.1), pretreatment counts were not considered critical to the primary experimental design. This study provides the only data on the long term impact of plowing on RIFA mound height, and the results suggest that plowing in the fall is an effective tool that producers can use to reduce the height of RIFA mounds to decrease equipment damage and increase hay harvest during the spring and summer.

Unlike mounds in plowed sites (Table 3.1), RIFA mounds in methoprene treated sites were not shorter than mounds in untreated sites (Table 3.2). Queens of RIFA colonies can recover from the effects of methoprene (Banks et al. 1983). Therefore, some RIFA queens in treated pastures could have recovered from the effects of methoprene and started colonies inside of the large, sturdy mounds that persisted in treated sites that were not plowed. Therefore, methoprene treatments in the absence of plowing are not useful to producers for reducing the height of RIFA mounds.

The area of RIFA mounds in plowed versus unplowed sites did not differ significantly through time, which is consistent with the results in Colby et al. (2008). The area of RIFA mounds also was not different in methoprene treated versus control sites. However, the mean mound area of plowed sites was 46 % less (statistically significant) than in unplowed sites when the data from May 2009, October 2009, and May 2010 were pooled within the statistical model (Table 3.3). The observed significant reduction in the overall RIFA mound area inversely corresponds to a possible increase in the area available in pastures for growing forage because the tops of RIFA mounds often are bare of grass. The results of this study suggest that plowing, rather than methoprene treatments, could increase the area in pastures that can be used to grow forage.

Colby et al. (2008) reported significant temporal reduction of RIFA mound volume, but no statistically significant temporal reduction of RIFA mound volume was observed in this study. However, in this study the mean volume of mounds calculated from pooled data collected from plowed and unplowed pastures in the May 2009, October 2009, and May 2010 sampling periods was 96 percent less in plowed than in unplowed sites (Table 3.3). Colby et al. (2008) measured the volumes of RIFA mounds monthly from pretreatment up to five months; whereas, in this study the first measurement of RIFA volume was taken six months after the sites had been plowed. Therefore, Colby et al. (2008) collected data points at a higher frequency during a time when the reduction of RIFA mound volume due to plowing was probably greatest, and as a result the statistical model showed temporal differences in RIFA mound volume.

The RIFA PI in plowed sites was not different than in unplowed sites (Table 3.4), but the RIFA PI in methoprene treated sites at 12 months was 34 % lower than at pretreatment (Table 3.5). The RIFA PI is an assessment of the reproductive status and the number of workers in an individual RIFA colony. The presence or absence of brood is the most definitive and heavily weighted factor of the RIFA PI. Estimates of the number of RIFA workers are somewhat subjective and not as heavily weighted in the RIFA PI. Plowing RIFA mounds likely kills a portion of the work force and brood of RIFA colonies, but if the queen is still alive and able to lay eggs, the reproductive status of the colony should not be changed. New worker brood and subsequent workers should be produced, and surveys of colonies recovering from a plowing event should likely detect brood; therefore, it is logical that the temporal effects of plowing on RIFA PI were insignificant. The RIFA PI was created to evaluate the effects of IGR's on RIFA colonies (Harlan et al. 1981). Hence, it is not surprising that RIFA PI in methoprene treated sites was significantly reduced.

Mound volume is directly related to the number of ants in a RIFA colony in terms of growth from incipiency (Tschinkel 1993) and is also a function of mound area and height. Colonies of the RIFA that rebuild a mound following a plowing event are likely to have suffered at least some level of worker and brood mortality. Since the old mound and a portion of workers and brood were probably lost due to plowing, a new mound should be built to fit the smaller space needed for the reduced recovering ant population within the mound. Red imported fire ant colonies invest a large proportion of energy into producing worker brood during the fall and reproductive brood during the spring while brood production is halted during the winter due to low temperatures (Tschinkel 2006). It is possible that RIFA colonies in plowed pastures are unable to fully recover from a fall plowing event before low winter temperatures halt brood production, and that the colonies maintain the small mound rather than rebuild since a large brood population is not present in the colony. Red imported fire ant colonies recovering from a fall plowing would have a reduced work force in the spring, which would lead to slow growth of the colonies. The observed significant temporal change in the height of RIFA mounds in plowed pastures (even though the earliest measurement of RIFA mound height were taken six months after plowing) suggests that the height of RIFA mounds is the most drastically affected mound dimension that contributes to the reduction of mound volume in response to plowing. The difference in mound volume in plowed versus unplowed pastures can be characterized by significant temporal changes in mound height coupled with insignificant temporal changes in mound area.

In study 1 (chapter 2), phorid flies were absent at SG and the RIFA population estimate (PI) in methoprene treated pastures was not different than the pretreatment population estimate after one year (Table 3.6 and 2.5). However, in study 2 at SG, two species of phorid flies, *Pseudacteon tricuspis* and *Pseudacteon curvatus*, were present and RIFA population estimates were 32 % lower than pretreatment population estimates after 1 year (Table 3.6). The prevalence of mature colonies in both treated and control pastures in study 1 (phorid flies absent) appeared to be higher than in study 2 (phorid flies present) (Figure 3.1). Thus, it is possible the presence of phorid flies could be interfering with growth of incipient or recovering RIFA colonies in pastures, or even dynamics of forager replacement in mature colonies.

The height of RIFA mounds is a major factor in the extent and frequency of damages to hay equipment, and the area of RIFA mounds is inversely proportional to the area that can be used to produce forage and subsequently hay in pastures. Plowing is an effective tool that producers can use to reduce RIFA related equipment damages and forage loss; however, chemical treatments are not. Of course, chemical treatments are useful for reducing RIFA populations in areas where animals or people are being attacked by RIFA. Since the effects of chemical treatments do not interact with the effects of plowing and do not reduce the height of RIFA mounds in pastures, using methoprene treatments is not an effective tool that producers can use to reduce RIFA related hay equipment damages. However, the presence of phorid flies may extend both the benefit of plowing and methoprene individually. Therefore in areas where phorid flies are present, producers could use plowing to reduce RIFA related damages in areas where equipment damage is the major concern. In cases where chemical treatments are needed to reduce RIFA related risks to animal and human health or of damages to electrical equipment, the presence of phorid flies may extend the temporal effects of chemical treatments on RIFA populations and potentially provide economic saving.

SUMMARY AND CONCLUSIONS

In study one, RIFA population estimates at sites treated with methoprene in the absence of phorid flies at Saint Gabriel returned to pretreatment levels within twelve months after treatment, but RIFA population estimates at sites in WP treated with methoprene in the presence of phorid flies were still significantly lower than pretreatment estimates at twelve months post treatment. Analysis of the methoprene treatment effects on RIFA social form indicated that methoprene equally affects all RIFA regardless of social form and that RIFA re-colonization of chemically treated areas is not significantly social form biased. The incidence of *K. solenopsae* infection in the RIFA populations was much greater in polygyne colonies and was not reduced in treated pastures at 12 months post treatment. No long-term effects of methoprene treatments on phorid fly populations were detected.

The results of the longitudinal comparison of RIFA PI data collected from methoprene treated and control sites at SG in study one when phorid flies were absent versus study two at SG when phorid flies were present indicated that methoprene treatments significantly reduced the RIFA PI for 12 months when phorid flies were present compared to 6 months when phorid flies were absent. The combined results of study one and study two suggest that the effects of methoprene treatments for control of RIFA populations of either social form are temporally extended in the presence of phorid flies, which do appear to be viable biological elements in integrated strategies. However, the value of *K. solenopsae* as an agent in integrated strategies when methoprene is the sole chemical treatment may be limited. In addition, the results of study two show that the effects of methoprene treatments and plowing do not interact and that methoprene treatments do not reduced the size of RIFA mounds in methoprene treated pastures.

However, plowing in the presence of phorid flies reduced the height of RIFA mounds in pastures for at least nine months.

The height of RIFA mounds is a major factor in the extent and frequency of damages to hay equipment, and the area of RIFA mounds is inversely proportional to the area that can be used to produce forage and subsequently hay in pastures. Therefore, plowing is an effective tool that producers can use to reduce RIFA related equipment damages and forage loss, but methoprene treatments are not. The presence of phorid flies may extend both the benefit of plowing and methoprene treatments individually. Therefore, in areas where phorid flies are present, producers could use plowing to reduce RIFA related damages in areas where equipment damage is the major concern. In cases where chemical treatments are needed to reduce RIFA related risks to animal and human health or of damages to electrical equipment, the presence of phorid flies may extend the temporal effects of chemical treatments on RIFA populations and potentially provide economic saving.

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