APPLIED ECOLOGY AND CONTROL OF IMPORTED FIRE ANTS AND ARGENTINE ANTS (HYMENOPTERA: FORMICIDAE)

by

BEVERLY ANNE WILTZ

(Under the Direction of Daniel R. Suiter)

ABSTRACT

The red imported fire ant, *Solenopsis invicta* Buren, and Argentine ant, *Linepithema humile* (Mayr), are invasive species that are major pests in urban, natural, and agricultural habitats. The goal of this dissertation was to study aspects the chemical sensitivity, behavior, and ecology of each species to enhance control options. In these studies, I: 1) provide recommendations for the optimal usage of various insecticides against each species, 2) evaluate deterrent and toxic effects of natural products, 3) develop a delivery system for ant toxicants that uses a pheromonal attractant to facilitate toxicant transfer by contact, and 4) determine which habitats within blackland prairies are most susceptible to invasion by imported fire ants.

Bifenthrin had properties best suited for use as barrier or mound treatments against both species. In laboratory assays, it was the fastest acting of the chemicals tested and was the only chemical that acted as a barrier to ant movement. Fipronil exhibited high horizontal toxicity and delayed topical toxicity, properties that are desirable in a broadcast treatment. Chlorfenapyr and thiamethoxam appeared best suited to use as mound treatments, as they had low horizontal toxicity and did not impede ant movement in barrier tests.

At least one of the four tested rates of basil, citronella, lemon, peppermint, and tea tree oils were repellent to both ant species. In continuous exposure assays, citronella oil was toxic to both species, and peppermint and tea tree oils were toxic to Argentine ants.

Of the semiochemicals tested, only triolen, a component of the red imported fire ant brood recognition pheromone, enhanced removal of treated granules by *S. invicta* workers. At a rate of 0.06% fipronil plus triolein, there was 90.5% mortality in laboratory colonies versus 46.5% with the same rate of fipronil without triolein.

Ground surveys and analysis of remotely sensed images were conducted for two sites in northeastern Mississippi, both of which contain *S. invicta* x *S. richteri* hybrid imported fire ants. Mound densities were highest and individual mounds were smallest in disturbed areas. Fire ants were not found in forest or chalk outcrop plots or in prairie containing > 26% tree cover.

INDEX WORDS: *Solenopsis* spp., Imported fire ant, *Linepithema humile*, Argentine ant, Chemical control, Repellant, Attractant, Pheromone, Remote sensing, Landscape ecology

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DEDICATION

This dissertation is dedicated to my parents, Paul and Rebecca Wiltz, and my grandmother, Sarah Mooney, for their love, prayers, and encouragement throughout this process.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

A paradox in the control of urban pest insects is that the public is concerned with the overuse of pesticides, yet has little tolerance for the pests themselves. Because of this zero-tolerance, concepts such as economic injury level and action threshold that define integrated pest management (IPM) in agricultural systems do not exist in the urban environment. Instead, what is sometimes referred to as "urban IPM" is simply the use of a collection of chemical and nonchemical control methods. The failure of IPM to be adopted for the control of household and structural pests is due to the difference in attitudes about the pests rather than to a lack of available nonchemical methods. Cultural or biological control methods exist for each of the major groups of household and structural pests. Reduction of harborages, food, and moisture sources are methods that can be applied to ant, termite, or cockroach control (Smith et al. 1995, Grace and Yates 1999, Rust et al. 2003). Physical barriers are effective against some ants and subterranean termites (Grace and Yates 1999, Rust 2003). Although natural enemies of ants, drywood termites, subterranean termites, and cockroaches are known (Suiter 1994, Grace 1997, Orr and Seike 1998, Porter 1998a, 1998b, Williams et al. 1999), biological control is of limited use for household and structural pests. In residential areas, people expect that pest control will be fast and will completely eliminate the pest population and may find the biological control agents as objectionable as the pests themselves (Thoms and Robinson 1987).

The best way to simultaneously manage urban pests and reduce pesticide usage and environmental impacts is to make alternative control methods available while implementing smart application methods when chemical toxicants are needed. These methods include proper placement and timing of pesticide application and the use of control methods that are more target-specific (Porter and Tschinkel 1987, Oi et al. 1994, Henderson et al. 1998, Hooper-Bùi et al. 2002, Vogt et al. 2003, Nuss et al. 2005).

The studies presented in this dissertation use the biology and ecology of imported fire ants, *Solenopsis* spp., and the Argentine ant, *Linepithema humile* (Mayr), to develop more targeted methods of controlling these pests, using both traditional insecticides and alternative methods. The objectives of this research were:

1) To determine the effects of some insecticides and natural products against the Argentine ant and the red imported fire ant, *Solenopsis invicta* Buren. Insecticides were evaluated in laboratory assays to determine repellency, topical toxicity, mobility impairment, horizontal effects on uncontacted individuals, and residual activity. Based on species-specific results, recommendations were made for the best use of each toxicant. Repellency and contact toxicity tests also were conducted on some essential oils with anecdotal reports of repellency. Results are presented in Chapters 2-4.
2) To enhance particle handling by ants through the use of semiochemicals, thereby facilitating horizontal transfer of a toxicant through ant-to-ant contact. Positive results were obtained using triolein, a component of the fire ant brood pheromone, with the addition of the toxicant fipronil, on corn cob granules. Results are presented in Chapter 5.
3) To identify habitat characteristics conducive to imported fire ant infestation, through ground surveys combined with the analysis of remotely-sensed images. Studies were conducted at two sites in northeastern Mississippi, containing blackland prairie remnants. Monogyne populations of the red x black imported fire ant hybrid, *S. invicta x richteri*, were present at

both sites. Analysis of fire ant mound distribution over varied land cover and topography identified the types of habitats most vulnerable to infestation. In addition to providing infestation data for poorly-studied prairie habitats, generalized methods and results can be applied to issues such as native species conservation and improved site selection for area-wide management programs. Results are presented in Chapter 6.

In this chapter I review literature relevant to this dissertation research. Where literature specifically related to the red imported fire ant and the Argentine ant exist, the review focuses primarily on these species. For imported fire ants and Argentine ants, I review literature related to distribution and dispersal, landscape and community ecology, pest status, and control. For the area of chemical ecology, I review literature on ant chemical ecology in general, although a large percentage of these studies have focused on the red imported fire ant. Spatial analysis has been used in relatively few ant studies. Because methods that have been applied to the study of other arthropods are of direct relevance to this dissertation, the use of spatial analysis in entomology in general is reviewed.

Distribution and dispersal

The red imported fire ant was first introduced into the United States in the Mobile, AL, area from central Brazil sometime around 1940 (Lofgren et al. 1975). It is now widespread throughout the southeastern United States from North Carolina through Oklahoma and in Puerto Rico and has been found in isolated locations in Virginia, Maryland, California, Arizona, and New Mexico (Williams et al. 2001, Sutherst and Maywald 2005). Since 2001, *S. invicta* has been reported in Australia, New Zealand, Malaysia, Taiwan, Hong Kong, and mainland China (ISSG 2005, Anonymous 2005).

Originating in South America, the Argentine ant has now been distributed to every continent except Antarctica (Hölldobler and Wilson 1990). It was introduced into the United States in the late 1800's in coffee shipments to New Orleans from Brazil (Newell 1908). Most infestations occur between 30° and 36° latitudes in both hemispheres. In California, Ward (1987) found that *L. humile* was usually confined to sites with permanent sources of water and was more likely to be found close to urban areas.

Both species are considered tramp ants. Tramp species are defined as being r-strategists, largely dispersed by human commerce, and living in close association with humans (Hölldobler and Wilson 1990, Tschinkel 1987). The high fecundity rates characteristic of tramp species are often associated with species that are polygynous, unicolonial, and reproduce by budding.

The red imported fire ant has several attributes that have enabled it to invade and prosper in all of the southeastern, and part of the southwestern, United States. *S. invicta* has a high fecundity rate, an omnivorous diet, and opportunistic behavior that allow it to shift among food sources. It rapidly establishes in disturbed areas such as grazed pastures, lawns, and regions disturbed by fires (Vinson 1994). Colonies of the red imported fire ant occur in two forms in the southeastern and south-central United States. It was originally believed that all colonies were monogynous. Polygyne colonies were first reported in the mid-1970's in Mississippi (Glancey et al. 1975) and Texas (Hung et al. 1974). Colonies of the polygyne form contain from several to hundreds of functional queens (Glancey et al. 1975). Colony founding occurs by nuptial flights and claustral founding in the monogyne form (Vargo and Porter 1989). In the polygyne form, newly-mated queens are adopted by other polygyne colonies, and new colonies are produced primarily through budding (Glancey and Lofgren 1988, Vargo and Porter 1989).

The success of the polygynous form of *S. invicta* can in part be attributed to its unicolonial structure and the large number of foragers in a colony. When queen numbers are high, the individuals in an area are more closely related, therefore, there is little or no intra-specific aggression. Because territorial boundaries exist more with monogyne populations of *S. invicta*, foraging ability and distance are limited relative to the polygyne form (Porter and Savignano 1990). In *S. invicta*, an increase in queen number results in a per queen reduction in fecundity, mounds are closer together, and the number of individuals per area is greater (Wojcik 1983, Lofgren and Williams 1984, Vinson and Sorensen 1986). Polygyny in the red imported fire ant is less common in South America. A possible factor limiting polygyny in South America is the presence of biological control agents. Briano et al. (1995) discovered that sexual individuals in *S. richteri* colonies were less frequent when colonies were infected with *Thelohania solenopsae* Knell, Allen, and Hazard, yet winged females were more abundant.

Haploid eggs are present in Argentine ant colonies throughout the year, providing the ability to develop reproductives at any time, and enabling the colony to reproduce in the absence of a queen (Aron 2001). Females can be inseminated by nestmates inside the nest, allowing for replacement of queens (Aron 2001). Although nuptial flights occur, the primary method of colony reproduction and founding is budding (Markin 1970). *L. humile* exhibits true polygyny because, although there is strong variation in individual oviposition rates, there is no clear dominance hierarchy. The number of queens varies seasonally, from 0.1 to 1.6 queens per 100 workers (Keller et al. 1989).

In introduced areas, a low degree of genetic variability in *L. humile* has been found (Tsutsi et al. 2000). Because of this lack of variability, there is no antagonism towards non-nestmates. Argentine ant workers frequently move among nests and are considered a super colony when multiple nests are located in an area. Polydomy may improve foraging efficiency (Hölldobler and Lumsden 1980) and the ability to defend resources (Davidson 1997). Markin (1968) found that Argentine ants exchanged over 50% of the individuals among the separate nest sites. Laboratory colonies were found to redistribute workers and brood among nests in response to spatial heterogeneity of food resources (Holway and Case 2001). Colonies relocate frequently in response to changes in weather or human activity (Markin 1968, Benois 1973). *L. humile* is omnivorous, with generalist feeding and nesting requirements (Majer 1994). Therefore, there are niche overlaps with many species.

Landscape and community ecology

The red imported fire ant is often abundant in lawns, pastures, old fields, roadsides, and other open, sunny habitats while it is rare or absent in late succession or climax communities such as mature deciduous or pine forests. When it is found in these communities, it is usually associated with local disturbances such as temporary ponds, dirt roads, and the margins of paved roads (Tschinkle 1986). In linear habitats such as roadsides and powerline cuts, mounds are most often found along ecotonal edges and are more common on the north (sunny) side of a clearing (Stiles and Jones 1998). Similarly, in South America, fire ants occur most often in disturbed areas such as along highways and in urban and cultivated areas (Wojcik 1983, Banks et al. 1985).

Fire ant mound densities are highly variable due to a combination of factors. Although the success of founding queens plays an important role in the future level of infestation, this process in itself is a result of a complex set of circumstances, including the density of established colonies and a number of biotic and abiotic factors. Soil type, soil fertility, cultural practices, and amount of ground cover have been correlated with mound densities in sugar cane fields (Ali et al. 1986). History of chemical control (Tschinkle 1986), competition from other ant species (Urbani and Kannowski 1974), and frequency of disturbance (Stiles and Jones 1998) may also affect density levels.

Questions have arisen about whether the abundance of *S. invicta* in disturbed habitats in the U.S. is comparatively greater than in similarly disturbed habitats in South America. The absence of natural enemies often allows exotic pests to reach population levels that are significantly higher than those found in their native areas. When *S. invicta* was introduced into the United States, its natural enemies remained in its original native range. *S. invicta* has an unusual ability to quickly resurge after environmental stress. Repeated environmental stress events, including pesticide applications, alternating with resurgence periods, lead to a larger population increase in fire ants and a strong suppression of native ant species (Buren 1983). Following World War II, there was a widespread habitat disturbance in the South and West as large numbers of people moved into these regions, constructing new homes, recreational, and industrial areas. This disturbance created an ideal situation for the establishment and spread of the newly-introduced *S. invicta* (Patterson 1994). In some areas of Brazil and Argentina, where climate and land use are similar to those in the United States, fire ant populations can be as high as in the United States, often exceeding 500 colonies per ha (Banks et al. 1985).

The degree of habitat disturbance affects the outcome of competition between fire ant species. The native fire ant *S. geminata* (F.) is able to co-exist with *S. invicta* in moderately-disturbed habitats (Tschinkel 1988). *S. geminata* is common throughout longleaf pine forest in northern Florida, but is nearly absent from heavily-disturbed or pond side sites occupied by *S. invicta* (Tschinkel 1988). *S. geminata* is rare throughout flatwood sites where the water table is close to the surface, and *S. invicta* is a common colonizer of clear-cut replanted areas and of graded roadsides. *S. invicta* also occurs at low densities in mature flatwoods, mostly along ungraded roads.

The polygyne form of the red imported fire ant may have the greatest negative effect on species diversity of ant communities. Polygyne colonies have slightly smaller workers and can contain several dozen egg-laying queens (Porter and Savignano 1990). These colonies have greater environmental impact because of higher nest density. Polygyne colonies often have mound densities 10 times as high as the monogyne form (300-1000 mounds/ha vs. 30-100 mounds/ha) (Vinson and Sorensen 1986).

The Argentine ant also thrives in disturbed areas (Majer 1994). This species has been found to be most successful in Mediterranean climates. With the exception of Australia, *L. humile* has frequently become the dominant ant species when it is introduced into areas with Mediterranean climates (Majer 1994). Although it has become established in subtropical and tropical areas, in these areas it is often out-competed or is able to coexist with other species (*Pheidole megacephala* (F.) in Bermuda and Hawaii) (Haskins and Haskins 1988, Reimer 1994).

In riparian woodlands in the lower Sacramento River Valley, Argentine ants were found along permanent streams more often than intermittent streams (Ward 1997). Gordon et al. (2000) found that in cold rainy weather or hot dry weather the Argentine ant invades houses to escape extreme conditions. It was not clear whether the ants were nesting inside the buildings or foraging from outside nests. In a California biological reserve, proximity to buildings was found to be a good predictor of Argentine ant occurrence (Human et al. 1998). Way et al. (1997) found that certain areas in Portugal were dominated by *L. humile* and others by native ants. The transition in Argentine ant presence corresponded with a change in soil types, and the authors suggested that certain soil types may hinder the successful establishment of *L. humile*.

Displacements of other ant species by *L. humile* and *S. invicta* have been reported throughout the introduced ranges of both species. Some authors cite species that are missing from areas containing one of the invaders, while others report a general decline in species richness.

At least nine native ant species were reportedly displaced by *S. invicta* (Table 1.1). In each case, the sites were disturbed, with most studies being conducted along roadsides. All of the displaced species are native (Creighton 1950). Camilo and Phillips (1990) found a negative correlation between *S. invicta* population density and the number of coexisting species. There were 21 ant species in undisturbed, un-infested plots and 14 ant species in disturbed, un-infested plots. In the presence of *S. invicta*, only nine species existed in undisturbed plots, and five species in disturbed plots. Porter and Savignano (1990) examined the ecological impacts of polygyne fire ant invasion on other ants and ground-dwelling arthropods in central Texas. Using pitfall traps and litter samples they calculated arthropod abundance and species richness, finding that *S. invicta* decimated indigenous ant fauna, primarily through competitive replacement.

Banks and Williams (1989) measured competitive interaction between *S. invicta* and *Paratrechina longicornis* (Latreille) on recruitment of individual workers to baits. They observed that although *P. longicornis* discovered the baits first, dominating the bait samples for the first 30 min, *S. invicta* recruited more foragers and their aggressive behavior resulted in displacement of other species within 60 min. Other *Paratrechina* spp. can successfully compete for food against *S. invicta* by discovering the food first and displacing *S. invicta* from it (Buren 1983).

Monomorium minimum (L.) is able to co-exist with *S. invicta* by using chemical defenses to repel *S. invicta* workers (Urbani and Kannowski 1974). *S. invicta* has been found to displace and out-compete native ant species of Brazil (Banks et al. 1985). Wojcik (1983) found no differences in the behavior of *S. invicta* in similar ecological habitats in North and South America.

At least 12 ant species have reportedly been displaced by *L. humile* (Table 1.2). With one exception, displacement was reported exclusively in Mediterranean climates (California, South Africa) or islands (Bermuda, Madeira). While *L. humile* had temporarily displaced imported fire ants in Mobile, AL (Wilson 1951), *S. invicta* has since regained dominance in the area (Glancey et al. 1976). *P. megacephala* and *S. saevissima* are introduced species, while the remainder of the displaced species are native (Creighton 1950). Where *L. humile* was present in riparian woodlands in California, there was a reduction in native epigaeic ant species collected (Holway 1998). Of the 16 species commonly collected in pitfall traps in uninfested areas, only one was found in the area containing *L. humile*.

Body size is often an important factor in deciding the outcome of competitive interactions. However, for social insects and other modular organisms the size of the group may be more important than the size of individuals (Buss 1981). Large colonies may be able to overcome superior defensive mechanisms of smaller colonies. It is difficult to determine if large colony size is a result of competitive superiority or vice-versa.

Chemical ecology

Chemicals involved in interactions between organisms are called semiochemicals. Semiochemicals play a central role in the organization of ant societies. Behavioral responses to chemical cues that are displayed by ants include alarm, attraction, recruitment, trail following, grooming, trophallaxis, nestmate recognition, caste determination, and control of oogenesis among competing reproductives (Hölldobler and Wilson 1990).

Allelochemicals are broadly classified as chemical mediators between organisms of different species. They can be subdivided into allomones, which benefit the organism sending the chemical signal, and kairomones, which benefit the receiver.

Some functions of allomones in ants include repellency and camouflage. Ants use their stings for defense, prey capture, and pheromone dispersal (Obin and Vander Meer 1985). Fire ants dispense venom through the air by raising and vibrating their gasters, a behavior called "gaster flagging". Foraging workers use air-dispensed venom to repel potential competitors in their foraging areas. Fire ant venom is unique in that it is rich in alkaloids and unusually low in protein (Attygalle and Morgan 1984).

Kairomones function in host or prey recognition. For example, some species of ant-decapitating flies in the genus *Pseudacteon* are attracted to their hosts by the odor of the ants' alarm pheromone. Studies of *Pseudacteon* spp. that are parasitoids of *Solenopsis* spp. have found that these parasitoids initially locate their hosts from a distance by olfaction and then switch to visual cues at close distances (Gilbert and Morrison 1997, Orr et al. 1997, Porter 1998a).

Chemicals that mediate behavior among individuals of the same species are called pheromones. Releaser pheromones are those that elicit immediate behavioral responses. Two common responses to releaser pheromones are alarm and trailing. Pheromones that result in delayed action by initiating a complex physiological response are called primer pheromones. They elicit responses such as caste determination and the inhibition of oogenesis. A single chemical compound can act as a pheromone, an allomone, or a kairomone, depending on the species of the emitter and receiver and which organism benefits from the interaction. Ants produce pheromones from a larger number of exocrine gland sources than any other hymenopteran group (Billen and Morgan 1998).

Sex pheromones are substances, usually emitted by females, that are used to attract individuals of the opposite sex for the purpose of mating. Sex pheromones have not been well studied in ants. Laboratory assays are difficult to conduct because, in many species, mating occurs in the air, with nuptial flights being triggered by specific environmental conditions. In monogyne *S. invicta*, males and females locate each other and mate at a height of about 50 to 150 m in the air, then the females return to the ground to initiate a colony (Markin et al. 1971). While Goodisman et al. (2000) observed polygyne *S. invicta* swarms at lower altitudes, these swarms contained mostly females and, based on the proportion of mated queens, probably occurred after mating.

Hölldobler and Bartz (1985) defined two principal mating systems for ants: the "male-aggregation syndrome", corresponding to the use of non-resource-based rendezvous sites, and the "female-calling syndrome," corresponding to the use of emergence site rendezvous sites. In both systems, pheromones play an important role in coordinating the departure of alates from the nest and the attraction of mates.

In the male aggregation syndrome, mating flights of males and females of a species are usually synchronized both within and between colonies (Hölldobler and Wilson 1990). The sexuals of many ant species aggregate at species-specific landmarks and at specific times of the day and year, with both sexes flying upwind to groups of males, attracted to the landmark and to pheromones emitted by the males (Hölldobler and Wilson 1990). Male mandibular gland pheromones are thought to induce flights by females in *Atta* and *Acromyrmex* species (Fowler 1982) and are the source of the attractant in several *Pogonomyrmex* species (Hölldobler 1976). For *Formica lugubris* Zetterstedt, the attractant, which is produced by the female's Dufour's gland, is composed of undecane, tridecane, and (*Z*)-4-tridecene (Walter et al 1993). Synthetic undecane elicits as strong a male response as Dufour's gland extract in this species. When virgin queens are near an aggregation, female-derived pheromones are assumed to attract mates or to stimulate males (Hölldobler 1976).

In *S. invicta*, the onset of nuptial flights is marked by heightened worker excitement and aggression. When environmental conditions are appropriate, workers open holes in the mound for alates to emerge. Before flying away from the nest, male and female alates move around on the ground and surrounding vegetation with frequent contact between the workers and alates (Morrill 1974). Obin and Vander Meer (1994) induced laboratory mating flights of *S. invicta* and demonstrated that workers preferentially entered, searched, and recruited nestmates to vials containing either an alate corpse or alate residue. Chemical cues from both male and female alates, but not workers, attracted workers, induced alarm – recruitment behaviors, and promoted alate retrieval by the workers. Alonso and Vander Meer (1997) determined that the alate mandibular glands are the source of these excitant pheromones.

In the female-calling syndrome, females are often wingless and do not travel far from the nest. Calling females stand on the ground or low vegetation, often assuming a characteristic position, with gasters raised and stings extruded, and release sex pheromones to call the winged males to them (Hölldobler and Haskins 1977). Sources of the male-attracting pheromones include the poison gland in *Xenomyrmex floridanus* Emery (Hölldobler 1971), the pygidial gland in *Rhytidoponera metallica* (Smith) (Hölldobler and Haskins 1977), and the mandibular gland in *Polyergus breviceps* Emery (Topoff and Greenberg 1988). In *Megaponera foetens* (F.), rather than locating queens, males disperse individually and locate conspecific nests using pheromone trails deposited by foragers (Longhurst and Howse 1979).

Queens control the colony through the production of eggs, the type of eggs produced, and influencing the behavior and physiology of workers and potential reproductives. Effects of queen pheromones include control of the development of sexuals (Vargo and Fletcher 1986), dealation and ovary development in winged virgin queens (Fletcher and Blum 1981), and ovary development among egg-laying queens in polygyne colonies (Vargo 1992).

Vargo and Laurel (1994) investigated the mode of action and perception of a queen pheromone that inhibits dealation and ovary development in virgin queens of *S. invicta*. After removal from the queen, virgin female alates exhibited ovary development and dealation. Virgin queens with their antennae removed dealated both in the presence and absence of a queen. Individuals from monogyne colonies were more responsive than those from polygyne colonies.

There is a positive correlation between a queen's fertility and her pheromonal influence (Fletcher and Blum 1983). In *S. invicta*, dealation corresponds to rapid changes in the endocrine system of queens, with these changes stimulating pheromone production and ovary development (Vargo 1999). Queen pheromones may act quantitatively in the regulation of queen number within colonies. Fletcher and Blum (1983) proposed that *S. invicta* workers maintain queen pheromone concentrations within an optimum range by adopting new queens or executing surplus queens. This phenomenon varies in its degree of queen regulation between polygyne and monogyne colonies. Execution of sexual brood by workers is significantly greater in queenright colonies than in queenless colonies, but does not differ between queenright colonies containing fertilized or unfertilized queens (Klobuchar and Deslippe 2002). When queens were removed from monogyne *S. invicta* colonies, worker aggression towards non-nestmate conspecifics decreased, while heterospecific aggression remained high (Vander Meer and Alonso 2002). Queenless workers from monogyne or polygyne colonies were not aggressive toward newly-mated queens. Following adoption of newly-mated monogyne queens, workers of both forms became aggressive towards non-nestmates. Vander Meer and Alonso (2002) proposed that a recognition primer pheromone produced by queens increases the sensitivity of workers to nestmate recognition cues.

Attraction or aggregation of workers to queens has been studied in several ant species, including *S. invicta* (Jouvenaz et al. 1974), *L. humile* (Keller and Passera 1989), and *Camponotus pennsylvanicus* (DeGeer) (Fowler and Roberts 1982). Because of the difficulty in distinguishing between attraction and aggregation, Cariou-Etienne and Passera (1993) used the term “queen power” to describe the combination of these effects.

Vander Meer et al. (1980) discovered that, in *S. invicta*, the attractants and queen recognition pheromones are stored in the poison sac and dispensed by the sting apparatus. The pheromone consists of minor non-alkaloid components of the poison sac contents. Queens have a small degenerated Dufour's gland and do not lay trails, but have fully developed poison sacs. The sting apparatus in queens is involved in egg-laying and in the application of queen pheromones and antimicrobial agents to eggs (Vander Meer and Morel 1995). The application of these chemicals increases egg survivorship, making the eggs more attractive to workers and providing protection against bacterial infection (Vander Meer 1983).

Brood pheromones elicit behavioral responses from workers that include brood recognition (Walsh and Tschinkel 1974), attraction (Glancey and Dickens 1988), and brood tending (Glancey et al. 1970). Glancey et al. (1970) found that *S. invicta* brood pheromone could be extracted from immature forms with hexane. Inanimate objects treated with the hexane extracts and scattered outside a nest cell were picked up and placed inside the cell with brood. Positive responses were obtained with extracts of worker and sexual larvae, but workers did not respond to pupal extracts. Walsh and Tschinkel (1974) criticized the study conducted by Glancey et al. (1970) for not providing a separate brood chamber and, therefore, not making a distinction between brood-tending and feeding responses. Walsh and Tschinkel (1974) modified the assay to include a nest cell with a well-defined brood chamber and conducted olfactometer studies to determine the volatility of the brood pheromone. They concluded that fire ant brood possess non-volatile contact pheromones distributed evenly over the cuticle and that the potency of the pheromone is reduced with the shedding of the pupal skin at eclosion. Unlike the earlier study, positive results also were obtained with sexual pupae. Bigley and Vinson (1975) isolated a brood-tending pheromone from sexual brood of *S. invicta* and identified triolein as a major component of this pheromone. Workers rapidly picked up filter paper discs treated with pupal extracts, triolein isolated from the brood, and synthetic triolein, transporting the treated discs in the colony. Lamon and Topoff (1985) found that chemical stimuli are utilized for the social facilitation of eclosion by *S. invicta*. Workers strip away pupal cuticle for eclosion, then consume the pupal cuticle. Inanimate objects treated with an extract of eclosing pupae were retrieved and placed in colony brood chambers with the appropriate age group, where they were attended by other workers.

The ability to discriminate nestmates from non-nestmates is key to colony maintenance and nest defense. Cues used to discriminate nestmates from non-nestmates may be environmentally derived, genetically based, or a combination of both (Obin and Vander Meer 1988). Using these cues, ants can accept or reject other individuals they encounter. Hydrocarbons have been shown to be a source of nestmate discrimination in several ant species, including *L. humile* (Liang and Silverman 2000), *Iridomyrmex purpureus* (Smith) (Thomas et al. 1999), and *Camponotus fellah* (dalla Torre) (Boulay et al. 2000). Environmental odors acquired from food or nesting material are used to assess colony membership in many wasps, bees, and some ants (Gamboa et al. 1986, Breed 1998, Hölldobler and Wilson 1990). Genetically-based systems are also important (Hölldobler and Wilson 1990, Beye et al. 1998, Stuart and Herbers 2000). Variation in cuticular hydrocarbon profiles has been shown to correlate with intercolonial aggression (Bonavita-Cougourdan et al. 1987).

Workers in monogyne colonies of *S. invicta* readily attack intruders from neighboring conspecific colonies. Obin and Vander Meer (1988) determined that environmentally correlated cues dominated a nestmate recognition cue hierarchy in monogyne field and laboratory colonies. They found that diet alone can significantly modify recognition labels of laboratory-reared workers. Aggression among non-nestmates was reduced by rearing laboratory colonies on the same diet, while aggression between colonies maintained on different diets did not differ significantly from field-collected colonies. Morel et al. (1990) found that, in field situations, workers from polygyne *S. invicta* colonies merge with relatively little aggression. Unlike monogyne workers, freshly-collected workers from polygyne colonies are not aggressive toward conspecifics of either social form.

In many ants, nestmate discrimination is well developed and workers aggressively defend territories, particularly against conspecifics, resulting in a form of colony structure known as multicoloniality (Hölldobler and Wilson 1977). Other species possess a unicolonial structure in which levels of intraspecific aggression are reduced or absent and colony boundaries are weak to nonexistent (Hölldobler and Wilson 1977). Unicoloniality is commonly observed in invasive ants, including *L. humile* (Passera 1994). Within native populations, Argentine ants typically appear more multicolonial, with intraspecific aggression occurring at smaller spatial scales (Suarez et al. 1999, Tsutsui et al. 2000). In introduced areas, intraspecific aggression is almost entirely absent, and Argentine ants form supercolonies (Markin 1968, Way et al. 1997, Tsutsui et al. 2000). Unicolonialty may have arisen in introduced populations as a result of reduced genetic diversity, with introduced populations not possessing levels of genetic diversity necessary to distinguish nestmates from non-nestmates (Tsutsui et al. 2000). A negative relationship exists between intraspecific aggression and the degree of genetic similarity in both native and introduced populations (Tsutsui et al. 2000). Other studies have shown that environmental factors also affect nestmate recognition in Argentine ants. The acquisition of cuticular hydrocarbons from prey such as the brown-banded cockroach, *Supella longipalpa* (F.), can induce intraspecific aggression between nestmates (Liang and Silverman 2000).

The trail pheromone was the first fire ant pheromone to be studied. Wilson (1959) tested extracts of potential glandular sources of the trail pheromone, finding that Dufour's gland extracts elicited trail following behavior. The trail pheromone functions as both a releaser and orienter of trail following. When a forager discovers a food source too large to carry, she lays a trail back to the nest. The trail pheromone passes from the Dufour's gland through the stinger to the ground (Wilson 1962b). As additional workers return from the food source they reinforce the trail. Because food size limits the number of ants that can feed, some workers return without food and do not reinforce the trail, making trail strength a function of food size (Wilson 1962a). Induction and orientation are differentially activated by the varying amount of Dufour's gland material released, with the inducer portion requiring approximately 250 times more worker equivalents for a response (Vander Meer et al. 1990).

Components of the *S. invicta* trail pheromone include Z,E-α-farnesene, E-E-α-farnesene, Z-E- homofarnesene, Z-Z-homofarnesene, and Z-Z-Z-allofarnesene (Vander Meer et al. 1981, Williams et al. 1981). Vander Meer (1983) found that similar trail-following behavior was elicited by Z,E-α-farnesene alone as by the Dufour's gland extract.

While trail pheromones have been studied more extensively in *S. invicta* than in other species, they are known for several other ant species. Trail pheromones have been identified from the venom of *Atta texana* (Buckley) (Tumlinson et al. 1971) and *Atta sexdens rubropilosa* Forel (Cross et al. 1979). Pharaoh ant, *Monomorium pharaonis* (L.), trail pheromones are present in both the poison and the Dufour's glands (Ritter et al. 1977), while *Lasius fuliginosus* (Latrielle) trail pheromones have been isolated from the hindgut (Huwyler et al. 1975).

The function of alarm pheromones is to warn colony members of an enemy; it is not used to ward off intruders (Vander Meer and Alonso 1998). Factors such as pheromone concentration, length of exposure, and context determine the reaction to alarm pheromones. Alarm pheromones are frequently terpenoids, ketones and aldehydes and can be released from the mandibular, anal, poison, Dufour's, pygidial, or frontal gland (Blum 1969, Kugler 1979). They are produced in large quantities and tend to be nonspecific, with some of the compounds found in multiple subfamilies (Blum 1969).

S. invicta has a complex alarm system. When in combat, the worker ant releases two substances: one from its head that acts as an alarm pheromone and another from the Dufour's gland that acts as a trail pheromone to orient other workers to the enemy (Wilson 1971). *Atta* spp. release an alarm pheromone from their mandibular glands. Many studies have attempted to determine the chemical components of this alarm pheromone. Hughes et al. (2001) examined two grass-cutting ant species, *Atta bisphaerica* F. and *Atta capiguara* Gonçalves, to determine if 4-methyl-3-heptanone, the concluded active alarm releaser, stimulates the same alarm response as crushed ant heads. They found that 4-methyl-3-heptanone is the compound that elicits the most alarm behavior and appears to be a common alarm pheromone compound among ants in general. Crushed ant heads stimulated greater levels of alarm behavior than the 4-methyl-3-heptanone, suggesting that one or more of the minor compounds may act synergistically with the main compound.

Some natural enemies have evolved ways to exploit these signals to find their hosts. Allan et al. (1996) found that the cursorial spider, *Habronestes bradleyi*, a specialist predator, is attracted to 6-methyl-5-hepten-2-one, the alarm pheromone of the of the meat ant *Iridomyrmex purpureus* (Smith). Feener et al. (1996) demonstrating that the parasitoid, *Apocephalus paraponerae* Borgmeier (Diptera: Phoridae), is able to detect 4-methyl-3-heptanone and 4-methyl-3-heptanol, alarm pheromones released from the mandibular glands of the giant tropical ant, *Paraponera clavata* (F.). Male parasitoids are attracted to these chemicals for feeding and location of mates, while the female parasitoids orient towards these chemicals for oviposition in the injured ants and to feed from their wounds.

Howard and Tschinkel (1976) demonstrated that the removal of dead ants from the nest is mediated entirely by contact chemical cues in *S. invicta*. They found that the necrophoric releaser is absent at the onset of death but appears rapidly and plateaus within ~1h. The rate of signal appearance was unaffected in heat or freeze-killed workers, indicating a non-enzymatic origin. Corpses that had been exhaustively extracted did not induce necrophoric behavior, but the extracts did when applied to filter paper. Wilson et al. (1958) found that oleic acid induced necrophoric behavior in the ants *Pogonomyrmex badius* (Latreille) and *S. saevissima*. They also noted that long-chain fatty acids are chemically among the most stable decomposition products and tend to accumulate disproportionately in ant corpses, potentially signaling the presence of aging corpses, as opposed to freshly-killed prey. Furthermore, Gordon (1983) discovered that the necrophoric response by *P. badius* depended on social context, with pieces of paper treated with oleic acid either being carried into the nest if a large percentage of the colony was foraging or away from the nest if a large percentage of the colony was involved in nest maintenance.

Although semiochemicals have been studied extensively for the monitoring and control of other insect pests (Grant 1991, Howell et al. 1992, Turchin and Odendaal 1996), few studies have been conducted on the use of semiochemicals for ant control. Vander Meer (1996) found that adding invictolide, a component of the fire ant queen recognition pheromone, to baits decreased bait discovery time and increased efficacy. Greenberg and Klotz (2000) found that Argentine ant trail pheromone enhances recruitment to and consumption of sucrose solutions and can potentially be used to enhance bait consumption. A variety of semiochemicals can potentially be used to enhance the handling or consumption of toxin-treated granules by eliciting predatory, necrophoric, or brood-tending responses. Vinson et al. (1967) found that trilinolein, pork brain lecithin, glycerin, linoleic acid, and linolenic acid induced feeding behavior by fire ants, while corn cob granules treated with oleic acid or palmitic acid were carried to the refuse pile, and those treated with linoleic acid, linolenic acid, or methyl or ethyl esters of linoleic acid were carried into the mound. Bigley and Vinson (1975) determined that triolein was a highly attractive component of the fire ant brood pheromone, with activity also being seen for diolein. In their assay, oleic acid was either ignored or placed in the trash piles. Bomar and Lockwood (1994a) found that volatiles emitted by grasshopper cadavers attracted other grasshoppers, both conspecifics and contraspecifics. Surface washes, extracts of ground grasshoppers, and fatty acids known to be found in grasshoppers were then tested for attractiveness (Bomar and Lockwood 1994b). In addition to attracting grasshoppers, linoleic acid, linolenic acid, and a combination of the two attracted a seed bug and 5 ant species in field trials. The addition of linoleic acid to carbaryl bran baits resulted in a greater reduction in total grasshopper density than baits without the fatty acid (Bomar and Lockwood 1994c).

Pest status

The status of the red imported fire ant ranges from that of a serious pest to a beneficial predator, depending on the circumstances. Some of the more important problems created by fire ants include hazards to human health from stings (Adams and Lofgren 1982), stinging of livestock, feeding on plants, damage to farm machinery (Adams 1986), and loss of hay and grazing areas. Fire ants are attracted to electrical fields, invading and short-circuiting electrical equipment and causing serious problem in relay switches that control traffic signals (Mackay et al. 1992). The red imported fire ant poses a serious threat to wildlife, killing poultry chicks, lizards, snakes, and ground-nesting birds (Vinson 1994). Parris et al. (2002) reported an increased presence of fire ants in loggerhead sea turtle nests in recent years resulting in increased hatchling mortality. Allen et al. (1995) found that the ants caused a 34% decrease in swallow nesting success and a decline in northern bobwhite quail populations. In the U.S., an estimated expense of $1 billion is incurred each year in control costs and damages to livestock, wildlife, and public health (Pimentel et al. 2005). The red imported fire ant has in some cases played an important role in reducing damage by crop pests. *S. invicta* has been reported as a predator of numerous pests including the boll weevil, *Anthonomus grandis* Boheman (Sterling 1978), soybean looper *Pseudoplusia includens* (Walker) (Whitcomb et al. 1972), velvetbean caterpillar, *Anticarsa gemmatalis* Hübner (Buschman et al. 1977), rice stink bug *Oebalus pugnax* (F.) (Newsom et al. 1960), and southern green stink bug, *Nezara viridula* L. (Whitcomb et al. 1972). There was a permanent reduction in lone star tick, *Amblyomma americanum* L. (Acari: Ixodidae), populations following the establishment of red imported fire ants in north-central and northwestern Louisiana and east Texas (Burns and Melancon 1977). Control of the red imported fire ant has been associated with increased damage by the sugarcane borer, *Diatraea saccharalis* (F.) (Reagan 1972).

In infested areas, the Argentine ant is often the dominant ant species because of its high reproductive capacity, aggressive foraging behavior, and lack of natural enemies (Holway 1999). Although they usually nest outdoors, Argentine ants invade homes in search of food, moisture, or warmth (Gordon et al. 2001). In a survey conducted in California, Knight and Rust (1990b) found that the Argentine ant was the most common urban pest ant (26% of all samples) collected by pest control personnel. Based on the percentage of re-treatments or call-backs for each species, Argentine ants were the most difficult species to control along the coast and in densely populated areas. *L. humile* is an important secondary pest in agriculture because it tends honeydew-producing homopteran insects and interferes with predators and parasites of mealybugs, soft scales, and mites (Markin 1968, Haney et al. 1987). Argentine ants cause further crop damage by chewing through plastic drip irrigation tubes (Chang and Ota 1990). They have been shown to displace native ants species and other arthropods in heavily-infested areas (Erickson 1971, Bond and Slingsby 1984, Ward 1987, Cole 1992).

Control

Following detection of the imported fire ant in the United States in the 1930's, there were many attempts at eradication (Williams et al. 2001). While failing to eliminate the fire ants, these attempts raised concern about the effects of large-scale insecticide broadcasts on non-target organisms (Carson 1962). More recently, the introduction of *S. invicta* into California has resulted in the implementation of spot-eradication programs (Bowen 2001).

Both contact insecticides and baits are used for fire ant control. Contact insecticides are used as drenches, broadcast treatments, and granules for the treatment of individual mounds. Toxic baits have been used since 1962 for control of fire ants (Lofgren et al. 1975). Baits are composed of a toxicant, an attractive food material, and a carrier. Their effectiveness depends upon the toxicant having sufficient delayed toxicity that the bait can be harvested by workers and distributed to other colony members via trophallaxis (Banks 1990).

Site-specific management programs were first described by Hamman et al. (1986) and later called the "Texas two-step method" (Merchant and Drees 2003). This method provides ant suppression for intermediate-sized areas by combining individual mounds treatments with broadcast bait treatments. Other treatment strategies include individual mound treatments for small areas and the application of long-residual contact insecticides for areas such as golf course tee boxes and greens where there is no tolerance for ant mounds (Drees et al. 2000).

Community-wide management programs have been implemented in several states, usually using a version of the two-step method (Miller et al. 1994, Drees et al. 1999, Hooper-Bùi et al. 2000). Such community-wide programs are essential for long-term control because fire ants quickly become re-established if adjacent areas are left untreated, particularly in areas where the polygyne form occurs. In a 4-ha pasture containing monogyne colonies, Callcott and Collins (1992) found that small colonies began to appear 4 months after treatment with fenoxycarb bait, and mature colonies containing over 10,000 workers with brood were detected after 12 to 15 months. Drees and Vinson (1990) found that 10 wk after treatment with chlorpyrifos drench, an average mound density of 486 mounds per ha was found in polygyne plots, while 73 mounds per ha were found in monogyne plots. Following injection with permethrin and a broadcast application of abamectin bait, Wiltz (1996) found that re-infestation by polygyne red imported fire ants began 14-35 days after treatment and that the rate of re-infestation was a function of mound density in the surrounding untreated area.

Some of its natural enemies, including *Thelohania solenopsae* Knell, Allen, and Hazard (Microsporidia: Thelohaniae) and *Pseudacteon* spp. (Diptera: Phoridae), have been isolated from the ant's native habitats in South America. *T. solenopsae*, which was also later isolated from Florida *S. invicta* colonies, is a microsporidian protozoan that infects immature ants, workers, and the queen, causing a gradual decline in a colony's population over a period of months (Williams et al. 1998, 1999). *Pseudacteon* spp. are phorid flies that only parasitize 1 to 3% of a colony, but have a greater impact by suppressing ant foraging behavior (Porter 1998a). Experimental releases of three *Pseudacteon* spp. and *T. solenopsae* have been made throughout the southeastern United States (Gilbert and Patrock 2002, Williams and Brenner 2001), along with trials to demonstrate the impact of integrating the use of natural enemies and large-scale bait broadcasts (Williams and Brenner 2001). Other insects that parasitize fire ants include *Solenopsis daguerrei* (Santschi) (Hymenoptera: Formicidae) and *Orasema* spp. (Hymenoptera: Eucharitidae) (Wojcik 1990). At least three species of nematodes have been found naturally parasitizing *Solenopsis* spp. in Argentina and Florida (Jouvenaz and Wojcik 1990). Fungi were among the first pathogens noticed to affect fire ant populations in South America. *Beauveria bassiana* (Balsamo) Vuillemin infects *S. invicta* naturally in field populations. While results using *B. bassiana* have been inconsistent (Oi et al. 1994), Bextine and Thorvilson (2002) demonstrated that fire ant mound activity was reduced following mound or broadcast treatments with *B. bassiana* pellets encapsulated in alginate and coated with peanut oil. With improved formulations to maximize the amount of fungi actually reaching the ants, fungi show promise as an alternative to chemical pesticides. Another protozoan, *Vairimorpha invictae* Jouvenaz and

Ellis (Microsporidia: Burernellidae), has been shown to infect *S. invicta* lab colonies (Oi et al. 2005). Natural infection rates in South America are lower than infection by *T. solenopsae* (Briano and Williams 2002). A recently discovered pathogen, *Mattesia* sp. (Neogregarinida: Lipotrophidae), was first identified in Florida during a 2001 survey for *T. solenopsae* (Pereira et al. 2002). Large workers and female alates had an atypical yellow-orange color head and sometimes thorax. Because of the characteristic appearance of infected individuals, the disease was called yellow-head disease. Although relatively little is known about this disease, it is promising as a biological control agent because it appears to be self sustaining and spreads rapidly.

The use of baits and the application of contact insecticides are the main control tactics for Argentine ants. Knight and Rust (1990a) found that chlorpyrifos G, diazinon G, chlorpyrifos WP and ME, cypermethrin EC and WP and heptachlor EC had high repellency and efficacy against Argentine ants. In field studies, chlorpyrifos spray provided an effective barrier for at least 30 days, while a combination of cyfluthrin spray and granules provided >80% reductions in ants trapped 60 days after treatment (Rust et al. 1996). While repellent or fast-acting insecticides are useful as barrier treatments, they do not provide the opportunity for foragers to spread the chemical to nestmates and the queen. Knight and Rust (1990a) found that if a chemical is highly repellent, mortality is low regardless of the toxicity level because few ants contact the chemical. Conversely, they found that low repellency treatments can cause high mortality even with only intermediate toxicity because of increased contact. Heavy irrigation, high temperatures, dense ground cover, sun exposure, and alkaline surface conditions can reduce the performance of barrier treatments (Rust et al. 2003). High volumes are required to penetrate some mulches. For example, Bello (1997) found that as much as 287 liters per 92.9 m^2 (75.8 gal / 1000 ft^2) of spray were required to penetrate 7.6 cm of shredded pine bark.

In orchards, a combination of techniques is used to exclude Argentine ants from trees. Tree banding has long been used as both a mechanical and chemical barrier. Barber (1920) reported on the use of a mixture of sulfur and sticky material as a tree band. Exclusion methods used more recently include the use of a sticky substance such as Tanglefoot™ or Stickem™ or an ant tape consisting of a single strand of cotton twine treated with farnesol and Stickem and covered with a strip of wax paper (Shorey et al. 1996). Sticky bands have the disadvantage of being ineffective when they become weathered or dirty (Phillips et al. 1987). Control has been achieved by combining tree circumference spraying and tree pruning (Moreno et al. 1987), nest spraying and tree pruning (Phillips 1986), or tree pruning, banding, and baiting (James et al. 1995).

While non-repellency is often considered an advantage in an insecticide, repellent materials would be a welcome alternative to exclude many pest species from areas where the use of toxic chemicals is not desired. Both plants and insects produce chemicals that are repellent to ants. Aromatic cedar mulch is repellent to red imported fire ants, Argentine ants, and odorous house ants (Thorvilson and Rudd 2001, Meissner and Silverman 2001, 2003). Leaf-cutting ants are deterred by terpenoids produced by many tropical plants (Hubbell et al. 1984, Howard 1987). Methyl palmitate, a component of the defensive secretion of *Polistes fuscatus* (F.), is repellent to several ant species (Henderson and Jeanne 1989). A barrier of farnesol, a component of the alarm pheromone of some ants (Schildknecht 1976), has been used to disrupt Argentine ant foraging in citrus trees (Shorey et al. 1992).

There are anecdotal reports of several essential oils, herbs, and spices being repellent to ants. While essential oils are potentially useful as ant repellants in sensitive areas, there are no reports of ant repellency tests in the scientific literature for most of these products. Some of the substances that have been reported to repel ants are catnip, eucalyptus, geranium, lemon balm, spearmint, tea tree, vetiver, cinnamon, and red and black pepper. Natural products have been more widely tested for repellency and control of other household and livestock pests. Nepetalacetone, a terpenoid found in catnip, is repellent to insects in at least 13 families, including Formicidae (Eisner 1964). Mint oil is repellent to American and German cockroaches and red imported fire ants (Appel et al. 2001, 2004). Catnip essential oil is repellent to subterranean termites (Peterson and Ems-Wilson 2003), while nookatone, a component of vetiver oil, is both a repellent and feeding deterrent to Formosan termites (Maestrello et al. 2000, 2002, Zhu et al. 2001, Nix et al. 2003). Although repellency tests were not conducted, Vogt et al. (2002) found that three formulations containing orange oil were toxic to fire ants. Orange oil contains the monoterpenoid d-limonene, which has a similar mode of action to that of pyrethrum, affecting sodium flux in the peripheral neurons (Ware 2000). Essential oils and monoterpenoids derived from them have been shown to have insecticidal activity against cockroaches (Appel et al. 2001, Ngoh et al. 1998), termites (Cornelius et al. 1997, Bläske and Hertel 2001, Zhu et al. 2001), mosquitoes (Watanbe et al. 1993), and ticks (Lwande et al. 1999).

Spatial analysis in entomology

Tobler’s first law of geography states that “All things are related, but nearby things are more related than distant things” (Tobler 1970). Geostatistics are used to describe this spatially-dependent component of variation in data. Spatially-independent data have the same variability regardless of location. However, spatial data are usually not spatially independent. Data values that are in close proximity have less variability than data values that are farther apart. Data analysis using geostatistics consists of two main steps. First, the extent to which values become more different as pairs of observations become spatially farther apart is quantified using variograms (also called semivariograms) (Manly 2001). The second step is the use of spatial interpolation models based on the variograms. A number of interpolation methods are available, with kriging being one of the most commonly used. Kriging assumes that there is a spatial correlation related to the distance or direction between two sample points. A mathematical function is fit to a specific number of points or all points within a specified radius to determine values for locations between the sampled points (ESRI 1996). Variograms and spatial interpolations can be constructed using traditional statistics, geostatistics, or GIS software. Geostatistics have been used to describe patterns of insect infestations and movement at a wide range of spatial scales. At local scales, Brenner et al. (1998) created contour maps to pinpoint the location of treatment failure for the German cockroach, *Blattella germanica* (L.), and used kriging to develop a targeted treatment plan based on the likelihood of cockroaches encountering the treated area. Arbogast et al. (2000) used contour analysis to examine spatial distributions of the hairy fungus beetle, *Typhaea stercorea* (L.) (Coleoptera: Mycetophagidae), in stored grain in relation to season, temperature, moisture content, and insecticide residue. The dispersion and movements of the western tarnished plant bug, *Lygus hesperus* Knight, adults and nymphs were studied in lentil fields (Schotzko and O'Keeffe 1989), and the colonization and spread of leafhoppers in orchards were investigated by Nestel and Klein (1995). On a regional scale, Williams et al. (1992) analyzed the distribution of sugarbeet wireworm, *Limonius californicus* (Mannerheim), in the northwestern United States, and geostatistical techniques were applied to pheromone trap catches to determine the distribution and expansion rate of the gypsy moth, *Lymantria dispar* (L.) (Sharov et al. 1995, 1996, 1997).

The term, “remote sensing”, is commonly associated with the use of satellite imagery and air photos, but is more broadly defined as all methods of observation of a target by a device some distance from it (Lillesand et al. 2004). Remote sensing in entomology has three main applications: monitoring environmental factors likely to influence insect behavior, detecting the effects of insects, and observing the insects themselves (Riley 1989). Analysis of satellite imagery and aerial photographs has been used to identify landscape characteristics associated with particular arthropod species. Frank et al. (1998) studied landscape features in a residential area in relation to the abundance of the tick *Ixodes scapularis* Say, a vector of Lyme disease, interpreting vegetation from aerial photographs. Analysis of aerial photographs also was used to describe *Anopheles* spp. density and diversity in relation to landscape features in agricultural and urban areas in northern Thailand (Overgaard et al. 2003).

Coulson et al. (1999) used remote sensing to study the effects of landscape heterogeneity on fire ant abundance in an area containing four major land cover types: grassland, cleared woodland, woodland, and bare soil. Fire ants were found in all habitat types, but numbers differed. Although no further analysis was presented, it was suggested that patch size, patch interface composition, and distance to water may be important factors in determining a site’s vulnerability to fire ant colonization. Aerial photographs were used to study the distribution of the leaf cutting ant, *Atta vollenweideri* Forel, in Paraguay (Jonkman 1976, 1979). Mounds were found in two regions, which differed in soil conditions and vegetation. Between the two areas, mounds differed in size, number of openings, and vegetative cover. The number of mounds was related to land use, with more mounds in grazed areas.

Methods have been developed for detecting the activity of insects from changes they produce in the appearance of plant foliage. One of the earliest applications of remote sensing in entomology was in the mapping of damage by forest pests. Defoliation by the hemlock looper was first sketch mapped by airborne observers in the 1930's (Chiang and Meyer 1974). Satellite imagery and aerial photography have recently been used for detection of tree damage by insects that include spruce budworm (Chalifoux et al. 1998), gypsy moths (Townsend et al. 2004), and hemlock wooly adelgid (Royle and Lathrop 2002).

Differences in reflectance of different wavelengths allow discrimination of various types of vegetation. For example, deciduous trees characteristically reflect almost twice the average near infrared radiation (NIR) of coniferous trees (Lillesand et al. 2004). The NIR spectral responses of vegetation are different for healthy versus damaged leaves, for leaves at various stages of senescence, and for leaf-on versus leaf-off conditions (Lillesand et al. 2004). The Normalized Difference Vegetation Index (NDVI), a scaled infrared-to-red ratio, is a widely used indicator of plant stress or damage. Sudbrink et al. (2003) found that beet armyworm, *Spodoptera exigua* (Hübner), infestations in cotton were associated with lower NDVI values representing zones of open or stressed canopy. Remote sensing has been used to detect developing or potential insect outbreaks. Bryceson (1989) used satellite imagery to identify areas that were likely to have egg beds of the Australian plague locust, *Chortoicetes terminifera* (Walker). The areas were detected by a change in the NDVI following March rains. A vegetation index was used in a similar way to detect locust habitats in Africa (Tappan et al. 1991).

Green et al. (1977) used aerial photography to detect imported fire ant mounds in Texas. They found that mounds could be detected on color, color infrared (CIR), and black and white infrared (BWIR) images. Because of seasonal variation in the characteristic appearance of mounds, the detection rate was highest in December and lowest in July. Vogt (2004) used airborne multispectral digital imagery to detect imported fire ant mounds in pastures. Up to 75% of the mounds present were detectable using false color infrared images.

Some types of analyses currently conducted using GIS pre-date the availability of GIS software. Intersections and differences between multiple data layers such as land cover, sample data, or time series were analyzed using acetate overlays. Wagner et al. (1979) used this technique to develop treatment priorities for a mosquito control district based on the locations of residential areas and mosquito breeding sites, as determined from remotely sensed images. GIS is a useful tool for modeling potential insect outbreaks based on sample data of the potential range of invasive species. Counts of trapped male gypsy moths were interpolated to created maps of predicted defoliation based on historical data (Gage 1991). Defoliation prediction maps could be used to identify areas where suppression is needed.

GIS has been used to create several models of the potential range expansion of the red imported fire ant (Thompson et al. 1999, Korzukhin et al. 2001, Sutherst and Maywald 2005). Most of the other studies reviewed here have utilized GIS software integrate, analyze, and map spatial data from a variety of sources, including databases and imagery. Functions of GIS include geostatistics, analysis of remotely sensed imagery, modeling, and cartography.

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Table 1.1. Ant species displaced by *Solenopsis invicta*.

Species displaced	Habitat	Location	Reference
Dolichoderinae			
Forelius pruinosus = *Iridomyrmex pruinosum*	Agricultural, Roadside	Florida	Whitcomb et al. (1992), Wojcik (1994)
Myrmicinae			
Monomorium viridum	Roadside	Florida	Wojcik (1994)
Paratrechina vividula	Roadside	Florida	Wojcik (1994)
Pheidole metallescens	Roadside	Florida	Wojcik (1994)
Pheidole floridana	Roadside	Florida	Wojcik (1994)
Pheidole morrisi	Roadside	Florida	Wojcik (1994)
Solenopsis geminata	Agricultural, Roadside	Alabama, Florida, Texas	Wilson (1958), Glancey et al. (1976), Whitcomb et al. (1992), Hung and Vinson (1978), Porter et al. (1988)
Pheidole spp.	Agricultural	Florida	Whitcomb et al. (1992)
Solenopsis xyloni	Roadside	Alabama, Texas	Wilson (1958), Glancey et al. 1976), Hung and Vinson (1978)

Table 1.2. Ant species displaced by *Linepithema humile*.

Species displaced	Habitat	Location	Reference
Ecitominae			
Neivamyrmex sp.	Scrub	California	Suarez et al. (1998)
Dolichoderinae			
Liometopum occidentale	Riparian	California	Ward (1987)
Tapinoma sessile	Riparian	California	Ward (1987)
Formicinae			
Anaplolepis custodiens	Scrub	South Africa	Bond and Slingsby (1984)
Formica occidentalis	Riparian	California	Ward (1987)
Myrmicinae			
Messor sp. (=*Veromessor*)	Scrub	California	Suarez et al. (1998)
Pheidole capensis	Scrub	South Africa	Bond and Slingsby (1984)
Pheidole grallipes	Roadside	California	Erickson (1972)
Pheidole megacephala[1]	Roadside	Madeira, Bermuda, Hawaii	Stoll (1898), Haskins and Haskins (1965), Crowell (1968), Lieberberg et al. (1975), Majer (1994)
Pogonomyrmex Californicus	Roadside	California	Erickson (1972)
Pogonomyrmex sp.	Scrub	California	Suarez et al. (1998)
Solenopsis saevissima	Roadside	Alabama	Wilson (1951)
Veromessor pergandii	Roadside	California	Erickson (1972)

[1] Later results (Haskins and Haskins 1988) indicate that equilibrium may exist between *L. humile* and *P. megacephala* in Bermuda.

CHAPTER 2

ACTIVITY OF BIFENTHRIN, CHLORFENAPYR, FIPRONIL, AND THIAMETHOXAM AGAINST ARGENTINE ANTS (HYMENOPTERA: FORMICIDAE)[1]

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[1] Wiltz, B. A. D. R. Suiter, and W. A. Gardner. Submitted to Journal of Economic Entomology.

ABSTRACT

Bifenthrin, chlorfenapyr, fipronil, and thiamethoxam were evaluated for activity against the Argentine ant, *Linepithema humile* (Mayr). Median lethal times (LT_{50}) following topical treatment were <30 min for bifenthrin, 118.8 min for chlorfenapyr, 168.7 min for fipronil, and 54.7 min for thiamethoxam. At least 50% of the ants were immobilized within 30 min of topical treatment with bifenthrin, 90 min with chlorfenapyr, and 120 min with thiamethoxam. Only 18% of fipronil-treated ants were immobilized 120 min after treatment. Mortality due to horizontal exposure to corpses of topically treated ants was evaluated at 10°, 20°, or 30°C, with 2, 5, 10, or 20% of the ants in a cohort treated. Mortality did not differ with temperature in the bifenthrin treatments, but increased with temperature in the chlorfenapyr treatments, with no treatment with either chemical yielding more than 30% mortality. Of the four chemicals, fipronil demonstrated the greatest horizontal activity, with 84% killed at 30°C when 20% were treated. At 10°C, mortality from thiamethoxam treatments did not differ from controls, while there was >50% mortality in each of the 30°C treatments. Seven days after exposure to insecticide-treated pine needles, mortality levels were 97% for bifenthrin, 36% for chlorfenapyr, 100% for fipronil, and 76% for thiamethoxam. Exposing 20% of the ants in a cohort to treated pine needles yielded 7d mortality levels of 50% for bifenthrin, 18% for chlorfenapyr, 65% for fipronil, and 31% for thiamethoxam. When ants were given a choice between crossing bridges treated with insecticide or water, only bifenthrin reduced the number of ants crossing.

Key words: *Linepithema humile*, insecticide, horizontal toxicity, contact toxicity, barrier

INTRODUCTION

The Argentine ant, *Linepithema humile* (Mayr), is a major household pest in the southeastern United States (Hedges 2000). In infested areas it is often the dominant ant species because of its high reproductive capacity, aggressive foraging behavior, and lack of natural enemies in its expanded range (Holway 1999). *L. humile* is an important secondary pest in agriculture because it tends honeydew-producing homopterans (Markin 1968) and has been shown to displace native ant species in heavily infested areas (Erickson 1971, Ward 1987). Although they usually nest outdoors, Argentine ants invade homes in search of food, moisture, or warmth (Gordon et al. 2001).

Traditional ant control tactics include the use of contact insecticides, either as a barrier (Rust et al. 1996), broadcast (Drees et al. 2000), or nest treatment (Williams and Lofgren 1983). In recent years there has been a resurgence in the use of baits, which are more target specific and control ants by using less toxicant (Klotz et al. 1997). Other ant control methods include habitat modification (Rust et al. 2003) and the use of natural products as repellent barriers (Shorey et al. 1992). Non-repellent insecticides offer promise as both contact insecticides and bait active ingredients because they go undetected by ants, allowing high degrees of horizontal transmission (Rust et al. 2003). The most effective control strategies often combine multiple treatment techniques. Control of Argentine ants in heavily infested areas can require a combination of nest treatments, perimeter treatments, and baits (Hedges 1998). Efficacy of contact insecticides is in large part a function of properties of the insecticide (Knight and Rust 1990) and environmental conditions (Rust et al. 2003). Some of the properties of the insecticide that contribute to total mortality are topical and contact toxicity, transferability to untreated individuals, residual activity, and repellency (Knight and Rust 1990, Ibrahim et al. 2003, Lofgren et al. 1989).

Different control strategies require toxicants with different sets of properties. To maximize efficacy of baits, the active ingredient should have delayed toxicity and be transferable and non-repellent (Stringer et al. 1964), while repellent or fast-acting toxicants are effective barriers (Pranschke et al. 2003). The purpose of this study was to evaluate contact insecticides for topical toxicity, knockdown rate, horizontal toxicity, residual activity, and barrier effects against the Argentine ant.

MATERIALS AND METHODS

Test insects. Argentine ant colonies were collected in Spalding Co., GA. Ants in their nesting material (mulch, leaf litter, or soil) were placed in plastic pans (58 x 43 x 15 cm, Sterilite Corporation, Townsend, MA) coated on the inside walls with a thin layer of Fluon™ (Northern Products, Inc., Woonsocket, RI) and provided water, 25% sugar water, frozen house crickets (*Acheta domesticus* (L.)), and nest cells. Nest cells were constructed from Petri dishes (100 x 25 mm) containing a 1.0 cm thick layer of hardened dental plaster (Castone; Dentsply International Inc., York, PA) to retain moisture. Dish sides and lids were painted black, and three 4-mm holes were drilled into the sides to allow ants to enter. As the nesting material in the pans dried, workers moved the entire colony into the moistened cells. Nest cells containing ants were transferred to clean 31 x 23 x 10 cm Fluon-lined plastic boxes (Pioneer Plastics, Dixon, KY), and colonies were maintained at room temperature (23° – 26°C) on a diet of water, 25% sugar water, and crickets.

Test chemicals. Laboratory studies were conducted to determine multiple effects of bifenthrin (Talstar Flowable; FMC Corporation, Philadelphia, PA), chlorfenapyr (Phantom; BASF, Research Triangle Park, NC), fipronil (Termidor SC; BASF, Research Triangle Park, NC), and thiamethoxam (thiamethoxam 25WG ; Syngenta Corp., Greensboro, NC). Bifenthrin, chlorfenapyr, and fipronil were evaluated at label concentration for ant control (0.06% fipronil, 0.06% bifenthrin, or 0.5% chlorfenapyr). Thiamethoxam, which is not labeled for ant control, was tested at a rate of 0.06%.

Toxicity from topical treatment. Each chemical was tested for ability to kill Argentine ants via topical treatment. For each treatment 10 groups of 20 workers were placed into a plastic box lined with absorbent paper, sprayed with one of the insecticidal suspensions or water (control) using a hand-held misting bottle (three sprays), and immediately transferred to dry Fluon-lined boxes. Ant mortality in each replicate was determined at half-hour intervals for up to 4 h post-treatment.

Mobility of topically-treated ants. Ants were topically treated, as above, by spraying 10 groups of ≈ 100 workers. Treated ants were transferred to dry containers, then from each group 10 randomly chosen ants were placed into each of four 30-ml, Fluon-lined, plastic cups. At half-hour intervals for 2 h, one of the cups was inverted onto the center of a 14-cm diameter circle drawn on a piece of paper, then removed. The number of ants remaining inside the circle was counted after 2 min. As ants walked out of the circle they were removed from the paper so they would not re-enter.

Horizontal toxicity. To test for mortality due to ant-to-ant contact, with limited possibility of mortality from a contaminated surface, we conducted an assay in which treated ants were allowed to die before being placed in containers with untreated ants.

Necrophoric behavior is common in ants (Wilson et al. 1958, Gordon 1983); therefore, handling of treated corpses by untreated nestmates should result in horizontal activity due to ant-to-ant contact. To determine if Argentine ants would remove the corpses of nestmates treated with each of the insecticides, we conducted an assay in which treated corpses were placed on a plastic disk in a box containing untreated ants. To obtain treated corpses, live Argentine ants were topically treated by spraying groups of approximately 1,000 ants then immediately transferring them to a dry box. Approximately 6 h after treatment, 30 treated corpses were transferred to 31 x 23 x 10 cm plastic boxes containing 300 untreated workers, a nest cell, sugar water, and water. To acclimate the untreated workers, they were placed in the test boxes 24 h before starting the assay. Immediately before adding treated corpses to the boxes, dead untreated workers were removed and replaced with live workers. To prevent contamination of the arena floor the treated ants were placed on a 5-cm diameter disk cut from the bottom of a plastic weigh boat. Control treatments received 30 ants killed by freezing. The number of dead ants remaining on the plastic disk was recorded after 1, 2, and 24 h. There were six replicates for each treatment.

If horizontal toxicity due to contact with treated individuals occurred, mortality was expected to increase with an increase in the percentage of ants in a cohort that were treated. Additionally, we expected ants to be more active at higher temperatures, thereby increasing the encounter rate and thus total mortality. A total of 300 ants was used for each replicate, with five, 10, or 20% of the total cohort treated and the remainder untreated. Additionally, a 2% treatment was included for fipronil-treated ants. Ants were treated as described above, then six, 15, 30, or 60 of the treated corpses were placed on plastic disks in boxes containing acclimated untreated workers, a nest cell, sugar water, and water. Control treatments received 60 ants killed by freezing. Boxes were covered with lids containing four, 6-mm holes and maintained at 10°, 20° or 30°C with a photoperiod of 12:12 (L:D) for 3 d when the number of dead ants was recorded in each replicate. There were six replicates for each treatment combination (6 replicates x 4 chemicals x 3 temperatures x 4 or 5 percentages treated = 306 total replicates).

Residual activity. The residual activity of each chemical was tested using treated pine needles, a common mulch substrate in the southeastern United States. Two fluon-lined plastic boxes (19 x 13 x 10 cm) were connected by a bridge comprised of insecticide-treated or control (water-treated) pine needles (Figure 2.1). Pine needles were treated by soaking for 15 min in one of the insecticide formulations or water, then allowed to dry for 1 h under a fume hood. The two ends of the bridge were constructed from 15 cm lengths of polyethylene tubing (6 mm (1/4") OD x 4 mm (0.17") ID) anchored in Castone-filled 30-ml plastic cups. A cotton plug was pushed 1 cm into the exposed end of the tubing to prevent ants from entering. A single bundle (3 needles) of treated pine needles was used to connect the two pieces of tubing. The treated portion of each bridge was approximately 15 cm long. Argentine ants were starved for 2 d, then for each replicate 100 ants were placed in an empty box (A) connected by the pine needle bridge to a second box (B). Box B contained a nest cell, water-soaked cotton ball, and sugar water-soaked cotton ball. This design forced ants to cross the treated bridge. Each day for 7 d, dead ants in each box were counted and removed, and water was added to the nest cells and cotton balls. On the last day, the number of live ants remaining in each box was counted. Tests were conducted at room temperature (23° – 26° C), with six replicates per treatment.

To test for horizontal activity following contact exposure, boxes connected by a pine needle bridge were set up as described above and groups of 20 ants were removed after crossing the bridge. Groups of 20 ants crossing a water-treated bridge were used as a control treatment. The 20 exposed ants were then placed in a box containing 80 untreated ants, a nest cell, water-soaked cotton ball, and sugar water-soaked cotton ball. Each day for 7 d, dead ants were counted, and water was added to the nest cells and cotton balls. Each treatment was replicated 6 times and conducted at room temperature (23° – 26° C).

Barrier tests. Tests were conducted to determine whether ants would cross insecticide-treated barriers to reach a food source. Choice tests were conducted in a three-chambered apparatus consisting of 19 x 13 x 10 cm Fluon-lined plastic boxes arranged linearly, with each of the end boxes connected to the center box by a paper bridge (Figure 2.2). The bridge to one box was treated with insecticide and the bridge to the other box with water. Bridges were constructed from 38 x 5 cm strips cut from legal sized manila file folders. Before installing bridges in the boxes, the center 10 cm section of each was treated by pipetting the insecticide or water onto the paper, brushing over the desired area, and air-drying for 1 h. Bifenthrin and fipronil were applied at label rates for broadcast ant treatment (5 gal 0.06% bifenthrin / 1,000 ft^2 = 1.0 ml / 50 cm^2 and 1.5 gal 0.06% fipronil / 1,000 ft^2 = 0.3 ml / 50 cm^2). Because chlorfenapyr and thiamethoxam do not have broadcast label rates for ants, rates of 0.3 ml 0.5% chlorfenapyr and 0.06% thiamethoxam were used. Because the suspensions did not penetrate the surface of the paper bridges, the paper was inverted and the reverse side treated in the same way. Water-water pairings were used as negative controls (0.3 ml water), and 0.6 ml 1.5% cinnamaldehyde (Cinnamite™; Mycotech Corporation, Butte, MT) was used as a positive control treatment.

Ants were starved for 2 d with access to only water, then 300 ants were placed in the center box (nest chamber), which contained only a moistened nest cell. The boxes on either side (food chambers) contained a nest cell, a sugar water-soaked cotton ball, and a water soaked cotton ball. To prevent a moisture differential that might influence ant movement, all nest cells were prepared by oven drying for 24 h at 50° C, cooling at room temperature and humidity for 2 h, then adding 6 ml water. The numbers of live and dead ants in each of the three chambers were recorded after 24 h. Tests were conducted at room temperature and replicated 10 times for each treatment.

Statistical analysis. Mortality from topical treatment was analyzed by probit analysis (PROC PROBIT; SAS Institute 1985). Data were not corrected for control mortality because control mortality was low and some of the time periods contained zero mortality values. Non-overlapping 95% confidence intervals for LT_{50}s were used to determine significant differences among insecticide treatments. For mobility, horizontal toxicity, and residual toxicity tests, percentages of immobilized or dead ants were transformed by arcsine of the square root. All analyses were performed on the transformed variable. Mobility and residual toxicity data were analyzed by one-way analysis of variance (ANOVA) (SAS Institute 1985), comparing chemical effects at each time period. Horizontal toxicity results were analyzed by two-way ANOVA for the main effects of temperature and percentage treated ants on mortality. Separate analyses were performed for each chemical. When main effects were significant, one-way ANOVA were performed. Means were separated with Tukey's Studentized range test. Barrier test data were analyzed by one-tailed paired t-tests to determine if fewer ants crossed an insecticide-treated surface than the paired water-treated surface.

RESULTS

Toxicity from topical treatments. Based on non-overlapping confidence intervals, the LT_{50}s for each of the four chemicals were significantly different. LT_{50}s (95% CI) from topical treatment were 118.8 min (110.9 – 126.9) for chlorfenapyr, 168.7 min (162.5 – 174.6) for fipronil, and 54.7 min (51.4 – 57.9) for thiamethoxam (Figure 2.3). An LT_{50} could not be determined for bifenthrin because 98.5% of the ants were dead before the first observation was made.

Mobility of topically-treated ants. One hundred percent of bifenthrin-treated ants were immobilized within 30 min of topical treatment (Figure 2.4). At each time interval, immobilization rates in the chlorfenapyr and thiamethoxam treatments were significantly lower than in the bifenthrin treatment, but significantly higher than in the fipronil treatment. The number of fipronil-treated ants immobilized differed from controls at 30 and 90 min after treatment. After 2 h, >80% of the fipronil-treated ants were able to walk out of the 14-cm diameter circles within 2 min.

Horizontal toxicity. Argentine ants removed the corpses of ants treated with each of the chemicals (Table 2.1). With the exception of the bifenthrin treatment, >90% of the treated ants were removed within the first hour. The removal rate of bifenthrin-treated ants was 81% after 24 h.

Mortality due to horizontal exposure did not differ with temperature in the bifenthrin treatments at any of the percentages of corpses treated (Table 2.2). Exposure to any percentage of treated corpses resulted in higher total mortality than in the control treatment, but increasing the percentage treated significantly increased mortality only at 20°C. For chlorfenapyr, mortality significantly increased with temperature but not with percentage of corpses treated. For both bifenthrin and chlorfenapyr, no treatment had more than 28% mortality of untreated ants. In fipronil trials, both temperature and percentage of treated ants affected mortality of untreated ants. The interaction of the two factors was also significant, with mortality increasing with percentage of treated ants at 10° and 30°, but not at 20°C. Fipronil had the greatest horizontal activity of the chemicals with 84% of the untreated ants killed at 30° when 20% were treated. At 20°, > 40% of the ants died when as few as 2% were treated. Response to thiamethoxam depended on temperature, percentage treated, and their interaction. At 10° none of the thiamethoxam treatments differed from control mortality. At 20°, mortality increased with percentage treated, while at 30°, treatments containing treated ants had higher mortality than controls, but did not differ from each other. Mortality was higher at 30°C than at 10° or 20°C, with >50% of untreated ants dying in each of the 30°C treatments. When 10% or 20% of the ants were treated, mortality increased with each increase in temperature.

Residual activity. Seven days after first exposure to treated pine needle bridges, Argentine ant mortality was 21% in the water, 97% in the bifenthrin, 36% in the clorfenapyr, 100% in the fipronil, and 76% in the thiamethoxam treatment (Figure 2.5). Throughout most of the observation period, mortality was highest in the fipronil and bifenthrin treatments, followed by thiamethoxam, then chlorfenapyr. Once ants had crossed bridges there was little travel back across the bridge. With the exception of the bifenthrin treatment, most of the mortality occurred with ants that successfully crossed the bridges. When ants were first introduced into the test arena, they were observed traveling short distances onto the pine needles and returning to the original box. This behavior continued for a minimum of 15 min before the first ants successfully crossed the bridge, resulting in some of the ants contacting the treated surface without completely crossing the bridges. For the bifenthrin treatment, there was significant mortality of ants that had not crossed the bridge, either by contact with the bridge or contact with other ants that had contacted the bridge. After 1d, an average of 74% of the ants were found dead in box A (did not cross bridge) and 16% in box B (crossed bridge) of the bifenthrin treatment, while few dead ants were found in box A of other treatments: 0.5% in the control, 0.8% in the chlorfenapyr, 11% in the fipronil, and 3% in the thiamethoxam treatment.

When 20% of the ants in a cohort were allowed to cross the treated bridges, 7 d mortality was 15% for controls, 50% for bifenthrin, 18% for chlorfenapyr, 65% for fipronil, and 31% for thiamethoxam (Figure 2.6). During the set-up of this test, rapid mortality occurred among ants attempting to cross the bifenthrin-treated bridges. Ants that had not crossed the bridge began dying within 30 min of introduction.

Barrier tests. In choice tests, only bifenthrin and the positive control cinnamaldehyde had fewer ants on the treated side than on the paired control bridge after 24h (Table 2.3). The bifenthrin treatment had 19% as many ants as the control, and the cinnamaldehyde treatment had 6% as many ants as the control. There was 80% mortality among ants that had crossed bifenthrin-treated bridges, while mortality was 18% in the chlorfenapyr treatment, 11% in the fipronil treatment, and 12% in the thiamethoxam treatment.

DISCUSSION

Along with baits, contact insecticide treatments are one of the main control tactics for pest ants. The efficacy of a contact insecticide is a function of several properties that include topical toxicity, knockdown rate, horizontal toxicity, contact toxicity, and effectiveness as a barrier. A variety of assays are used to evaluate individual properties of contact insecticides. However, a combination of properties rather than any one property alone is a better indicator of efficacy and of the best use for a toxicant.

Speed of kill and knockdown are two key factors used to determine the best application for an insecticide. Assuming equivalent residual activity and bioavailability, faster acting chemicals would be more effective as a barrier, while slower acting active ingredients allow more opportunity for ant-to-ant transfer. Delayed-action toxicants used in ant baits require the insect to survive long enough to return to the nest and engage in trophallaxis. For contact toxicants, it may be enough to have the exposed individuals survive to return to the nest. When combined with transferability, even a slight delay in knockdown or mortality can contribute to the toxicant's horizontal activity.

In our study, bifenthrin was faster acting than the other chemicals when applied topically, immobilizing 100% of the ants within 30 minutes. Spraying Argentine ant trails with bifenthrin kills foraging ants quickly, thus most die before they can return to the nest. Chlorfenapyr, fipronil, and thiamethoxam exhibited some degree of delayed toxicity, with at least 50% of the ants remaining alive and motile 1 h after topical treatment. Fipronil was the slowest acting, requiring >2 h to immobilize 50% of the topically-treated ants. Each of these three chemicals act slowly enough for exposed ants to return to the nest, allowing for potential horizontal activity due to contamination of nesting material, interaction with nestmates, and necrophoresis.

Horizontal activity occurs through contact between treated and untreated individuals (Ferster et al. 2001, Ibrahim et al. 2003, Shelton and Grace 2003), cannibalism (Smith and Lockwood 2003), contact with contaminated surfaces (Buczkowski and Schal 2001), and trophallaxis (Banks et al. 1978, Knight and Rust 1991, Hooper-Bùi and Rust 2000). While many studies have examined horizontal activity due to trophallaxis as it relates to baiting, other forms of horizontal activity have not been widely studied in ants. Subterranean and drywood termite mortality due to contact between treated and untreated individuals has been demonstrated in several studies, which showed that horizontal activity depends on caste, insecticide concentration, exposure time, and exposure method (Ferster et al. 2001, Ibrahim et al. 2003, Shelton and Grace 2003). Smith and Lockwood (2003) found that cannibalism increased the efficacy of diflubenzuron and fipronil against grasshoppers. Both topical and residual delivery of fipronil exhibit horizontal toxicity to German cockroaches, *Blatella germanica* (L.), via ingestion of excreted insecticide residues, cannibalism, and contact with contaminated substrates (Buczkowski and Schal 2001). We found that the test chemicals did not deter untreated ants from removing treated corpses from the nest area. Initial removal of bifenthrin-treated corpses occurred more slowly than in the other treatments, with 45% removal 1 h after introduction, versus >90% in the chlorfenapyr, fipronil, and thiamethoxam treatments. However, by 24 h, >80% of the bifenthrin-treated corpses had been removed. Handling of treated corpses demonstrated that there is potential for horizontal activity due to necrophoresis and interaction with live donors (i.e., grooming and trophallaxis). Soeprono and Rust (2004) evaluated horizontal toxicity of bifenthrin, fipronil, and cyfluthrin to Argentine ants. Two sets of bioassays were conducted. In the first, donor ants were treated by contact with treated sand and placed with untreated ants at one of two temperatures. In the other tests, donor ants were allowed to die before being placed with untreated ants at a single temperature. As in our study, they found that treatment with fipronil caused the highest rate of horizontal mortality. In live donor tests they found a trend toward higher mortality in the fipronil treatment at 27-29°C than at 21–23°C. Our study corroborates this increase in horizontal toxicity of fipronil with temperature, extending the lower temperature range to 10°C. While we did not find a difference in mortality among temperatures for bifenthrin-treated ants, Soeprono and Rust (2004) found higher mortality at the higher temperatures. This difference between the two studies may be explained by the fact that in their tests treated ants remained alive for a period of time after introduction to the test arena. At higher temperatures the treated ants may have been more active, allowing more opportunity for contact with untreated ants and contamination of the box floor.

Of the chemicals we tested, chlorfenapyr had the lowest horizontal toxicity to Argentine ants. Other results presented here suggest that this may be due to low contact toxicity rather than an aversion to handling the treated corpses, since removal rates of chlorfenapyr-treated corpses was 94% after 1 h. Horizontal mortality due to thiamethoxam was highly temperature dependent, with mortality at 10° not differing from controls, but all 30° treatments having at least 50%

mortality of recipient ants. In practical terms, lower horizontal activity at lower temperatures may not be as important as laboratory results suggest. In winter, several Argentine ant nests in an area combine to form larger colonies (Newell and Barber 1913), making nest treatments an effective way to target large outdoor populations. Horizontal activity of toxicants can play a more important role in warmer weather when populations are more diffuse, increasing the difficulty of locating all nests for treatment.

Mortality due to contact with treated surfaces is an important factor contributing to a chemical's ability to exclude ants from structures and containers. When applied as bands around the perimeters of homes, chlorpyrifos, cyfluthrin, and cypermethrin sprays and cyfluthrin spray + cyfluthrin granules provided >80% reduction in the number of Argentine ants trapped 7 d after treatment, and the combination of cyfluthrin spray and granules provided >80% reduction after 60 d (Rust et al. 1996). Costa and Rust (1999) found that a soil-mix treatment of fipronil prevented Argentine ants from establishing colonies in potted plants. While ants initially began moving brood into the pots, foraging on the plants ceased within 1 d and mortality was ≥ 93% after 1 wk. In a similar study using red imported fire ants, *Solenopsis invicta* Buren, Oi and Williams (1996) found that when granular bifenthrin was applied in potting mix at its LC_{50}, LC_{90}, or maximum label rate (25 ppm), 92, 32, or 6% of the pots were infested with fire ants 48 h after exposure. After 2 wk of exposure, all pots were considered uninfested by quarantine standards. Because pots were initially infested, they concluded that fire ant exclusion from the pots was due to mortality, not repellency. In our study, contact with bifenthrin-treated pine needles resulted in almost immediate mortality of Argentine ants. Within 1 d, 74% of the ants died without successfully crossing pine needle bridges, and an additional 16% died after crossing the bridges. One-day mortality was similar in the fipronil treatment, but 70% crossed the pine needles before dying while 14% died without crossing.

Chlorfenapyr was the slowest acting in contact toxicity tests, with mortality not differing from controls during the first 5 d. Mortality due to contact with thiamethoxam-treated pine needles occurred more slowly than with bifenthrin or fipronil, but cumulative mortality on days 3-7 was greater than in the chlorfenapyr treatment and reached 76% by day 7.

While repellent substances are useful as barriers, Knight and Rust (1990) found that if a chemical is highly repellent, mortality is low regardless of its toxicity level because few ants contact the chemical. Conversely, they found that non-repellent treatments cause high mortality, even with actives that are only of intermediate toxicity, because of increased contact. Many repellency tests measure the response of individual ants or the number of ants crossing a barrier over a short period of time. Knight and Rust (1990) used a tube with the center section treated to connect nest and feeding chambers. They counted the number of ants crossing the treated area during 15 30-s intervals within 1 h of set-up, then removed the treated section and recorded mortality of exposed ants after 48 h. Our assay differs from this and similar tests in that it measures a group response rather than the movement of individual ants. By counting the number of ants in each feeding chamber after 24 h instead of the number crossing the barriers, we incorporate repellency and recruitment effects. Because there were no food or water in the center chambers, ants successfully crossing bridges should recruit more foragers to one of the end containers unless they are killed or immobilized quickly. Of the chemicals we tested, we found that only the bifenthrin treatment had fewer ants cross than its paired control bridge. Other studies have shown bifenthrin to be non-repellent to fire ants (Oi and Williams 1996, Richman and Hooper-Bùi 2003). Although we found bifenthrin to act as a barrier to Argentine ants, our combined results suggest that this effect is due to its rapid action rather than repellency. Our findings of 100% knockdown within 30 min and 100% mortality within 2.5 h may explain the lack of recruitment across bifenthrin-treated bridges. While repellent or fast-acting insecticides are useful as barrier treatments, they do not provide the opportunity for foragers to spread the chemical to nestmates. These results are in contrast with those seen in the fipronil treatment. Our pine needle bridge assay demonstrated that bifenthrin and fipronil caused similar 24 h mortality due to contact. However, ants crossing the fipronil bridges survived to recruit more ants across the bridges. When ants were given a choice between bridges treated with bifenthrin or water, ants continued to be recruited to the water side, but were killed too quickly to be recruited to the bifenthrin side.

Summary. Bifenthrin has properties best suited for use as a barrier treatment against Argentine ants. It was the fastest acting of the four chemicals in contact toxicity assays. Ants crossed the untreated bridge when provided a choice or died when provided no alternative to a bifenthrin treated bridge. Chlorfenapyr was most effective when applied topically. It did not deter ants from walking across treated surfaces or nestmates from handling the corpses of treated ants. However, both contact and horizontal toxicities were less than those of other chemicals tested. Topical toxicity of fipronil was delayed relative to the other chemicals. Horizontal toxicity from topical or contact exposure was the greatest of the four chemicals. Contact exposure caused >80% mortality within 1 d and 100% within 5 d but was sufficiently slow to allow ants to cross the treated pine needles. Thiamethoxam also resulted in significant mortality due to contact with topically or contact exposed ants. Horizontal activity was positively correlated with temperature. Like fipronil, thiamethoxam acted slowly enough to allow exposed ants to return to the nest and transfer toxicant to nestmates.

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Table 2.1. Percentage (mean ± SE) of insecticide-killed corpses removed by untreated nestmates. Each of 6 replicates contained 300 untreated (live) ants and 30 treated corpses.

Treatment	N	1h	2h	24h
Bifenthrin	6	45.0 ± 9.1	46.7 ± 8.9	80.6 ± 6.5
Chlorfenapyr	6	94.4 ± 1.6	96.1 ± 1.0	97.2 ± 1.0
Fipronil	6	96.7 ± 1.7	98.9 ± 1.1	99.4 ± 0.6
Thiamethoxam	6	98.9 ± 1.1	99.4 ± 0.6	100.0 ± 0.0
Control	6	90.6 ± 2.6	96.7 ± 2.1	100.0 ± 0.0

Table 2.2. Percentage cohort mortality (mean ± SE, *n*=6) of untreated Argentine ants after 3 days exposure to insecticide-killed corpses.

		Temperature (°C)		
Treatment	% treated	10	20	30
Bifenthrin	0	7.6 ± 1.5bA	6.2 ± 0.7cA	7.2 ± 1.4bA
	5	15.6 ± 4.3abA	11.1 ± 1.6bcA	19.2 ± 1.6aA
	10	23.1 ± 3.7aA	21.1 ± 2.0abA	17.2 ± 3.8aA
	20	26.2 ± 5.7aA	27.4 ± 6.5aA	23.4 ± 1.8aA
Chlorfenapyr	0	5.8 ± 0.8bB	6.5 ± 0.1bAB	10.4 ± 2.0bA
	5	9.7 ± 1.5abB	14.0 ± 1.2aB	22.8 ± 1.3aA
	10	12.0 ± 2.4abB	13.5 ± 1.5aB	22.0 ± 1.7aA
	20	15.6 ± 3.4aB	17.4 ± 1.5aAB	26.7 ± 3.5aA
Fipronil *	0	6.6 ± 0.8cA	8.3 ± 1.0bA	7.0 ± 2.1dA
	2	13.7 ± 1.5bC	42.6 ± 3.7aA	26.0 ± 1.4cB
	5	14.9 ± 1.7bB	46.0 ± 3.5aA	54.3 ± 3.2bA
	10	18.3 ± 1.8abC	48.6 ± 4.3aB	64.4 ± 4.3bA
	20	24.2 ± 2.1aC	42.8 ± 2.4aB	84.2 ± 2.7aA
Thiamethoxam *	0	5.9 ± 0.6aA	8.5 ± 1.6cA	6.0 ± 1.5bA
	5	6.6 ± 0.4aB	18.4 ± 2.4bB	50.4 ± 10.8aA
	10	7.0 ± 1.1aC	23.5 ± 1.9abB	51.2 ± 9.4aA
	20	6.0 ± 0.8aC	31.9 ± 3.2aB	71.5 ± 3.6aA

For each chemical, means within a column followed by the same lowercase letters, and within a row followed by the same uppercase letters, do not differ significantly (Tukey's HSD, $P < 0.05$). * = Interaction between temperature and percentage treated is significant (Two-way ANOVA, $P < 0.05$).

Table 2.3. Number of ants (mean ± SE) remaining on treated and control sides of barrier choice tests after 24 h. Each replicate (*n*=10) contained a total of 300 ants. Numbers do not total 300 because some ants did not cross either bridge. Numbers of ants on treated and control sides were compared by 1-tailed paired t-tests.

Treatment	# treated side	# control side	T	P
Bifenthrin	26.1 ± 4.6	138.1 ± 18.6	-5.569	0.0002
Chlorfenapyr	56.1 ± 22.4	127.4 ± 27.3	-1.586	0.0735
Fipronil	107.5 ± 24.8	40.8 ± 17.5	1.865	0.9525
Thiamethoxam	58.3 ± 18.1	73.3 ± 17.9	-0.502	0.3137
Control	82.7 ± 24.2	100.6 ± 24.4	-0.405	0.3476
Cinnamaldehyde	7.7 ± 2.2	123.9 ± 34.5	-3.341	0.0043

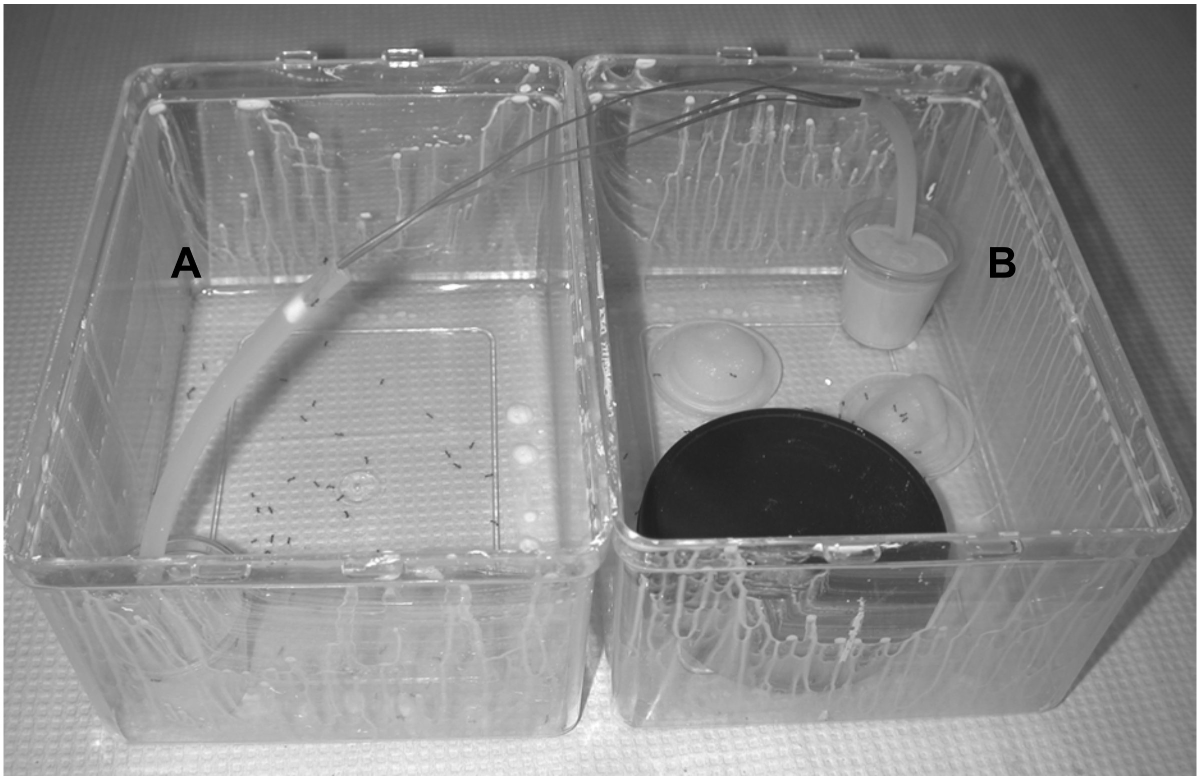

Figure 2.1. Apparatus used for Argentine ant contact toxicity assay. Ants were placed in container A and forced to cross a treated pine needle bridge to reach food, water, and harborage.

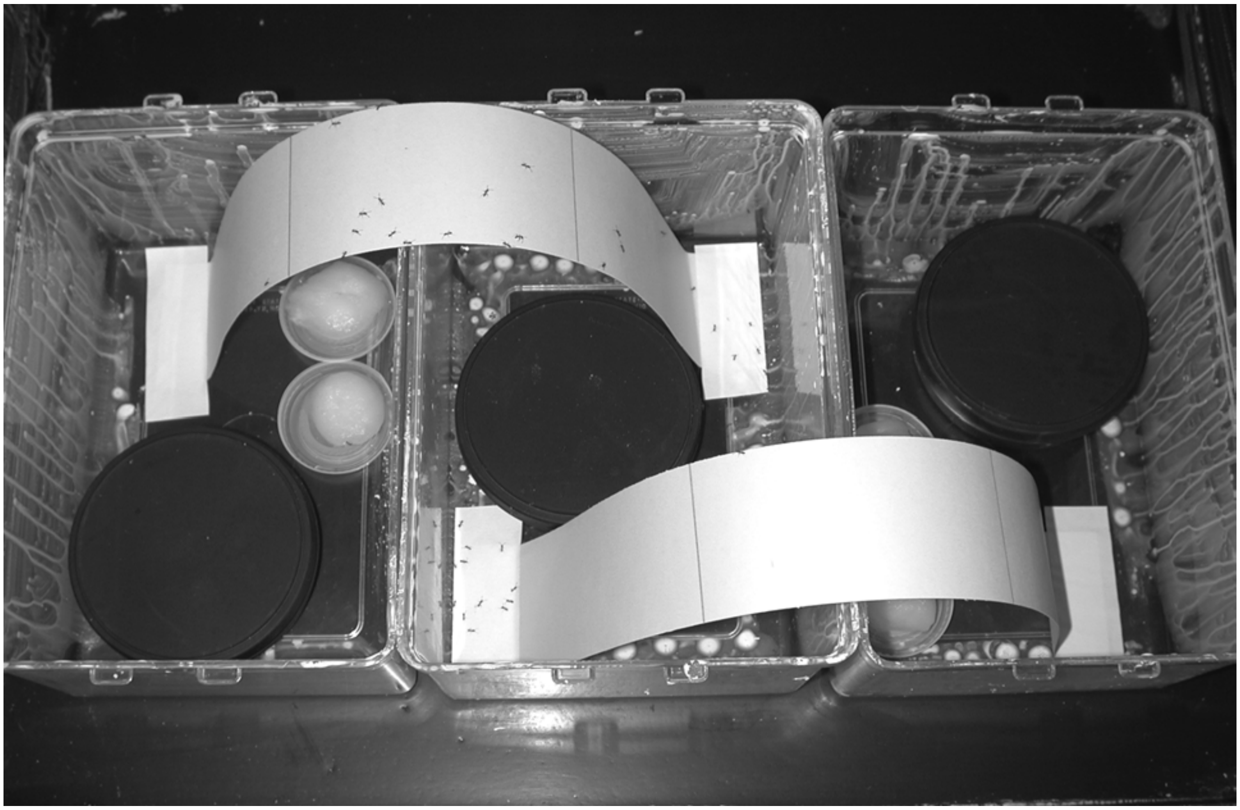

Figure 2.2. Apparatus used for Argentine ant barrier assay. Ants were placed in the center container. One of the bridges was treated with water and the other with a test chemical, providing ants with a choice of crossing insecticide or water (control)-treated barriers to reach food, water, and harborage.

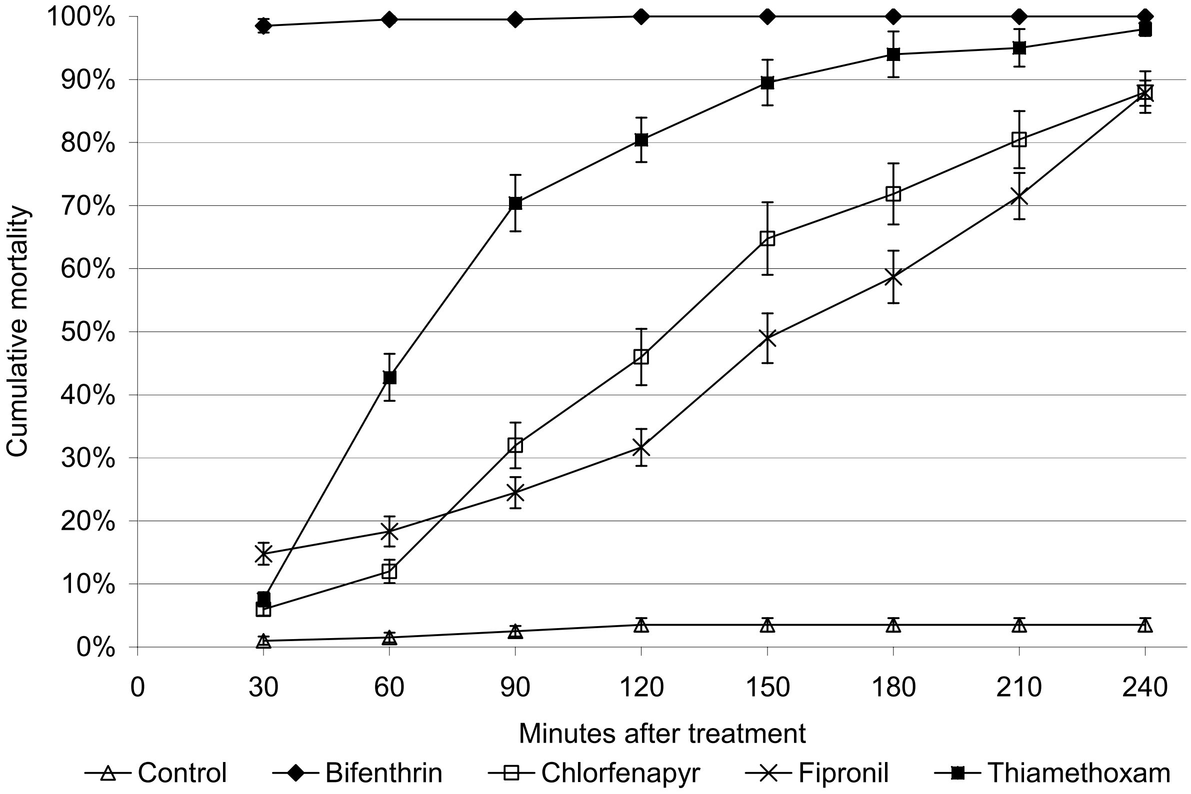

Figure 2.3. Argentine ant mortality from topical application of bifenthrin, chlorfenapyr, fipronil, thiamethoxam, or water (control). Each point represents the mean ± SE of *n* = 10 replicates, 20 ants per replicate.

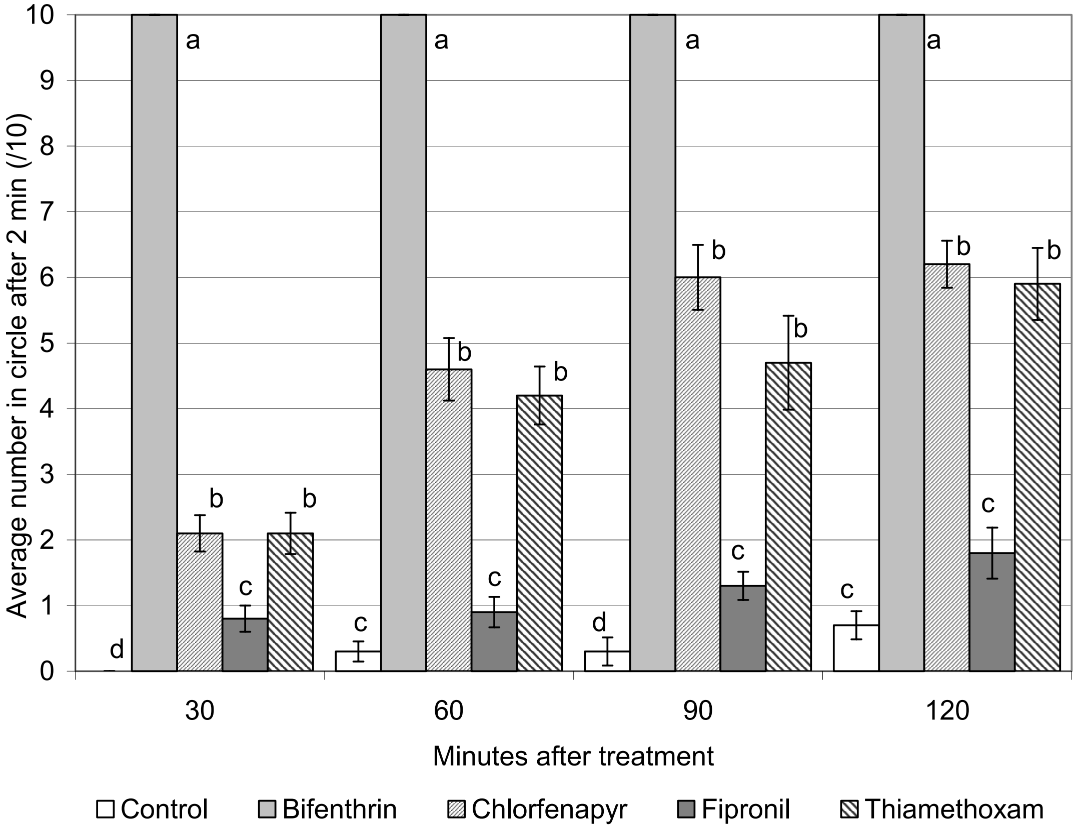

Figure 2.4. Number of Argentine ants unable to walk out of a 14 cm diameter circle following topical application of bifenthrin, chlorfenapyr, fipronil, thiamethoxam, or water (control). Each replicate contained 10 ants and each bar represents the mean ± SE of 10 replicates. Within each time period, means with the same letter are not significantly different (Tukey's HSD, $p<0.05$).

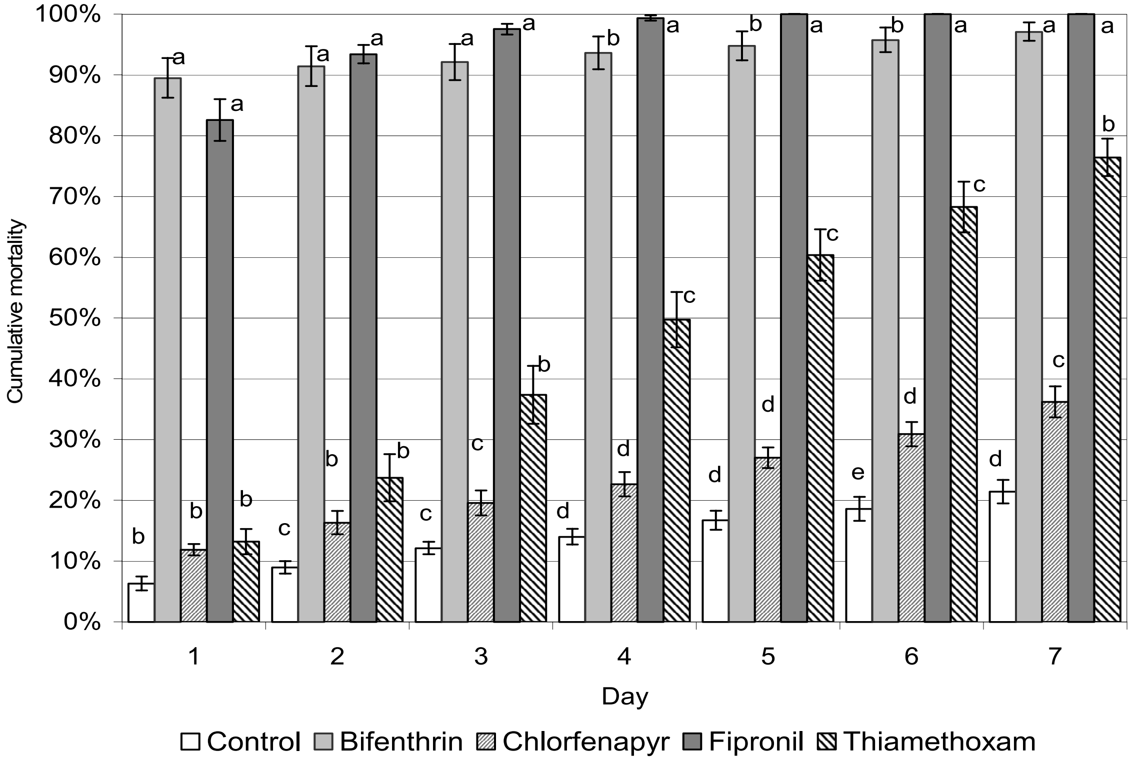

Figure 2.5. Contact toxicity (mean ± SE cumulative daily mortality) to Argentine ants crossing insecticide-soaked pine needle bridge. Within each time period, means with the same letter are not significantly different (Tukey’s HSD, $p<0.05$).

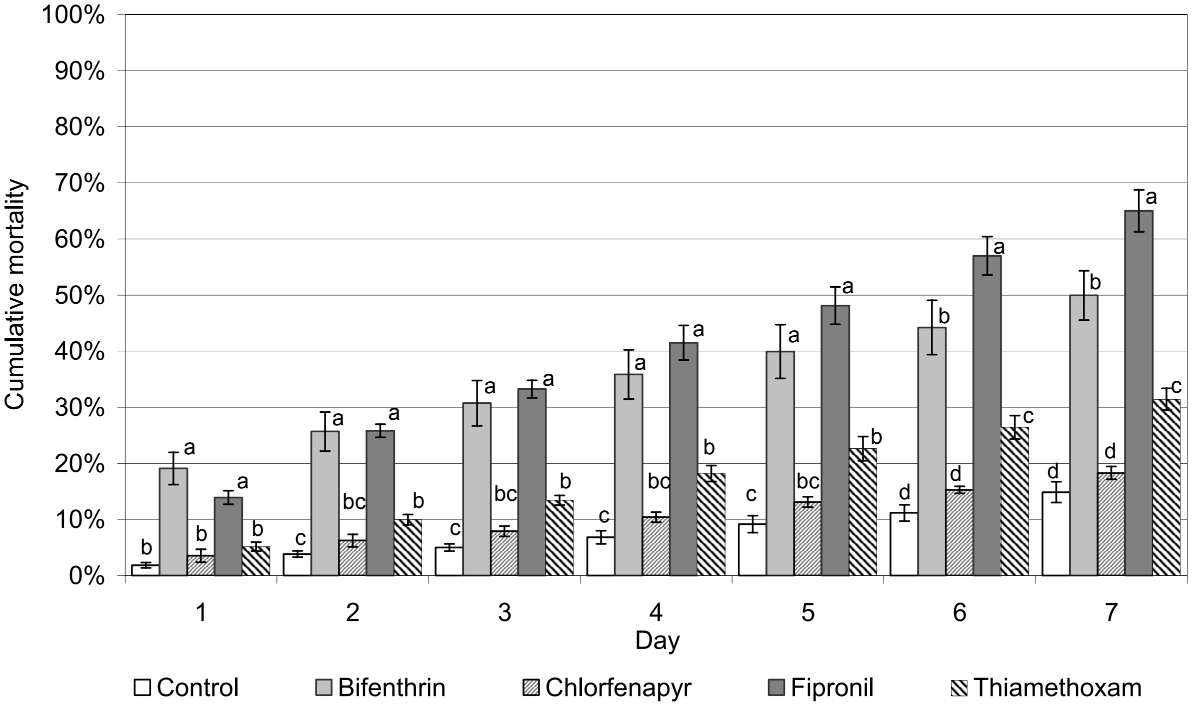

Figure 2.6. Horizontal toxicity (mean ± SE cumulative daily mortality) to Argentine ants after 20% of the ants in a cohort of 100 were exposed by crossing an insecticide-soaked pine needle bridge. Within each time period, means with the same letter are not significantly different (Tukey's HSD, $p<0.05$).

CHAPTER 3

ACTIVITY OF BIFENTHRIN, CHLORFENAPYR, FIPRONIL, AND THIAMETHOXAM AGAINST RED IMPORTED FIRE ANTS (HYMENOPTERA: FORMICIDAE)[2]

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ABSTRACT

Bifenthrin, chlorfenapyr, fipronil, and thiamethoxam were evaluated for activity against the red imported fire ant, *Solenopsis invicta* Buren. Median lethal times (LT_{50}) following topical treatment were 59.0 min for bifenthrin, 248.3 min for chlorfenapyr, 709.8 min for fipronil, and 210.7 min for thiamethoxam. For bifenthrin, 100% of the ants were immobilized within 30 min of topical treatment. The percentage of ants immobilized after 2h was 50% after treatment with chlorfenapyr, 15% with fipronil, and 92% with thiamethoxam. Mortality due to exposure to the corpses of topically treated nestmates was evaluated at 10°, 20°, or 30°C, with 5, 10, or 20% of the ants in a cohort treated. Bifenthrin had the greatest horizontal activity of the chemicals tested, with 95% mortality of the untreated ants at 20°C when 20% were treated. For chlorfenapyr, the only treatments having higher mortality than controls were the 20% treatments at either 10°C or 30°C. Horizontal activity of fipronil was lower than that of bifenthrin, but higher than that of chlorfenapyr or thiamethoxam. The highest mortality rate (54%) occurred at 20° when 20% of the ants were treated. Mean mortality due to thiamethoxam was similar to that with chlorfenapyr. Significant mortality occurred in all of the 20°C and 30°C treatments, but none of the 10°C treatments. In barrier tests where ants were given a choice between crossing bridges treated with insecticide or water, only bifenthrin had fewer ants on the treatment side than the paired control side.

Key words: *Solenopsis invicta*, insecticide, horizontal toxicity, barrier

INTRODUCTION

The red imported fire ant, *Solenopsis invicta* Buren, is an important pest throughout most of the southeastern United States, parts of several western states, and Puerto Rico (Hedges 2000, Williams et al. 2001). Since 2001, *S. invicta* has been reported in Australia, New Zealand, Malaysia, Taiwan, Hong Kong, and mainland China (Invasive Species Specialist Group 2005, Anonymous 2005). Problems caused by fire ants include hazards to humans (deShazo et al. 1999), livestock (Jones et al. 1996), wildlife (Lockley 1995), electrical equipment (MacKay et al. 1992), farm machinery, and crops (Thompson et al. 1996). Annual damage and control costs are estimated at approximately $1 billion (Pimentel et al. 2005).

Ant control tactics include the use of contact insecticides, either as a barrier (Rust et al. 1996), broadcast (Drees et al. 2000), or mound treatments (Williams and Lofgren 1983), and the use of baits, which are more target specific and control ants by using less toxicant (Klotz et al. 1997). While individual mound treatments provide rapid control of colonies, ants frequently relocate following treatment (Francke 1983, Vogt and Appel 1996). The most effective control strategies often combine multiple treatment techniques, e.g., the two-step method for fire ant control includes mound treatments and broadcast applications of granular baits (Merchant and Drees 2003).

Insecticide efficacy depends on properties of the insecticide (Knight and Rust 1990), environmental conditions (Rust et al. 2003), and the target insect species (Gibson and Scott 1989). A more complete knowledge of the properties of different toxicants would aid in the selection of the best active ingredient for a specific application. For example, repellent or fast-acting toxicants provide effective barriers (Pranschke et al. 2003), while slower-acting toxicants increase the potential for horizontal movement of the active ingredient by recruitment across treated areas. The purpose of this study was to evaluate contact insecticides for topical toxicity, knockdown rate, horizontal toxicity, and barrier effects against the red imported fire ant to better assess the potential of bifenthrin, chlorfenapyr, fipronil, and thiamethoxam in ant control programs.

MATERIALS AND METHODS

Test insects. Red imported fire ant colonies were collected in Spalding Co., GA. Ants were separated from soil by connecting two 19 x 13 x 10 cm Fluon™ (Northern Products, Inc., Woonsocket, RI) -lined plastic boxes (Pioneer Plastics, Dixon, KY) with paper bridges (10 x 50 cm strips of cardstock, with the ends taped to the bottom of each box). Soil containing red imported fire ants was placed in one box and nest cells, food, and water in the other. Nest cells were constructed from 100 x 25 mm Petri dishes containing a 1.0 cm thick layer of hardened dental plaster (Castone; Dentsply International Inc., York, PA) to retain moisture. Dish sides and lids were painted black and three 4 mm holes were drilled into the sides to allow ants to enter. As soil dried, workers moved the entire colony to the nest cells. Nest cells containing ants were transferred to clean 31 x 23 x 10 cm Fluon-lined plastic boxes (Pioneer Plastics, Dixon, KY) and colonies were maintained at room temperature (23° – 26°C) on a diet of water, 25% sugar water, and frozen house crickets (*Acheta domesticus* (L.)).

Test chemicals. Laboratory studies were conducted to determine multiple effects of bifenthrin (Talstar Flowable; FMC Corporation, Philadelphia, PA), chlorfenapyr (Phantom; BASF, Research Triangle Park, NC), fipronil (Termidor SC; BASF, Research Triangle Park, NC), and thiamethoxam (thiamethoxam 25WG; Syngenta Corp., Greensboro, NC) on worker acticvity. Bifenthrin, chlorfenapyr, and fipronil were evaluated at label concentration for ant control (0.06% fipronil, 0.06% bifenthrin, or 0.5% chlorfenapyr). Thiamethoxam, which is not labeled for ant control, was tested at a rate of 0.06%.

Toxicity from topical treatment. Each chemical was tested for its ability to kill red imported fire ants via topical treatment. For each treatment 10 groups of 20 workers were placed into a plastic box lined with absorbent paper, sprayed with one of the insecticidal suspensions or water (control) using a hand-held misting bottle (three sprays), and immediately transferred to dry Fluon-lined boxes. Ant mortality in each replicate was determined at half-hour intervals for up to 6 h post-treatment.

Mobility of topically-treated ants. Ants were topically treated, as above, by spraying 10 groups of ≈ 100 workers. Treated ants were transferred to dry containers, then from each group 10 randomly chosen ants were placed into each of four 30-ml, Fluon-lined, plastic cups. At half-hour intervals for 2h, one of the cups was inverted onto the center of a 14-cm diameter circle drawn on a piece of paper, then removed. The number of ants remaining inside the circle was counted after 2 min. As ants walked out of the circle they were removed from the paper so they would not re-enter.

Horizontal toxicity. To test for mortality due to ant-to-ant contact, while limiting the possibility of mortality from a contaminated surface, we conducted an assay in which treated ants were allowed to die before being placed in containers with untreated ants.

Necrophoric behavior is common in ants (Wilson et al. 1958, Gordon 1983); therefore, handling of treated corpses by untreated nestmates should result in horizontal activity due to ant-to-ant contact. To determine if red imported fire ants would remove the corpses of nestmates treated with each of the insecticides, we conducted an assay in which treated corpses were placed on a plastic disk in a box containing untreated ants. To obtain treated corpses, live red imported fire ants were topically treated by spraying groups of approximately 1,000 ants then immediately transferred to a dry box. Approximately 6 h after treatment, 30 treated corpses were transferred to 31 x 23 x 10 cm plastic boxes containing 300 live untreated workers, a nest cell, sugar water, and water. To acclimate the untreated workers, they were placed in the test boxes 24 h before starting the assay. Immediately before adding treated corpses to the boxes, dead untreated workers were removed and replaced with live workers. To prevent contamination of the arena floor, the treated ants were placed on a 5-cm diameter disk cut from the bottom of a plastic weigh boat. Control treatments received 30 ants killed by freezing. The number of ants remaining on the plastic disk was recorded after 1, 2, and 24 h. There were six replicates for each treatment.

If horizontal toxicity due to contact with treated individuals occurred, mortality was expected to increase with an increase in the percentage of ants in a cohort that had been treated and killed by the toxicant. Additionally, we expected ants to be more active at higher temperatures, thereby increasing the encounter rate and thus total mortality. A total of 300 ants (corpses and live ants combined) was used for each replicate, with five, 10, or 20% treated and the remainder untreated. Ants were treated as described above, then 15, 30, or 60 of the treated corpses were placed on plastic disks in boxes containing acclimated, untreated workers, a nest cell, sugar water, and water. Control treatments received 60 ants killed by freezing. Boxes were covered with lids containing four, 6-mm holes and maintained at 10°, 20° or 30°C with a photoperiod of 12:12 (L:D) for 3 d when the number of dead ants was recorded in each replicate. There were six replicates for each of the treatment combinations (6 replicates x 4 chemicals x 3 temperatures x 4 percentages treated = 288 total replicates).

Barrier tests. Tests were conducted to determine whether ants would cross insecticide-treated barriers to reach a food source. Choice tests were conducted in a three-chambered apparatus consisting of 19 x 13 x 10 cm Fluon-lined plastic boxes arranged linearly, with each of the end boxes connected to the center box by a paper bridge (Figure 3.1). The bridge to one box was treated with insecticide and the bridge to the other box with water. Bridges were constructed from 38 x 5 cm strips cut from legal sized manila file folders. Before installing bridges in the boxes, the center 10-cm section of each was treated by pipetting the insecticide or water onto the paper, brushing over the desired area, and air-drying for 1 h. Bifenthrin and fipronil were applied at label rates for broadcast ant treatment (5 gal 0.06% bifenthrin / 1,000 ft^2 = 1.0 ml / 50 cm^2 and 1.5 gal 0.06% fipronil / 1,000 ft^2 = 0.3 ml / 50 cm^2). Because chlorfenapyr and thiamethoxam do not have broadcast label rates for ants, rates of 0.3 ml 0.5% chlorfenapyr and 0.06% thiamethoxam were used. Because the suspensions did not penetrate the surface of the paper bridges, the paper was inverted and the reverse side treated in the same way. Water-water pairings were used as negative controls (0.3 ml water), and 0.6 ml 1.5% cinnamaldehyde (Cinnamite™; Mycotech Corporation, Butte, MT) was used as a positive control treatment.

Ants were starved for 2 d with access to only water, then 300 ants were placed in the empty center box (nest chamber). The boxes on either side (food chambers) contained a nest cell, a sugar water-soaked cotton ball, and a water soaked cotton ball. To prevent a moisture differential that might influence ant movement, all nest cells were prepared by oven drying for 24 h at 50° C, cooling at room temperature and humidity for 2 h, then adding 6 ml water. The numbers of live and dead ants in each of the three chambers were recorded after 24 h. Tests were conducted at room temperature and replicated 10 times for each treatment.

Statistical analysis. Mortality from topical treatment was analyzed by probit analysis (PROC PROBIT; SAS Institute 1985). Data were not corrected for control mortality because control mortality was low and some of the time periods contained zero mortality values. Non-overlapping 95% confidence intervals for LT_{50}s were used to determine significant differences among insecticide treatments. For mobility and horizontal toxicity tests, percentages of immobilized or dead ants were transformed by arcsine of the square root. All analyses were performed on the transformed variable. Mobility data were analyzed by one-way analysis of variance (ANOVA) (SAS Institute 1985), comparing chemical effects at each time period. Horizontal toxicity results were analyzed by two-way ANOVA for the main effects of temperature and percentage treated ants on mortality. Separate analyses were performed for each chemical. When main effects were significant, one-way ANOVA were performed. Means were separated with Tukey's Studentized range test. Barrier test data were analyzed by one-tailed paired t-tests to determine if fewer ants crossed an insecticide-treated surface than the paired water-treated surface.

RESULTS

Toxicity from topical treatments. Based on non-overlapping of confidence intervals, LT_{50}s for each of the four chemicals were significantly different. LT_{50}s (95% CI) from topical treatment were 59.0 min (55.9 – 62.1) for bifenthrin, 248.3 min (240.8 – 256.3) for chlorfenapyr, 709.8 min (490.7 - 1927) for fipronil, and 210.7 min (194.3 – 228.7) for thiamethoxam (Figure 3.2).

Mobility of topically-treated ants. All of bifenthrin-treated ants were immobilized within 30 min of topical treatment (Figure 3.3). At each time period, bifenthrin had the highest number immobilized, followed by thiamethoxam, chlorfenapyr, fipronil, and control. After 30 min, immobilization rates were not significantly different among treatments with chlorfenapyr, fipronil, or the control. After 60 min, there was no significant difference in immobilization rate between chlorfenapyr and fipronil or between fipronil and the control. After 90 min, fipronil did not differ from the control. After 120 min, all treatments were significantly different, with 15% of fipronil-treated, 50% of chlorfenapyr-treated, and 92% of thiamethoxam-treated ants remaining in the circle.

Horizontal toxicity. Removal of insecticide-killed corpses by *S. invicta* was inconsistent (Table 3.1). For each treatment, >50% of the ants were removed within 24 h. However, removal varied widely even among control replicates. In some replicates, the number of corpses on a plastic disk increased rather than decreased between observations. Because ants repeatedly move the same corpses, sometimes replacing them, the results of this test indicate only that the ants handle the treated corpses. No attempt was made to quantify differences because the actual amount of handling could not be determined.

Bifenthrin exhibited the greatest horizontal activity of the chemicals tested, with 95% of the untreated ants killed at 20°C when 20% of the ants were treated and >60% mortality at 20°C when 10% were treated and 30°C when 20% were treated (Table 3.2). Temperature, percentage treated, and their interaction significantly affected red imported fire ant mortality. At 10°C, increasing the percentage treated did not increase mortality, although bifenthrin treatments caused higher mortality than the controls. At 20° and 30°C, mortality increased with percentage of ants treated. With 5% treated, temperature did not significantly affect mortality. When 10 or 20% were treated, the highest mortality occurred at 20°. For chlorfenapyr, the only treatments having higher mortality than controls were the 20% treatments at 10° and 30°C. Mortality was not affected by temperature. Horizontal activity of fipronil was generally lower than that of bifenthrin, but higher than that of chlorfenapyr and thiamethoxam. As with bifenthrin, the highest mortality rate (54%) occurred at 20°C when 20% of the ants were treated. For fipronil, mortality generally increased with percentage treated, but only differed with temperature when 20% were treated. Mean mortality due to thiamethoxam exposure was similar to that seen with chlorfenapyr. None of the 10°C thiamethoxam treatments caused significantly higher mortality than the controls, while mortality in the 20°C treatments was higher than in controls, but did not change with percentage treated. At 30°C, mortality increased with percentage treated. Mortality increased with temperature, with the difference being significant when 5% or 20% of the ants were treated.

Barrier tests. In choice tests, only bifenthrin and the positive control cinnamaldehyde had fewer ants on the treated side than on the paired control side after 24 h (Table 3.3). The bifenthrin treatment had 43% as many ants as the control, and the cinnamaldehyde treatment had 18% as many ants as the control. There was 94% mortality among ants that had crossed bifenthrin-treated bridges, while mortality was 16% in the chlorfenapyr treatment, 6% in the fipronil treatment, and 5% in the thiamethoxam treatment.

DISCUSSION

Speed of kill and knockdown are two key factors used to determine the best application for an insecticide. In our study, bifenthrin was faster acting than the other chemicals when applied topically, immobilizing 100% of the ants within 30 min and killing >90% within 2 h, versus <10% immobilization and <20% mortality with each of the other three chemicals. We observed similar trends for activity against the Argentine ant, *Linepithema humile* (Mayr) (Chapter 2). However, our data indicate that red imported fire ants may be more tolerant than Argentine ants to all chemicals tested. For example, bifenthrin immobilized 100% of Argentine ants within 30 min versus <25% by the other chemicals tested. In addition, bifenthrin killed 99% of Argentine ants within 30 min versus <15% mortality with the other chemicals. Assuming equivalent residual activity and bioavailability, a faster acting chemical like bifenthrin would be more effective as a barrier, while slower acting active ingredients allow more opportunity for horizontally-induced toxicity by allowing more time for ant-to-ant interaction. When used in a bait, a toxicant must allow the insect to survive long enough to return to the nest and engage in trophallaxis. For contact toxicants, it may be sufficient to have the exposed individuals survive to return to the nest. When combined with horizontal movement, even a slight delay in knockdown or mortality can contribute to the toxicant's activity. Fipronil, chlorfenapyr, and thiamethoxam each act slowly enough for exposed ants to return to the nest, allowing for potential horizontal movement of the active ingredient due to contamination of nesting material, interaction with nestmates, and necrophoresis. Fipronil is the only active ingredient that is currently available in both contact and bait (CHIPCO Firestar, Bayer Environmental Science, Montvale, NJ) formulations for red imported fire ant control.

Horizontal activity occurs when a toxicant is moved from treated to untreated individuals, resulting in mortality of untreated individuals. Most of the research on horizontal toxicity to ants focuses on the transfer of toxic baits (Banks et al. 1978, Knight and Rust 1991, Hooper-Bùi and Rust 2000). In addition to trophallaxis, other mechanisms for movement of toxicant include contact between treated and untreated individuals (Ferster et al. 2001, Ibrahim et al. 2003, Shelton and Grace 2003, Soeprono and Rust 2004), cannibalism (Smith and Lockwood 2003), and contact with contaminated surfaces (Buczkowski and Schal 2001).

We did not find any evidence that red imported fire ants were deterred from removing corpses treated with any of the test chemicals from the nest area. Removal rates were highly variable for all treatments, including the controls. However, determining the actual amount of handling of treated corpses by fire ants was impossible because corpses were sometimes returned to their original location after they had been removed. For Argentine ants, removal rates were higher (81 – 100%) and were more consistent among replicates (Chapter 2). For both species, removal of bifenthrin-treated corpses was less than that of other treatments after 1 h, but similar after 24 h. Despite inconsist results with red imported fire ants, we demonstrated that some amount of handling of treated corpses occurred in all treatments, indicating a potential for horizontal activity due to necrophoresis and interaction with live donors (i.e., grooming and trophallaxis). For bifenthrin, horizontal mortality following contact with treated corpses differed with temperature. The overall trend was towards highest mortality at the intermediate temperature of 20°C. At 10°, treating any percentage of the ants resulted in higher mortality than the controls, while increasing the percentage of sprayed ants did not increase mortality, while at the higher temperatures, total mortality increased with the percentage of ants treated. These findings may be due to temperature-related red imported fire ant activity, because foraging is the greatest between 21 and 35°C, while limited foraging occurs between 10 and 15°C on sunny days (Lofgren et al. 1975). Additionally, bifenthrin (type I pyrethroid) has a negative temperature coefficient, with greater efficacy at lower temperatures (Ware and Whitacre 2004), which could in part account for higher mortality at 20°C than at 30°C. Bifenthrin had the greatest horizontal toxicity of the four chemicals tested, a fact that appears to contradict the idea that slower acting toxicants result in greater horizontal activity. However, for a transfer opportunity to be lost because of rapid kill, the exposure would have to occur away from the nest. In our horizontal toxicity test, treated corpses were placed at the nest site and were removed by untreated nestmates. As with bifenthrin, there was a trend towards higher mortality at 20° in the fipronil treatments. However, the only statistically significant differences occurred among percentages treated, and not temperatures for fipronil. In general, chlorfenapyr and thiamethoxam treatments caused lower mortality due to horizontal contact than the corresponding temperatures and percentages treated with bifenthrin or fipronil. Mortality of red imported fire ants exposed to chlorfenapyr-treated nestmates increased with percentage treated, but was not affected by temperature, while horizontal toxicity of thiamethoxam increased with temperature and at 30°C increased with percentage treated. While results presented here indicate that there is a trend toward higher horizontal activity against red imported fire ants at 20°C than at 10° or 30°C, we found that activity against Argentine ants tended to increase with temperature (unpublished data). Fipronil and thiamethoxam were more active against Argentine ants, while bifenthrin was more active against red imported fire ants and chlorfenapyr had relatively low horizontal activity against either species. Differences between the species can be attributed to differences in insecticide sensitivity and behavior. In topical toxicity tests we found that all of the chemicals tended to be more active against Argentine ants than against red imported fire ants. Holway et al. (2002) found that Argentine ants reached maximum abundance at baits at 34°C, which is at the upper end of the 21 to 35°C foraging temperature range reported for red imported fire ants (Lofgren et al. 1975). In our tests, Argentine ants were more consistent than red imported fire ants in their removal of corpses. Twenty-four hour corpse removal rates for Argentine ants exceeded 80% for all treatments while corpse removal by red imported fire ants averaged 54 to 71%. Because bifenthrin had the highest topical toxicity of the chemicals tested, horizontal mortality may be possible at lower ant activity and interaction levels for bifenthrin than for the other chemicals.

In addition to the mound and broadcast treatments used for red imported fire ant control, barrier treatments are sometimes needed to exclude the ant from sensitive areas such as nursing homes, animal rearing facilities, and electrical equipment. While repellent substances are useful as barriers, Knight and Rust (1990) found that if a chemical is highly repellent, mortality is low regardless of its toxicity because few ants contact the chemical. Conversely, they found that non-repellent treatments cause high mortality, even with active ingredientss that are only of intermediate toxicity, because of increased contact. Therefore, use of repellent chemicals is best restricted to situations where a barrier is required. Many assays have been developed to measure the response of individual ants or the number of ants crossing a barrier over a short period of time (1 h or less) (Knight and Rust 1990, Pranschke et al. 2003). Our assay differs in that it measures a group response rather than the movement of individual ants. By counting the number of ants in each feeding chamber after 24 h instead of the number crossing the barriers, we incorporate repellency and recruitment effects to test for a chemical's effectiveness as a barrier. Because there was no food or water in the center chambers, foragers successfully crossing bridges should recruit more ants unless they are killed or immobilized quickly. Of the chemicals we tested, we found that only the bifenthrin treatment had fewer ants cross than its paired control bridge. Other studies have shown bifenthrin to be non-repellent to red imported fire ants (Oi and Williams 1996, Richman and Hooper-Bùi 2003). Although we found bifenthrin to act as a barrier to red imported fire ants, the combined results presented here suggest that this effect is due to its rapid action rather than repellency. Our findings of 100% knockdown within 30 min and 100% mortality within 2.5 h likely explain the lack of recruitment across bifenthrin-treated bridges. These results are consistent with our findings for Argentine ants (Chapter 2).

Although bifenthrin may not be repellent to red imported fire ants, it does provide an effective barrier. Treatment zones of 2.0 or 3.0 m excluded red imported fire ants for 7 wk after treatment, possibly by killing foragers too quickly for the establishment of recruitment trails (Pranschke et al. 2003). While repellent or fast acting insecticides are useful as barrier treatments, they do not provide the opportunity for foragers to spread the chemical to nestmates.

Summary. Bifenthrin had the highest topical and horizontal toxicity of the four chemicals tested. In a barrier choice test, only bifenthrin had fewer ants on the treatment side than the paired control side. These properties are best suited to barrier or mound treatments. Because bifenthrin is fast-acting, foragers would not have time to return to the nest to transfer toxicant. This conclusion is supported by the Loftin et al. (2003) field trial, which showed that while broadcast treatments with both bifenthrin and fipronil reduced foraging populations of red imported fire ants between 3 and 14 d after treatment, only the fipronil treatment maintained a significant population reduction after 65 d.

Of the four chemicals, fipronil was the slowest acting in topical toxicity tests. Horizontal toxicity was lower than that of bifenthrin, but higher than chlorfenapyr or thiamethoxam. Fipronil has properties suited for mound or broadcast treatment. Because it is non-repellent and relatively slow acting, it is not effective as a barrier.

Chlorfenapyr and thiamethoxam had intermediate topical toxicity, low horizontal toxicity, and did not provide a barrier to red imported fire ants. Other active ingredients have properties better suited to red imported fire ant control.

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Table 3.1. Percentage of corpses (mean ± SE) of insecticide-treated red imported fire ants removed by untreated ants. Each replicate contained 300 untreated ants and 30 treated ants.

Treatment	N	1h	2h	24h
Bifenthrin	6	2.6 ± 3.0	37.8 ± 2.5	71.1 ± 5.6
Chlorfenapyr	6	17.8 ± 4.9	34.4 ± 11.0	54.4 ± 13.6
Fipronil	6	33.9 ± 8.6	45.6 ± 13.4	78.3 ± 11.1
Thiamethoxam	6	16.7 ± 3.2	38.3 ± 8.9	63.3 ± 16.7
Control	6	10.6 ± 3.5	31.7 ± 9.2	58.9 ± 17.4

Table 3.2. Percentage cohort mortality (mean ± SE, *n* = 6) of untreated red imported fire ants after 3 days exposure to insecticide-killed corpses.

		Temperature (°C)		
Treatment	% treated	10	20	30
Bifenthrin *	0	1.1 ± 0.4bA	1.3 ± 0.7dA	2.7 ± 0.8cA
	5	20.0 ± 3.7aA	30.7 ± 6.9cA	28.3 ± 2.6bcA
	10	22.6 ± 4.7aB	61.3 ± 5.6bA	47.5 ± 10.8abAB
	20	34.4 ± 5.3aB	95.4 ± 2.3aA	63.5 ± 14.3aB
Chlorfenapyr	0	2.5 ± 0.7bA	3.4 ± 1.0aA	3.1 ± 1.2bA
	5	7.2 ± 2.1abA	12.0 ± 5.0aA	3.7 ± 1.1bA
	10	5.5 ± 1.0abA	13.0 ±3.9aA	22.6 ± 11.0abA
	20	11.5 ± 3.0aA	16.0 ± 6.0aA	34.5 ± 11.7aA
Fipronil	0	2.0 ± 0.5cAB	1.2 ± 0.2bB	4.0 ± 1.1cA
	5	5.0 ± 1.0bA	29.0 ± 11.2abA	15.7 ± 3.7bA
	10	8.3 ± 0.6aA	34.0 ± 13.3abA	24.0 ± 1.6abA
	20	10.3 ± 1.2aB	54.0 ± 15.9aA	35.2 ± 5.1aAB
Thiamethoxam	0	3.6 ± 0.6aA	2.8 ± 1.2bA	2.8 ± 0.5cA
	5	3.9 ± 1.4aB	11.0 ± 2.0aA	12.0 ± 2.4bA
	10	10.2 ± 4.1aA	16.9 ± 2.8aA	18.4 ± 2.4abA
	20	10.4 ±3.8aB	21.7 ± 3.2aAB	27.0 ± 4.9aA

For each chemical, means within a column followed by the same lowercase letters, and within a row followed by the same uppercase letters, do not differ significantly (Tukey's HSD, $P < 0.05$).

* = Interaction between temperature and percentage treated is significant (ANOVA, $P < 0.05$).

Table 3.3. Red imported fire ants crossing insecticide-treated bridges as a proportion of total ants crossing treated and control bridges (mean ± SE), with paired t-test comparison of the total number of ants on each side.

Treatment	n	Proportion on treated side	T	P
Bifenthrin	10	0.30 ± 0.02	-6.308	0.0001
Chlorfenapyr	10	0.43 ± 0.09	-0.769	0.4615
Fipronil	10	0.57 ± 0.05	1.250	0.2427
Thiamethoxam	10	0.42 ± 0.06	-1.362	0.2063
Control	10	0.56 ± 0.10	0.783	0.4538
Cinnamaldehyde	10	0.15 ± 0.08	-4.481	0.0015

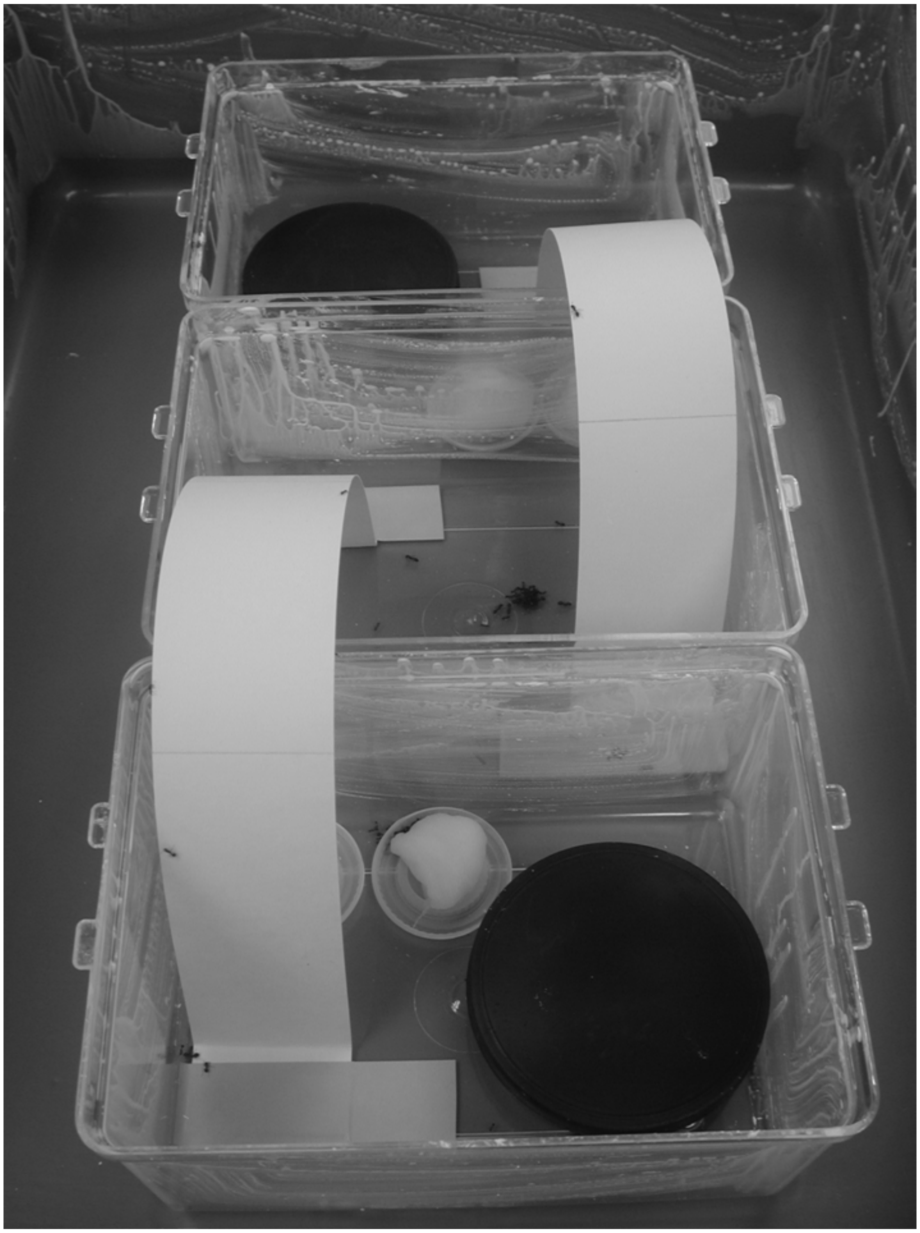

Figure 3.1. Apparatus used for red imported fire ant barrier assay. Ants were placed in the center container. One of the bridges was treated with water and the other with a test chemical, providing ants with a choice of crossing insecticide or water (control)-treated barriers to reach food, water, and harborage.

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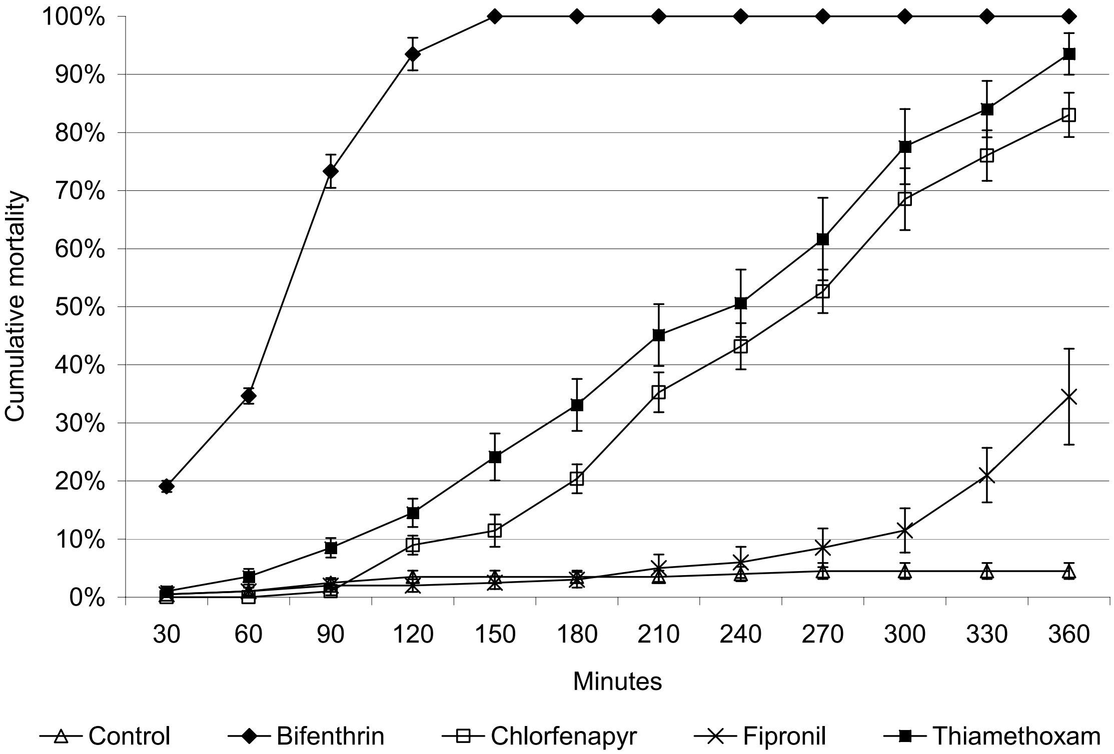

Figure 3.2 Red imported fire ant mortality from topical application of bifenthrin, chlorfenapyr, fipronil, thiamethoxam, or water (control). Each point represents the mean ± SE of *n* = 10 replicates, 20 ants per replicate.

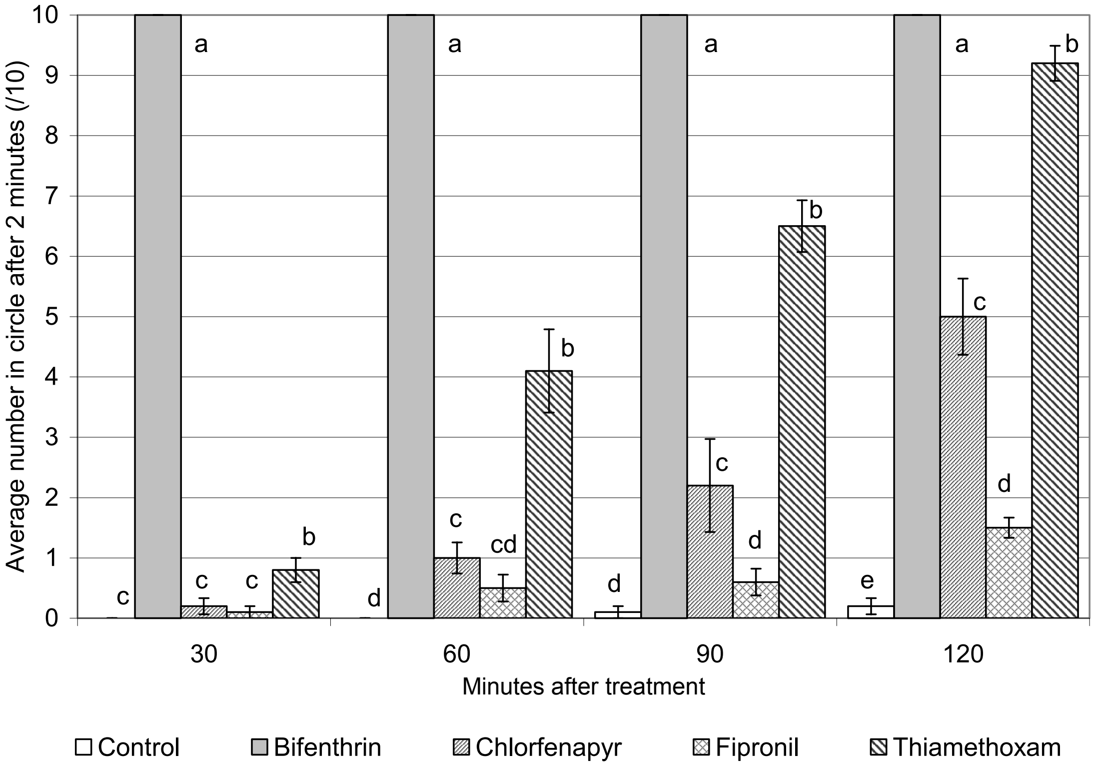

Figure 3.3. Number of red imported fire ants unable to walk out of a 14 cm diameter circle following topical application of bifenthrin, chlorfenapyr, fipronil, thiamethoxam, or water (control). Each replicate contained 10 ants and each bar represents the mean ± SE of 10 replicates. Within each time period, means with the same letter are not significantly different (Tukey's HSD, $p<0.05$).

CHAPTER 4

DETERRENCY AND TOXICITY OF ESSENTIAL OILS TO ARGENTINE ANTS AND RED IMPORTED FIRE ANTS (HYMENOPTERA: FORMICIDAE)[3]

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[3] Wiltz, B. A. D. R. Suiter, and W. A. Gardner. For submission to Journal of Entomological Science.

ABSTRACT

Laboratory assays were conducted to evaluate deterrency and contact toxicity of six essential oils to the Argentine ant, *Linepithema humile* (Mayr), and the red imported fire ant, *Solenopsis invicta* Buren. In choice tests, both Argentine ants and fire ants crossed barriers treated with multiple rates of basil, citronella, lemon, peppermint, or tea tree oil less frequently than paired control barriers. Eucalyptus oil did not prevent movement of either species at any of the rates tested. In continuous exposure assays citronella killed 100% of exposed Argentine ants in 75 min. Argentine ant mortality at 24 h was 89.8% with peppermint oil and 85.7% with tea tree oil, with the remaining treatments having mortality levels not significantly different from the controls. After 24 h of continuous exposure, red imported fire ant mortality was 50.6% with citronella oil, the only treatment in which mortality was significantly greater than in the controls.

Key words: *Linepithema humile, Solenopsis invicta*, essential oil, repellency, contact toxicity

INTRODUCTION

The Argentine ant, *Linepithema humile* (Mayr), and red imported fire ant, *Solenopsis invicta* Buren, are important invasive species in natural, agricultural, and urban settings. While the primary control strategies for ants are broadcast and individual mound treatments and the use of toxic baits, barrier treatments are sometimes needed to exclude ants from structures, equipment, or fruit trees (Shorey et al. 1992, Drees et al. 2000, Vega and Rust 2001). The Argentine ant is a secondary pest in vineyards and citrus groves because it disrupts the control of honeydew-producing homopteran insects by aggressively interfering with their predators and parasitoids (Prins et al. 1990). Although they usually nest outdoors, Argentine ants sometimes invade homes in search of food, moisture, or warmth (Gordon et al. 2001). Red imported fire ants frequently invade areas such as nursing homes, small animal traps, and electrical equipment, making repellent or toxic barriers necessary (Chabreck et al. 1986, MacKay et al. 1992, deShazo et al. 1999). Homeowners have become increasingly concerned about the use of traditional insecticides around the home and schools and, therefore, are interested in less toxic or "natural" substances for ant control, either as a toxicant or a repellent barrier (Drees and Lennon 1998, Potter and Bessin 1998).

Both plants and insects produce chemicals that are repellent to ants. Aromatic cedar mulch is repellent to red imported fire ants, Argentine ants, and odorous house ants (Thorvilson and Rudd 2001, Meissner and Silverman 2001, 2003), leaf-cutting ants are deterred by terpenoids produced by many tropical plants (Hubbell et al. 1984), and cat thyme is repellent to the Pharaoh ant, *Monomorium pharaonis* (L.) (Eisner et al. 2000). Methyl palmitate, a component of the defensive secretion of *Polistes fuscatus* (F.), is repellent to several ant species (Henderson and Jeanne 1989). A barrier of farnesol, a component of the alarm pheromone of some ants (Schildknecht 1976), has been used to disrupt Argentine ant foraging in citrus trees (Shorey et al. 1992).

There are anecdotal reports of several essential oils, herbs, and spices being repellent to ants and other insects. However, there are no reports of ant repellency tests in the scientific literature for most of these products. In tests against other urban and medical pests, essential oils and their components that have been found to be repellent include mint, cedarwood, vetiver, and catnip oils, nookatone, citronellal, citral, geraniol, and eugenol (Eisner 1964, Grace and Yamamoto 1994, Cornelius et al. 1997, Ngoh et al. 1998, Appel et al. 2001, Henderson et al. 2001, Zhu et al. 2001, Maistrello et al. 2001, Peterson and Ems-Wilson 2003). Essential oils and monoterpenoids derived from them have been shown to have insecticidal activity against cockroaches (Appel et al. 2001, Ngoh et al. 1998), termites (Cornelius et al. 1997, Bläske and Hertel 2001, Zhu et al. 2001), mosquitoes (Watanbe et al. 1993), and ticks (Lwande et al. 1999). Vogt et al. (2002) found that three formulations containing orange oil were toxic to red imported fire ants.

The purpose of this study was to determine the repellency and toxicity of essential oils against Argentine ants and red imported fire ants. Repellency was determined using treated paper bridges in a choice test; toxicity was evaluated in continuous exposure tests with treated filter paper.

MATERIALS AND METHODS

Test insects. Argentine ant and red imported fire ant colonies were collected in Spalding Co., GA. Argentine ants in their nesting material (mulch, leaf litter, or soil) were placed in plastic pans (58 x 43 x 15 cm, Sterilite Corporation, Townsend, MA), coated on the inside walls with a thin layer of Fluon™ (Northern Products, Inc., Woonsocket, RI) and provided water, 25%

sugar water, frozen house crickets (*Acheta domesticus* (L.)), and nest cells constructed from 100 x 25 mm Petri dishes containing a 1.0 cm thick layer of hardened dental plaster (Castone; Dentsply International Inc., York, PA) to retain moisture. Dish sides and lids were painted black, and three 4-mm holes were drilled into the sides to allow ants to enter. As the nesting material in the pans dried, workers moved the entire colony into the moistened cells. Red imported fire ants were separated from soil by connecting two 19 x 13 x 10 cm Fluon-lined plastic boxes (Pioneer Plastics, Dixon, KY) with paper bridges (10 x 50 cm strips of cardstock, with the ends taped to the bottom of each box). Soil containing red imported fire ants was placed in one box and nest cells, food, and water in the other, without soil. As soil dried, workers moved the entire colony to the nest cells. For both species, nest cells containing ants were transferred to clean 31 x 23 x 10 cm Fluon-lined plastic boxes (Pioneer Plastics, Dixon, KY) and colonies maintained at room temperature (23° – 26°C) on a diet of water, 25% sugar water, and crickets.

Test chemicals. Essential oils of six plant species were evaluated for deterrency and toxicity to Argentine ants and red imported fire ants: sweet basil, *Ocimum basilicum* L. (Wyndmere Naturals, Inc. Minneapolis, MN); citronella, *Cymbopogon nardus ceylon* (L.) (Now Foods, Bloomingdale, IL); eucalyptus, *Eucalyptus globulous* Labillardiere (Now Foods, Bloomingdale, IL); lemon, *Citrus limon* L. (Wyndmere Naturals, Inc. Minneapolis, MN); peppermint, *Mentha piperita* L. (Now Foods, Bloomingdale, IL); and tea tree, *Melaleuca alternifolia* (Maiden and Betche) Cheel (Now Foods, Bloomingdale, IL).

Deterrency tests. Tests were conducted to determine whether ants would cross essential oil barriers to reach a food source. Choice tests were conducted in a 3-chambered apparatus consisting of 19 x 13 x 10 cm Fluon-lined plastic boxes arranged linearly, with each of the end boxes connected to the center box by a paper bridge. Bridges were constructed from 38 x 5 cm strips cut from legal sized manila file folders. Before installing bridges in the boxes, the center 10 x 5 cm section of each was treated by pipetting the test solution onto the paper, then allowed to air dry for 1 h. The bridge to one box was treated with one of the essential oils dissolved in hexane (1 ml total volume) and the bridge to the other box with 1 ml hexane. Trials for each of the essential oils were conducted at different times. Therefore, a separate group of controls were run for each of the essential oils. Six treatments were tested for each trial: four rates of the essential oil (1, 20, 100, or 500 µl), a negative control (hexane), and a positive control (0.6 ml 1.5% cinnamaldehyde (Cinnamite™; Mycotech Corportation, Butte, MT)).

Ants were starved for 2 d with access to only water, then 100 ants were placed in the center box (nest chamber). The boxes on either side (feeding chambers) contained a nest cell, a sugar water-soaked cotton ball, and a water-soaked cotton ball. To prevent a moisture differential that might influence ant movement, all nest cells were prepared by oven drying for 24 h at 50°C, cooling at room temperature and humidity for 2 h, then adding 6 ml water. The numbers of live and dead ants in each of the 3 chambers were recorded after 24 h. Tests were replicated 10 times for each treatment and species.

Toxicity tests. Continuous exposure toxicity tests were conducted in Petri dishes containing treated filter paper. Disposable Petri dishes (100 x 25 mm) were prepared by coating the sides with Fluon to prevent ant escape and force the ants to remain on the treated paper. For each replicate, 25 µl of one of the essential oils dissolved in 1 ml hexane was pipetted onto a filter paper circle (90 mm diameter, Whatman #1), for a rate of 0.4 µl essential oil / cm^2. The paper was then dried under a fume hood for 1 h and placed in the bottom of a Petri dish. Control filter papers were treated with 1 ml hexane. In each of 10 replicates per species, 10 ants were placed on top of the treated filter paper. Dishes were left uncovered to allow assessment of mortality due to contact toxicity, as opposed to fumigation. To prevent desiccation, while forcing ants to remain on the treated filter paper, water was provided in a plastic cap, cut from the top of a 0.5 ml microcentrifuge tube and placed on top of the filter paper. Mortality was assessed every 15 min for 2 h, then a final time after 24 h.

Statistical analysis. For the deterrency assays, paired t-tests were performed to compare the total number of ants (dead + alive) in treated and control sides of choice tests and to compare mortality on each side. Because ants occasionally escaped from Petri dishes in continuous exposure toxicity tests, total ant numbers used for mortality calculations were determined by adding the total number dead in each dish to the number alive after 24 h. Cumulative mortality was transformed by the arcsin of the square root and for each time period one-way analysis of variance (ANOVA) (SAS Institute 1985) were performed on the transformed variable to determine differences among treatments. Means were separated using Tukey's Studentized range test.

RESULTS AND DISCUSSION

Tables 4.1 and 4.2 summarize the results of the deterrency tests. Both Argentine ants and red imported fire ants crossed barriers treated with multiple rates of basil, citronella, lemon, peppermint, or tea tree oil less frequently than paired control barriers. Eucalyptus oil did not prevent movement of either species at any of the rates tested. Significant differences between the numbers of ants in the two chambers never occurred for the negative control (0 µl treatment) and always occurred for the deterrent control (Cinnamite).

Essential oils had little or no contact toxicity to either species following limited exposure from crossing treated bridges. With each essential oil treatment, the average number of ants that died after crossing a treated bridge was ≤2.6 Argentine ants and ≤4.5 red imported fire ants. As a percentage of the ants that had crossed the treated bridges, mortality rates were ≤17.8% for Argentine ants and ≤24.3% for fire ants. Mortality levels were significantly higher on treated sides than the paired control sides for Argentine ants in the 100 and 500 μl lemon treatments and for red imported fire ants in the 100 and 500 μl basil and lemon treatments (Table 4.3). While significant, rates at these concentrations do not translate to an appreciable level of control under field conditions. The fact that mortality rates are low confirms that deterrency, not mortality, is responsible for the low numbers of ants crossing treated bridges. We have previously found that this assay evaluates a chemical's efficacy as a barrier by combining deterrency and recruitment effects (Chapters 2 and 3). We found that fewer ants crossed bridges treated with bifenthrin than the paired control bridges. Because we observed mortality levels of 80% among Argentine ants and 94% among fire ants crossing bifenthrin-treated bridges, we attributed at least part of the barrier effect to a lack of recruitment. In the current assays, low mortality after crossing treated bridges indicate that differences between sides are due to deterrency, not toxicity.

In continuous exposure assays citronella was the only oil to kill 100% of Argentine ants, requiring 75 min to do so (Table 4.4). Mortality after 24 h was 89.8% with peppermint oil and 85.7% with tea tree oil, with the remaining treatments having mortality not significantly different from the control. After 24 h of continuous exposure, red imported fire ant mortality was 50.6% with citronella oil, the only treatment in which mortality was significantly greater than the control (Table 4.5). Toxicity tests were conducted at a single rate of 25 μl per filter paper, the equivalent volume per area of the 20 μl treatment in the deterrency choice test. This rate was selected because, in general, it was the lowest effective rate in the deterrency tests.

With the exception of peppermint oil, this is the first report of effects of each of these essential oils against ants. Appel et al. (2004) found that mint oil granules (Earth Care Naturals, Insect Killer Granules, Spectrum Group, St. Louis, MO) were both toxic and repellent to red imported fire ants. While the mint species used in this formulation was not specified, active components are likely the same as those in the peppermint oil we tested. Phytochemicals in *Mentha* spp. that have insecticidal and repellent activity against insects include limolene, menthone, and menthol (USDA 2005). Deterrent or toxic effects of mint oils or monoterpenoids found in mint oils also have been reported for the American cockroach, *Periplaneta americana* (L.), German cockroach, *Blattella germanica* (L.) (Appel et al. 2001), human head louse *Pediculus humanus capitis* De Geer (Yang et al. 2004), housefly, *Musca domestica* (L.), red flour beetle *Tribolium castaneum* (Herbst), and southern corn rootworm *Diabrotica undecimpunctata howardi* Barber (Rice and Coats 1994). Mint was found to be ineffective in field trials against the Japanese beetle, *Popillia japonica* Newman (Held et al. 2003), and *Anopheles gambiae* Giles (Barasa et al. 2002). Citronella was the only oil that we found to be repellent and toxic to both the Argentine ant and fire ant. While citronella is best known as a mosquito repellent, Lindsay et al. (1996) reported that citronella candles and incense were ineffective at reducing the biting pressure of mosquitoes (primarily *Aedes* spp. at the study site). Another citronella-based repellent, Pyranha, had no effect on Africanized honey bee *Apis mellifera* L. attack behavior when delivered in a stream of air directed toward the colony entrance (Schmidt et al. 2003). Tea tree oil, the third substance that we found to be both deterrent and toxic is toxic to both the whitefly *Trialeurodes vaporiorum* Westwood (Choi et al. 2003) and the human head louse (Yang et al. 2004). Basil essential oil, which was deterrent but did not have residual toxicity to fire ants or Argentine ants, is toxic to the two spotted spider mite *Tetranychus urticae* Koch, the whitefly *Bemisia tabaci* Genn. (Aslan et al. 2004), and the human head louse (Yang et al. 2004) and both toxic and somewhat repellent to bean weevils, *Acanthoscelides obtectus* (Say) (Papachristos and Stamopoulos 2002). Lemon oil, which we also found to be deterrent, but not toxic, has not been widely tested against other species, but when delivered as a fumigant is not toxic to *T. vaporiorum*, *T. urticae*, or the predatory mite *Phytoseiulus persimilis* (Choi et al. 2003, 2004). Eucalyptus oil was the only essential oil we tested that did not exhibit repellent or toxic effects against *S. invicta* or *L. humile*. Likewise, Zhu et al. (2001) found that it was not active against the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. However, activity has been demonstrated against several other species, including the human head louse (Yang et al. 2004), bean weevil (Papachristos and Stamopoulos 2002, 2004), maize weevil, *Sitophilus zeamais* Motschulsky, and the confused flour beetle, *Tribolium confusum* du Val (Tapondjoua et al. 2005).

In some situations, the use of deterrent or toxic natural products may be more desirable than contact insecticides or baits or may be used in conjunction with these methods to provide additional protection. Based on the results of this study, basil, citronella, lemon, peppermint, and tea tree oils offer promise as potential deterrent barriers. In addition, in continuous exposure tests, citronella oil was toxic to both Argentine ants and fire ants, and peppermint and tea tree oils demonstrated mild toxicity to Argentine ants. Additional testing is needed to determine dose responses for oils that demonstrated toxic effects.

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Table 4.1. Number (mean ± SE) of Argentine ants on treated and control sides of essential oil barrier choice tests after 24 h. Each replicate contained a total of 100 ants. Numbers do not total 100 because some ants did not cross either bridge. Data were compared using paired t-tests.

Trial	Treatment (µl)	n	# treated side	# control side	T	P
Basil	0	10	43.2 ± 8.0	53.4 ± 8.6	-0.618	0.5522
	1	10	44.8 ± 9.6	48.9 ± 9.9	-0.211	0.8375
	20	10	26.1 ± 8.4	71.1 ± 9.0	-2.600	0.0288
	100	10	17.0 ± 3.6	81.6 ± 3.6	-9.405	<0.0001
	500	10	8.1 ± 2.2	88.2 ± 3.4	-15.483	<0.001
	Cinnamite	10	9.5 ± 2.5	84.6 ± 2.7	-15.044	<0.0001
Citronella	0	10	48.6 ± 13.1	50.2 ± 12.4	-0.063	0.9512
	1	10	31.3 ± 9.0	60.0 ± 9.1	-1.584	0.1477
	20	10	24 ± 10.3	74.6 ± 10.9	-2.396	0.0402
	100	10	5.1 ± 2.6	95.4 ± 4.3	-13.521	<0.0001
	500	10	1.4 ± 0.7	99.3 ± 2.4	-34.214	<0.0001
	Cinnamite	10	4.1 ± 1.1	95.3 ± 2.4	-27.959	<0.0001
Eucalyptus	0	10	55.1 ± 9.1	47.3 ± 9.4	0.426	0.6804
	1	10	43.8 ± 5.7	43.6 ± 5.3	0.019	0.9854
	20	10	34.5 ± 10.3	63.4 ± 9.8	-1.445	0.1825
	100	10	59.8 ± 10.5	42.8 ± 10.9	0.798	0.4453
	500	10	37.1 ± 10.4	59.5 ± 10.4	-1.076	0.3100
	Cinnamite	10	10.2 ± 1.8	93.1 ± 1.0	-41.058	<0.0001

Table 4.1. Continued.

Lemon	0	10	51.8 ± 7.6	44.6 ± 7.4	0.481	0.6421
	1	10	39.4 ± 7.9	49.6 ± 8.3	-0.635	0.5411
	20	10	13.1 ± 6.5	77.9 ± 8.1	-4.448	0.0016
	100	10	7.9 ± 0.9	85.4 ± 1.3	-39.728	<0.0001
	500	10	8.3 ± 0.07	84.1 ± 1.8	-31.366	<0.0001
	Cinnamite	10	7.0 ± 0.6	88.9 ± 0.8	-64.456	<0.0001
Peppermint	0	10	42.4 ± 8.9	52.9 ± 9.1	-0.585	0.5731
	1	10	34.3 ± 6.2	53.4 ± 6.4	-1.528	0.1608
	20	10	25.5 ± 3.8	67.1 ± 3.7	-5.645	0.0003
	100	10	11.7 ± 3.6	82.5 ± 2.9	-10.931	<0.0001
	500	10	8.5 ± 2.1	86.5 ± 2.2	-18.699	<0.0001
	Cinnamite	10	7.8 ± 1.0	86.5 ± 1.5	-33.794	<0.0001
Tea tree	0	10	40.8 ± 8.4	58.0 ± 8.4	-1.027	0.3310
	1	10	48 ± 10.8	43.6 ± 10.4	0.208	0.8400
	20	10	20.5 ± 8.3	75.2 ± 8.1	-3.364	0.0083
	100	10	14.0 ± 6.9	80.9 ± 7.4	-4.768	0.0010
	500	10	5.3 ± 1.0	90.8 ± 1.5	-34.954	<0.0001
	Cinnamite	10	7.6 ± 1.0	90.2 ± 1.9	-33.102	<0.0001

Table 4.2. Number (mean ± SE) of red imported fire ants on treated and control sides of essential oil barrier choice tests after 24 h. Data were compared using paired t-tests.

Trial	Treatment (µl)	n	# treated side	# control side	T	P
Basil	0	10	38.5 ± 9.6	49.5 ± 10.1	-0.604	0.5607
	1	10	36.8 ± 4.8	50.1 ± 5.9	-1.268	0.2367
	20	10	33.1 ± 4.9	66.1 ± 4.9	-3.370	0.0083
	100	10	18.1 ± 1.0	79.2 ± 2.3	-23.490	<0.0001
	500	10	7.0 ± 1.0	81.5 ± 3.6	-23.195	<0.0001
	Cinnamite	10	5.6 ± 0.9	87.3 ± 4.1	-23.716	<0.0001
Citronella	0	10	49.2 ± 8.9	53.8 ± 9.3	-0.256	0.8041
	1	10	28.6 ± 5.0	55.9 ±4.9	-2.823	0.0200
	20	10	39.4 ± 5.7	63.1 ± 7.3	-1.911	0.0883
	100	10	28.1 ± 6.8	72.6 ± 7.0	-3.270	0.0097
	500	10	14.0 ± 2.8	78.6 ± 5.5	-10.146	<0.0001
	Cinnamite	10	18.5 ± 2.5	88.0 ± 2.3	-16.481	<0.0001
Eucalyptus	0	10	49.7 ± 7.3	43.8 ± 7.4	0.402	0.6969
	1	10	40.2 ± 6.8	47.5 ± 5.5	-0.597	0.5655
	20	10	53.4 ± 9.0	46.4 ± 8.9	0.403	0.6963
	100	10	52.0 ± 8.9	47.1 ± 9.5	0.269	0.7939
	500	10	48.1 ± 9.9	50.7 ± 11.2	-0.124	0.9042
	Cinnamite	10	8.9 ± 1.9	89.6 ± 2.4	-26.289	<0.0001

Table 4.2. Continued.

Lemon	0	10	48.6 ± 6.5	41.3 ± 6.5	0.562	0.5877
	1	10	36.9 ± 7.6	47.9 ± 7.5	-0.729	0.4845
	20	10	39.9 ± 7.0	54.4 ± 6.5	-1.077	0.3095
	100	10	30.2 ± 2.8	59.6 ± 2.7	-5.932	0.0002
	500	10	14.1 ± 1.3	77.6 ± 1.6	-26.128	<0.0001
	Cinnamite	10	8.1 ± 0.5	88.0 ± 1.3	-65.262	<0.0001
Peppermint	0	10	47.3 ± 8.4	42.5 ± 8.5	0.286	0.7814
	1	10	29.1 ± 6.5	62.7 ± 5.3	-2.909	0.0173
	20	10	33.7 ± 6.6	61.1 ± 6.4	-2.199	0.0555
	100	10	7.6 ± 1.5	87.0 ± 1.6	-34.225	<0.001
	500	10	6.8 ± 1.7	87.1 ± 2.4	-21.512	<0.0001
	Cinnamite	10	10.3 ± 1.4	85.9 ± 2.3	-21.840	<0.0001
Tea tree	0	10	44.1 ± 6.5	50.4 ± 6.3	-0.497	0.6312
	1	10	42.8 ± 8.4	40.8 ± 8.0	0.123	0.9050
	20	10	30.2 ± 7.2	61.3 ± 7.3	-2.143	0.0607
	100	10	10.9 ± 1.1	79.3 ± 1.4	-32.592	<0.0001
	500	10	10.5 ± 1.8	80.9 ± 2.2	-18.366	<0.0001
	Cinnamite	10	8.6 ± 0.6	84.5 ± 0.8	-69.000	<0.0001

Table 4.3. Essential oil repellency choice tests having significantly higher mortality on treated side than control side. Data were compared using paired t-tests (α=0.05).

Species	Treatment	Rate	Treated mortality (mean ± SE)	Control mortality (mean ± SE)	T	P
L. humile	Lemon	100	17.5 ± 2.8	4.3 ± 0.6	4.557	0.0014
		500	17.8 ± 3.8	5.4 ± 1.0	2.885	0.0180
S. invicta	Basil	100	10.6 ± 1.5	4.4 ± 0.6	3.753	0.0045
		500	24.3 ± 9.0	4.9 ± 0.8	2.300	0.0470
	Lemon	100	6.4 ± 2.3	4.2 ± 1.4	2.443	0.0372
		500	13.5 ± 7.4	3.1 ± 1.3	4.970	0.0008

Table 4.4. Mean ± SE cumulative mortality of Argentine ants continuously exposed to filter paper treated with 25 µl essential oil. Within columns, means followed by the same letter are not significantly different (α = 0.05, Tukey's Studentized Range Test).

	Time (min.)								
Treatment	15	30	45	60	75	90	105	120	24h
Basil	1.0 ± 1.0b	5.0 ± 2.2b	6.0 ± 2.2b	8.0 ± 2.5bc	9.0 ±2.8bc	9.0 ± 2.8bc	9.0 ± 2.8bc	9.0 ± 2.8bc	10.0 ± 2.6c
Citronella	22.1 ± 5.3a	44.3 ± 5.5a	67.4 ± 6.3a	86.7 ± 3.8a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
Eucalyptus	1.0 ± 1.0b	2.0 ± 1.3b	3.0 ± 1.5b	4.0 ± 0.0c	6.0 ± 3.4c	7.0 ± 3.3c	7.0 ± 3.3c	7.0 ± 3.3c	9.1 ± 3.2c
Lemon	4.1 ± 2.2b	5.1 ± 2.3b	6.1 ± 3.1b	6.1 ± 3.1bc	7.1 ± 3.0bc	10.2 ± 3.2bc	10.2 ± 3.0bc	10.2 ± 3.0bc	11.2 ± 2.8c
Peppermint	7.0 ± 2.6b	11.0 ± 2.8b	16.0 ± 4.8b	19.0 ± 4.6b	20.0 ± 4.9b	21.0 ± 4.8b	21.0 ± 4.8b	21.0 ± 4.8b	89.8 ± 3.4b
Tea tree	4.0 ± 2.7b	8.0 ± 2.5b	9.0 ± 2.3b	12.1 ± 2.5bc	13.0 ± 2.5bc	14.9 ± 2.9bc	14.9 ± 3.3bc	14.9 ± 3.3bc	85.7 ± 2.8b
Control	0.0 ± 0.0b	2.0 ± 1.3b	3.0 ± 1.5b	4.0 ± 1.6c	5.1 ± 1.7c	6.1 ± 2.2c	6.1 ± 2.2c	6.1 ± 2.2c	6.1 ± 2.2c

Table 4.5. Mean ± SE cumulative mortality of red imported fire ants continuously exposed to filter paper treated with 25 µl essential oil. Within columns, means followed by the same letter are not significantly different (α = 0.05, Tukey's Studentized Range Test).

	Time (min.)								
Treatment	15	30	45	60	75	90	105	120	24h
Basil	0.0 ± 0.0a	0.0 ± 0.0a	1.0 ± 1.0a	1.0 ± 1.0a	1.0 ± 1.0a	2.0 ± 1.3a	2.0 ± 1.3ab	2.0 ± 1.3ab	6.2 ± 1.7b
Citronella	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	3.0 ± 2.1a	4.0 ± 3.1a	6.1 ± 3.1a	9.1 ± 3.8a	50.6 ± 6.3a
Eucalyptus	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0b	0.0 ± 0.0b	2.0 ± 1.3b
Lemon	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0b	0.0 ± 0.0b	3.0 ± 2.1b
Peppermint	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0b	0.0 ± 0.0b	1.0 ± 1.0b
Tea tree	1.7 ± 1.7a	1.7 ± 1.7a	1.7 ± 1.7a	1.7 ± 1.7a	1.7 ± 1.7a	1.7 ± 1.7a	1.7 ± 1.7ab	1.7 ± 1.7b	3.3 ± 3.3b
Control	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0b	0.0 ± 0.0b	1.0 ± 1.0b

CHAPTER 5

A NOVEL DELIVERY METHOD FOR FIRE ANT TOXICANTS[4]

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[4] Wiltz, B. A., D. R. Suiter, W. A. Gardner, and C. W. Berisford. For submission to Sociobiology.

ABSTRACT

Described here is a new delivery method for ant toxicants consisting of an inert carrier, an attractant, and a toxicant. Unlike baits, this system does not contain a food source, but uses ant-to-ant contact rather than trophallaxis as the mechanism for horizontal dispersal of the toxicant through the colony. Of the six potential attractants evaluated, we found that only triolein, a reported component of the *Solenopsis invicta* Buren brood pheromone, increased removal of treated filter paper by fire ant workers in laboratory studies. When placed on corn cob granules, removal by workers was optimized at a rate of 60 µl triolein per gram. In laboratory assays, mortality of fire ants offered corn cob granules treated with a combination of 0.06% (w/w) fipronil and triolein (0.15 µl / granule) was 90.5 ± 0.9%, versus 46.5 ± 3.7% when granules were treated with the same rate of fipronil without triolein. In both laboratory and field trials, the removal of corn cob granules treated with a combination of fipronil and triolein was greater than removal of untreated granules but less than removal of granules treated with triolein alone.

Key words: fire ant, horizontal toxicity, attractant, pheromone, triolein

INTRODUCTION

Control strategies for ants and other social insect pests differ from those for non-social insects because the queen(s) must be killed in order to eliminate the colony. In part, this has been addressed through the use of toxic baits, which capitalize upon the foraging and food distribution activities of ants. The toxicant is transferred to all members of the colony through trophallaxis. An effective ant bait contains an active ingredient that exhibits delayed toxicity, is non-repellent, and is effective over a range of concentrations (Stringer et al. 1964, Williams 1983). Commercially available ant baits use an oil, sugar, or protein source, or combination therof, that acts as a phagostimulant and sometimes as a solvent for the active ingredient. In some cases, the toxicant and attractant are absorbed onto an inert carrier. Less toxicant is usually required to eliminate ant colonies with baiting than with other strategies.

Despite the widespread use of baits for ant control, there remains a need for effective attractants for baiting because most currently available baits are not consistently accepted by most ant species. Although baiting has been practiced for control of the red imported fire ant, *Solenopsis invicta* Buren, for over 40 years (Williams et al. 2001), problems remain because of seasonal changes in bait preferences (Stein et al. 1990). Additionally, baits generally lack species specificity because of differences in food preferences among ant species.

In earlier studies, we found that the red imported fire ant and the Argentine ant, *Linepithema humile* (Mayr), exhibit high levels of mortality due to contact between untreated ants and corpses treated with some toxicants (Chapters 2 and 3). We propose that the combination of a toxicant and a behavior-modifying chemical(s) applied to an inert carrier can be effective at killing ants via physical contact with handling as the primary mechanism of toxicant transfer. While the concept is applicable to the control of other social insects, the red imported fire ant was selected as a model for this study because it is a major economic pest, its chemical ecology has been extensively studied, and all life stages are readily available.

Pheromones have been reported to enhance recruitment to or consumption of baits by ants. Vander Meer (1996) found that invictolide, a component of the fire ant queen recognition pheromone, decreased the discovery time and increased the number of bait granules discovered by fire ant workers. The addition of Argentine ant trail pheromone to a sucrose solution enhanced consumption by workers, demonstrating a potential method of improving liquid bait consumption and specificity (Greenberg and Klotz 2000). Chemicals that could potentially entice ants to collect treated granules include those that elicit necrophoric or brood tending behaviors, such as the fatty acids linoleic acid, linolenic acid, and oleic acid. All are long-chain fatty acids, which are chemically among the most stable decomposition products and tend to accumulate disproportionately in corpses, potentially signaling the presence of aging corpses, as opposed to freshly-killed prey. For example, Bomar and Lockwood (1994a,b,c) found that the decomposition products linoleic acid and linolenic acid attracted multiple grasshopper species, a seed bug and five ant species. They further demonstrated that the addition of linoleic acid increased the efficacy of baits against grasshoppers. Oleic acid induces necrophoric behavior in imported fire ants and the harvester ant *Pogonomyrmex badius* (Wilson et al. 1958).

The purpose of this study was to demonstrate that a behavior-modifying chemical could be used to enhance particle handling by ants, thereby increasing total mortality relative to particles with a toxicant but no attractant.

MATERIALS AND METHODS

This study consisted of four parts: screening potential attractants on filter paper, determining the optimum concentration of attractants on corn cob granules, quantifying mortality of lab colonies due to handling granules treated with attractant and toxicant, and evaluating removal of treated granules by field colonies of the red imported fire ant.

Test insects. Red imported fire ants used for the laboratory studies were collected in Spalding County, GA. Ants were separated from soil by placing in a 19 x 13 x 10 cm Fluon™ (Northern Products, Inc., Woonsocket, RI) -lined plastic box (Pioneer Plastics, Dixon, KY), connected to a second box by a paper bridge (10 x 50 cm strips of cardstock, with the ends taped to the bottom of each box). The second box contained nest cells (100 x 25 mm Petri dishes containing a 1.0 cm thick layer of hardened dental plaster (Castone; Dentsply International Inc., York, PA), with 4 mm holes drilled into the sides to allow ants to enter), food, and water. As soil dried, workers moved the entire colony to the nest cells. Nest cells containing ants were transferred to clean 31 x 23 x 10 cm Fluon-lined plastic boxes (Pioneer Plastics, Dixon, KY). Colonies were maintained at room temperature (23° – 26°C) on a diet of water, 25% sugar water, and frozen house crickets (*Acheta domesticus*).

Attractant screening. Initial screenings were conducted using small lab colonies. A nest cell containing approximately 5 g of workers with brood was placed at one end of a 19 x 13 x 10 cm Fluon-lined plastic box. Two-choice tests were conducted by placing treated filter paper circles (Whatman #3, punched with a 3 mm (1/8”) hole punch) on scoops (approximately 30 x 20 mm bottom with 20 mm handle) cut from plastic weigh boats and placing the scoops at the opposite end of the box from the nest cell. This provided a flat surface from which to evaluate the removal of filter paper circles and allowed choices to easily be placed into the box simultaneously.

Six potential attractants were evaluated: house cricket washes, oleic acid, linoleic acid, linolenic acid, diolein, and triolein. Test solutions were expected to elicit handling through one of three types of responses: food handling, necrophoric, or brood-tending (Table 5.1). Some chemicals or extracts were evaluated at multiple rates or in multiple solvents, resulting in a total of 16 treatments. Unless otherwise indicated, all chemicals were dissolved in hexane. Each replicate consisted of ten filter paper circles treated with 5 μl of one of the test materials, paired with a control of ten filter paper circles treated with the same solvent used on the treatment side. Before applying the treatments, either treated or control circles were marked with a pencil so the treatments could be distinguished from each other within the box. At 10 min intervals for 1 h, the number of filter papers removed and the number transported into the nest were recorded. Tests were replicated nine times for each treatment.

Rate determination. Triolein was selected for further testing based on the positive results of the filter paper assays above. A no-choice test was conducted to determine the rate of triolein that elicited the greatest response when applied to corn cob granules. Each of six lab colonies was provided 10 granules treated with 1 of 8 rates of triolein: 0.01, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, or 1.0 μl. Controls were treated with 10 μl hexane. For this test and all that follow, granules were cleaned prior to use by triple washing with hexane and air drying for at least 1 d.

Triolein was dissolved in hexane to a total volume of 10 μl per granule and applied to individual 10-14 mesh corn cob granules (The Andersons, Maumee, OH) using a Hamilton syringe. This granule size was selected because it reportedly is the preferred food particle size for red imported fire ants (Hooper-Bùi et al. 2002). Treated granules were air dried for 30 – 60 min., then 10 granules were placed on a plastic scoop in a 19 x 13 x 10 cm Fluon-lined plastic box containing a nest cell with approximately 5 g fire ant workers + brood. The time at which each granule was removed and the time it was transported into the nest were recorded, for up to 30 min.

Laboratory evaluation with toxicant. Based on the results of the previous test, a rate of 0.15 µl triolein per granule was selected for evaluation on granules with a toxicant. Fipronil (Termidor SC, 9.1% fipronil, BASF, Research Triangle Park, NC) was used as the active ingredient for this assay because it is non-repellent, slow-acting, and has high contact and horizontal toxicity (Chapter 3). Granules were treated with 0.01, 0.02, 0.03, 0.04, 0.05, or 0.06% fipronil (w / w) + 0.15 µl triolein per granule, 0.15 µl triolein per granule, 0.06% fipronil, or hexane (control). Granules for all fipronil treatments were treated by mixing the appropriate volume of Termidor SC in water to make a total volume of 2 ml, then adding to 1 g corn cob granules in a glass Petri dish. Granules were dried 24 h under a fume hood, with occasional stirring to facilitate drying. Triolein was then applied to individual dry granules as described above, with the triolein being applied to dry fipronil-treated granules in treatments containing both chemicals. Controls were treated with 20 µl hexane. Assays were conducted in 19 x 13 x 10 cm Fluon-lined plastic boxes, each containing a nest cell with 3g fire ant workers and a water-soaked cotton ball on a 45 mm plastic lid. The water source was placed on top of the nest cell and a weigh boat scoop containing 50 treated granules was placed at the end of the box. Boxes were covered with lids containing two, 6-mm holes and maintained at 24°C with a photoperiod of 12:12 (L:D) for 3 d when the numbers of dead and live ants and the positions of granules (not moved, on box floor, or in nest cell) were recorded.

Granule removal by field colonies. Removal of granules by field colonies was evaluated using corn cob granules treated with either fipronil + triolein, triolein alone, or hexane (control). Fipronil was applied to granules at a rate of 0.06%, and granules were dried under a fume hood for 24 h before treatment with triolein. Triolein was applied at a rate of 60 µl per g granules, the equivalent of the 0.15 µl rate used in the previous tests. In both the fipronil and no fipronil treatments, triolein was dissolved in hexane, then sprayed onto the granules in a glass dish using an aromatherapy atomizer (Aura Cacia, Urbana, IA). Granules were sprayed 5 times, swirled to mix, and repeated until all of the triolein solution was used, to provide a uniform coating to all granules without removing the fipronil. Field trials were conducted 1 to 2 h after treatment with triolein. Treatments were randomly assigned to mound numbers in advance. Each mound encountered that had actively foraging ants was then assigned a sequential number. To quantify granule removal, 50 treated granules were placed on a 50 mm diameter plastic disk adjacent to a fire ant foraging trail. The number of granules remaining on the disk was recorded at 15 min intervals for 1 h. All tests were conducted in the morning (7:30 – 9:30 a.m.) or evening (6:00 – 8:00 p.m.) when ants were actively foraging. Air temperatures ranged from 21 - 24° C. Ten colonies per treatment were evaluated.

Statistical analysis. Results of filter paper choice tests were analyzed by paired t-tests. For the fipronil-treated granule assay, mortality and granule location data for all treatments were compared by one-way analysis of variance (ANOVA), and means were separated with Tukey's Studentized range test (SAS Institute 1985). Mortality was analyzed by probit analysis (PROC PROBIT; SAS Institute 1985) to determine an LC90 of fipronil on granules with triolein added. For field trials, the cumulative numbers of granules removed in each treatment was compared at each time period by ANOVA, and means were separated with Tukey's Studentized range test.

RESULTS

Attractant screening. Only one treatment, 1% triolein, significantly increased filter paper removal versus the paired controls (Table 5.2). At each time period, the cumulative number of filter paper circles removed and the cumulative number carried into the nest cell were greater for the triolein treatment than the controls. Fewer filter papers with either linoleic acid, linolenic acid, or diolein were removed than controls for at least one time period.

Rate determination. Two of the triolein rates tested, 0.10 and 0.15 µl triolein per granule, enticed ants to move all 10 treated granules into the nest after 30 min (Table 5.3). Of these rates, the mean time for all granules to be removed and transported into the nest was slightly lower in the 0.15 µl treatment than in the 0.1 µl triolein treatment. Therefore, the 0.15 µl rate was selected for subsequent tests.

Laboratory evaluation with toxicant. Ants were observed transporting treated corn cob granules into nest cells within minutes of placement in the boxes. However, few granules in any of the treatments remained inside the nest after 3 d. When 3 g fire ant workers were provided corn cob granules treated with fipronil and 0.15 µl triolein per granule, the LC90 (95% CI) was 0.059% (0.053 – 0.066%) fipronil (w/w). More important than the actual rate, we found that the addition of triolein to granules treated with fipronil doubled the efficacy of the granules (Table 5.4). Fire ant mortality increased to 90.5% when triolein was added to granules with 0.06% fipronil, versus 46.5% ant mortality with the same rate of fipronil alone. Mortality from the 0.06% fipronil only treatment did not differ from the 0.01, 0.02, or 0.03% fipronil plus triolein treatments.

Granule removal by field colonies. At all time periods, removal of triolein-treated granules by field colonies of red imported fire ants was greater than the removal of control granules ($P<0.05$) (Table 5.5). Removal of granules treated with fipronil and triolein was greater than removal of controls at all time periods except 30 min, but was less than removal of triolein treated granules at 45 and 60 min.

DISCUSSION

We demonstrated that the addition of the attractant triolein to corn cob granules 1) enhanced the number of granules transported by foragers into laboratory and field colonies of *S. invicta* and 2) enhanced mortality relative to granules treated with an active ingredient alone. This technique has potential to provide a more species specific ant control method and to alleviate the problem of variable food preferences that sometimes makes control of some species by baiting difficult.

Areas for future studies include evaluation of various toxicants, identification of compounds that elicit handling behaviors in other species, and evaluation of species specificity. In preliminary field trials using 1, 5, or 25 g of granules treated with 0.06% fipronil plus triolein, we did not observe any colony mortality 10 d after treatment (unpublished data). At this time, many of the granules remained, or had been returned, outside the nest. These results suggest two areas that must be investigated further. First is the stability of triolein. While granules treated with triolein were removed by ants in both lab and field trials, few remained in the nest after 3 d in the lab assay. Bigley and Vinson (1975) found that while 100% of triolein treated disks were inside fire ant colonies after 30 min, only 75% remained after 4 h. Another factor that may impact field efficacy is the active ingredient formulation. Removal by lab and field colonies was less for triolein treated granules with Termidor SC than without Termidor SC. We previously showed that fipronil residues are non-repellent to fire ants and that Argentine ants crossed fipronil treated barriers more frequently than paired control barriers (Chapters 2 and 3). Therefore, we suspect that the reduction in granule handling could be due to the accumulation of Termidor SC inert ingredients on the surface of corn cob granules. However, we were not able to test this hypothesis because technical grade fipronil was not available for evaluation.

Numerous possibilities exist for the use of other chemicals that may induce handling in either red imported fire ants or other ant species. Obin and Vander Meer (1994) demonstrated that *S. invicta* workers preferentially entered, searched, and recruited nestmates to vials containing either an alate corpse or alate residue. Alonso and Vander Meer (1997) later determined that the alate mandibular glands are the source of these excitant pheromones. Vander Meer et al. (1980) discovered that in *S. invicta*, the attractants and queen recognition pheromones are stored in the poison sac and dispensed by the sting apparatus. The pheromone consists of minor non-alkaloid components of the poison sac contents. Components of the *S. invicta* trail pheromone include Z,E-α-farnesene, E-E-α-farnesene, Z-E- homofarnesene, Z-Z-homofarnesene, and Z-Z-Z-allofarnesene (Vander Meer et al. 1981, Williams et al. 1981). Vander Meer (1983) found that similar trail-following behavior was elicited by Z,E-α-farnesene alone as by the Dufour's gland extract. While trail pheromones have been studied more extensively in *S. invicta* than in other species, they are known for several other ant species, including *Monomorium pharaonis* (L.) (Ritter et al. 1977), *Lasius fuliginosus* (Latrielle) (Huwyler et al. 1975), *Atta texana* (Buckley) (Tumlinson et al. 1971), and *Atta sexdens rubropilosa* Forel (Cross et al. 1979). Triolein is not reported as a pheromone of other ant species (El-Sayed 2005) and does not enhance filter paper handling by *L. humile* at 0.1 or 1.0% (unpublished data). However, more work must be done to evaluate species specificity. Despite the lack of field efficacy with the current formulation, positive results obtained in the lab studies and granule removal by field colonies indicate that this technique may be an effective means of non-repellent toxicant delivery.

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Table 5.1. Treatments used for screening of potential red imported fire ant attractants.

Treatment	Solvent	Rate	Suspected activity	Reference
whole cricket (Acheta domesticus) wash	hexane 75% hexane: 25% ether 50% hexane: 50% ether 25% hexane: 75% ether ether	¼ cricket equivalent	food handling	
oleic acid	hexane	0.1% 1.0%	food handling or necrophoric	Wilson et al. 1958 Gordon 1983
linoleic acid	hexane	0.1% 1.0%	food handling or necrophoric	Vinson et al. 1967
linolenic acid	hexane	0.1% 1.0%	food handling or necrophoric	Vinson et al. 1967
diolein	hexane	0.1% 1.0%	brood tending	Bigley and Vinson 1975
triolein	hexane	0.1% 1.0%	brood tending	Bigley and Vinson 1975

Table 5.2. Cumulative percentage treated (TR) and control (C) filter paper circles removed by red imported fire ants in choice test (mean ± SE). “Nest” = filter papers in nest at observation time and “total” = total of filter papers that have been moved to locations inside or outside of the nest. N = 9.

		Time (minutes)					
Treatment		10	20	30	40	50	60
Cricket (hexane wash)	TR nest	0	0	0	0	0	0
	C nest	0	0	0	0	0	1.1 ± 1.1
	TR total	1.1 ± 1.1	1.1 ± 1.1	1.1 ± 1.1	1.1 ± 1.1	1.1 ± 1.1	1.1 ± 1.1
	C total	0	0	0	0	0	1.1 ± 1.1
Cricket (75% hexane wash)	TR nest	0	0	0	0	1.1 ± 1.1	1.1 ± 1.1
	C nest	0	0	0	0	0	0
	TR total	0	0	1.1 ± 1.1	2.2 ± 1.5	3.3 ± 2.4	3.3 ± 2.4
	C total	0	0	0	0	0	1.1 ± 1.1
Cricket (50:50)	TR nest	0	0	0	0	0	0
	C nest	0	0	0	0	0	1.1 ± 1.1
	TR total	4.4 ± 3.5	4.4 ± 3.5	4.4 ± 3.5	4.4 ± 3.5	4.4 ± 3.5	6.7 ± 3.7
	C total	0	0	1.1 ± 1.1	1.1 ± 1.1	1.1 ± 1.1	1.1 ± 1.1
Cricket (75% ether wash)	TR nest	2.2 ± 2.2	4.4 ± 4.4	7.8 ± 6.6	7.8 ± 6.6	10.0 ± 8.9	10.0 ± 8.9
	C nest	0	0	1.1 ± 1.1	1.1 ± 1.1	1.1 ± 1.1	3.3 ± 2.4
	TR total	3.3 ± 2.1	5.6 ± 4.4	10.0 ± 7.8	10.0 ± 7.8	12.2 ± 10.0	12.1 ± 10.0
	C total	0	0	2.2 ± 2.2	3.3 ± 3.3	4.4 ± 3.4	6.7 ± 4.4

Table 5.2. Continued.

Treatment		Time (minutes)					
		10	20	30	40	50	60
Cricket (ether wash)	TR nest	0	0	0	1.1 ± 1.1	2.2 ± 2.2	2.2 ± 2.2
	C nest	0	0	0	1.1 ± 1.1	1.1 ± 1.1	1.1 ± 1.1
	TR total	1.1 ± 1.1	3.7 ± 1.7	5.6 ± 1.8	6.7 ± 2.4	8.9 ± 3.5	10. 0 ± 3.7
	C total	0	0	0	1.1 ± 1.1	1.1 ± 1.1	2.2 ± 2.2
0.1% oleic	TR nest	0	0	0	0	0	0
	C nest	0	0	0	0	0	0
	TR total	0	0	0	0	0	0
	C total	0	1.1 ± 1.1	2.2 ± 1.5	2.2 ± 1.5	2.2 ± 1.5	2.2 ± 1.5
1.0% oleic	TR nest	0	0	0	0	0	0
	C nest	0	0	0	0	0	0
	TR total	1.1 ± 1.1	3.3 ± 3.3	4.4 ± 3.4	4.4 ± 3.4	4.4 ± 3.4	4.4 ± 3.4
	C total	0	0	0	0	0	1.1 ± 1.1
0.1% linoleic	TR nest	0	0	0	1.1 ± 1.1	1.1 ± 1.1	1.1 ± 1.1
	C nest	2.2 ± 2.2	5.6 ± 2.4	5.6 ± 2.4	5.6 ± 2.4	5.6 ±2.4	6.7 ± 2.9
	TR total	8.9 ± 3.8*	11.1 ± 5.1*	16.7 ± 5.8*	22.2 ± 7.0*	25.6 ± 8.0*	30.0 ± 8.9*
	C total	52.2 ± 11.5	76.7 ± 12.1	77.8 ± 12.3	81.1 ± 11.7	81.1 ± 11.7	83.3 ± 10.1

Table 5.2. Continued.

		Time (minutes)					
Treatment		10	20	30	40	50	60
1.0% linoleic	TR nest	6.7 ± 4.4	8.9 ± 6.1	8.9 ± 6.1	8.9 ± 6.1	8.9 ± 6.1	8.9 ± 6.1
	C nest	1.1 ±1.1	7.8 ± 6.7	7.8 ± 6.7	7.8 ± 6.7	7.8 ± 6.7	7.8 ± 6.7
	TR total	46.7 ± 7.1	53.3 ± 10.0*	54.4 ± 10.2*	61.1 ± 10.3*	64.4 ± 94.4*	71.1 ± 10.0*
	C total	54.4 ± 7.1	85.6 ± 7.7	87.8 ± 7.6	90.0 ± 7.8	91.1 ± 7.8	92.2 ± 7.8
0.1% linolenic	TR nest	0	0	0	0	0	0
	C nest	0	0	0	0	0	0
	TR total	32.2 ±13.8	34.4 ± 14.1*	41.1 ± 15.2	44.4 ± 14.9	46.7 ± 14.1	64.4 ± 10.2
	C total	55.6 ± 11.7	73.3 ± 11.1	78.9 ± 11.8	84.4 ± 8.9	85.6 ± 8.7	92.2 ± 6.6
1.0% linolenic	TR nest	11.1 ± 11.1	11.1 ± 11.1	1.1 ± 11.1	11.1 ± 11.1	12.2 ± 11.0	13.3 ± 10.9
	C nest	11.1 ± 11.1	11.1 ± 11.1	11.1 ± 11.1	12.2 ± 11.0	12.2 ± 11.0	12.2 ± 11.0
	TR total	45.6 ± 20.4	67.8 ± 18.9	74.4 ± 17.6	75.6 ± 17.7	84.4 ± 17.4	90.0 ± 16.7
	C total	52.2 ± 19.8	66.7 ± 19.1	73.3 ± 18.3	78.9 ± 18.3	82.2 ± 18.2	85.6 ± 17.5

Table 5.2. Continued.

		Time (minutes)					
Treatment		10	20	30	40	50	60
0.1% diolein	TR nest	0	0	1.1 ± 1.1	1.1 ± 1.1	1.1 ± 1.1	1.1 ± 1.1
	C nest	1.1 ± 1.1	1.1 ± 1.1	1.1 ± 1.1	1.1 ± 1.1	1.1 ± 1.1	1.1 ± 1.1
	TR total	8.9 ± 3.1	11.1 ± 11.1	24.4 ± 6.5	33.3 ± 6.9	36.7 ± 6.9	42.2 ± 8.5
	C total	6.7 ± 2.9	12.2 ± 4.9	17.8 ± 6.2	25.6 ± 8.5	27.8 ± 9.2	31.1 ± 10.7
1.0% diolein	TR nest	4.4 ± 4.4	4.4 ± 4.4	11.1 ± 6.5	13.3 ± 6.5	14.4 ± 6.7	14.4 ± 6.7
	C nest	0	2.2 ± 1.5	2.2 ± 1.5	4.4 ± 3.4	7.8 ± 4.3	7.8 ± 4.3
	TR total	26.7 ± 10.3	36.7 ± 12.2	50.0 ± 11.1	60.0 ± 12.4	62.2 ± 12.6*	65.6 ± 11.2*
	C total	31.1 ± 6.3	57.8 ± 8.1	74.4 ± 8.0	83.3 ± 6.0	90.0 ± 7.1	93.3 ± 6.5
0.1% triolein	TR nest	5.6 ± 4.4	6.7 ± 4.4	6.7 ± 4.4	6.7 ± 4.4	6.7 ± 4.4	6.7 ± 4.4
	C nest	0	0	1.1 ± 1.1	1.1 ± 1.1	1.1 ± 1.1	2.5 ± 1.5
	TR total	25.6 ± 10.3	36.7 ± 9.3	43.3 ± 9.4	51.3 ± 10.9	52.2 ± 10.2	65.6 ± 10.2
	C total	20.0 ± 7.3	27.8 ± 9.8	38.9 ± 11.6	55.6 ± 12.8	65.6 ± 13.2	70.0 ± 13.3

Table 5.2. Continued.

Treatment		Time (minutes)					
		10	20	30	40	50	60
1.0% triolein	TR nest	17.8 ± 6.2*	18.9 ± 6.1*	24.4 ± 7.3*	27.8 ± 6.8*	27.8 ± 6.8*	27.8 ± 6.8*
	C nest	0	1.1 ± 1.1	2.2 ± 1.5	3.3 ± 2.4	4.4 ± 2.4	4.4 ± 2.4
	TR total	43.3 ± 8.0*	52.2 ± 8.1*	71.1 ± 7.5*	78.9 ± 6.8*	81.1 ± 5.4*	84.4 ± 5.0*
	C total	11.1 ± 3.9	20.0 ± 6.7	28.9 ± 7.2	41.1 ± 8.7	55.6 ± 10.8	62.2 ± 10.0

* Paired treated and control counts are significantly different by paired t-tests (α = 0.05).

Table 5.3. Thirty min. removal of corn cob granules treated with various rates of triolein. Small lab colonies (n = 6) were provided 10 granules and removal times were recorded. Times until 10 were removed are the average of the times the final granule was removed in each replicate. A replicate was assigned a value of 30 if not all granules were removed.

Rate (µl per granule)	Number of granules removed (mean ± SE)	Number of granules in nest (mean ± SE)	Time until 10 removed	Time until 10 in nest
0	0.5 ± 0.22	0	> 30	> 30
0.01	2.2 ± 1.4	1.2 ± 1.2	> 30	> 30
0.05	9.0 ± 1.0	8.7 ± 1.1	> 14	> 20.2
0.10	10.0 ± 0	10.0 ± 0	8.0 ± 2.8	11.5 ± 2.6
0.15	10.0 ± 0	10.0 ± 0	7.5 ± 1.6	10.5 ±1.0
0.20	8.8 ± 1.0	8.5 ± 1.0	> 17	> 19.7
0.25	10.0 ± 0	8.8 ± 1.0	13.2 ± 1.3	> 20.0
0.30	9.5 ± 0.5	8.3 ± 1.3	> 15	> 20.3
1.0	4.2 ± 1.5	2.5 ± 1.1	> 30	> 30

Table 5.4. Percentage of corn cob granules in nest, removed from plastic disk, and fire ant mortality after 48 h exposure to treated granules. Groups of 3 g fire ant workers were provided 50 corn cob granules treated with 0.15 µl triolein per granule, one of six rates of fipronil + 0.15 µl triolein, or 0.06% fipronil with no triolein. Means were compared by ANOVA. Means within a column followed by the same letter are not significantly different (Tukey's Studentized range test, α = 0.05).

Treatment	N	In nest	Removed	Mortality
Control	7	0.9 ± 0.6ab	29.8 ± 3.8c	0.6 ± 0.1f
Triolein	7	2.1 ± 1.2ab	85.0 ± 3.8a	0.6 ± 0.1f
0.01% fipronil	7	3.0 ± 1.1a	69.8 ± 7.3ab	29.3 ± 6.3e
0.02% fipronil	7	0b	70.7 ± 6.3ab	46.9 ± 7.5de
0.03% fipronil	7	0b	60.2 ± 10.2ab	51.2 ± 6.1cd
0.04% fipronil	7	0b	76.3 ± 3.9ab	68.5 ± 5.9bc
0.05% fipronil	7	0.6 ± 0.6ab	76.0 ± 6.2ab	77.0 ± 2.7ab
0.06% fipronil	7	0.3 ± 0.3b	77.8 ± 4.3ab	90.5 ± 0.9a
0.06% fipronil, no triolein	7	0b	55.3 ± 3.5bc	46.5 ± 3.7de

Table 5.5. Mean (±SE) number of treated granules removed by field colonies. Means were compared by ANOVA. Means within a column followed by the same letter are not significantly different (Tukey's Studentized range test, α = 0.05).

Treatment	N	15 min	30 min	45 min	60 min
Control	10	3.9 ± 1.3b	9.0 ± 1.9b	11.6 ± 2.4c	13.5 ± 2.2c
Triolein	10	11.7 ± 2.2a	27.2 ± 4.2a	33.6 ± 3.6a	37.4 ± 3.4a
Fipronil + triolein	10	10.9 ± 1.8a	18.6 ± 1.2ab	23.8 ± 1.5b	26.9 ± 1.9b

CHAPTER 6

IMPORTED FIRE ANT (HYMENOPTERA: FORMICIDAE) LANDSCAPE ECOLOGY IN MISSISSIPPI BLACKLAND PRAIRIE REMNANTS[5]

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[5] Wiltz, B. A., J. T. Vogt, D. R. Suiter, and W. A. Gardner. For submission to Journal of Insect Science.

ABSTRACT

Blackland prairie remnants in northeast Mississippi were surveyed for the presence of imported fire ants, *Solenopsis* spp., in relation to land cover characteristics. Ground surveys were conducted at two sites and remotely sensed images of the sites were analyzed. Imported fire ants were found in prairie, dirt road, roadside, powerline, and lawn habitats and were absent from chalk outcrop and forest habitats. Mound densities of 53.6 mounds / ha and 54.5 mounds / ha at the respective sites were lower than the densities reported in other studies. Infested and uninfested prairie habitats differed in mature trees as a percentage of land cover in prairie patches. Fire ant presence or absence did not differ with patch size or distance to disturbed habitats.

Key words: imported fire ant, landscape ecology, remote sensing

INTRODUCTION

Blackland prairies are characterized by a thin layer of black soil high in organic matter covering a chalk or marl substrate (Foti 1989). They contain three primary plant communities: open prairie, prairie cedar woodland, and chalk outcrops (Leidolf and McDaniel 1998). The blackland prairies of Mississippi and Alabama once covered an area several hundred miles long and 25 miles wide (Harper 1943). Today only small prairie relics remain in abandoned pastures, along roadsides, in powerline rights-of-way, and in public and private natural areas (Schauwecker 2001).

The black imported fire ant, *Solenopsis richteri* Forel, and the red imported fire ant, *Solenopsis invicta* Buren, were first introduced into the Mobile, AL area around 1918 and the late 1930's, respectively (Lofgren 1986). Initially the two were believed to be the same species until Buren (1972) revised the taxonomy of imported fire ants, thus separating them into two species. It was later discovered that a hybrid of the two species exists (Vander Meer *et al.* 1985). In the United States, *S. invicta* ranges from North Carolina through Oklahoma and has been found in isolated locations in Virginia, Maryland, California, Arizona, and New Mexico (Williams *et al*. 2001, Sutherst and Maywald 2005). *S. richteri* occurs in a band from northeastern Mississippi to northwestern Alabama and the hybrid *S. invicta* x *richteri* is found from eastern Mississippi eastward into northwestern Georgia (Diffie *et al.* 1988).

In addition to being pests in the urban environment, imported fire ants kill livestock and wildlife, including poultry chicks, lizards, turtles, snakes, and ground-nesting birds (Vinson 1994; Allen *et al.* 1995; Parris *et al.* 2002) and have been reported to displace at least nine native ant species (Whitcomb *et al.* 1972; Glancey *et al.* 1976; Porter *et al.* 1988; Wojcik 1994). In general, imported fire ants tend to be abundant in open, sunny habitats such as lawns, pastures, old fields, and roadsides, while they are rare or absent in late succession or climax communities (Tschinkle 1986). In linear habitats, such as roadsides and powerline rights-of-way, mounds are most often found along ecotonal edges and are more common on the north side of clearings (Stiles and Jones 1998). Mound densities are highly variable due to a combination of factors including soil type, soil fertility, cultural practices, ground cover, history of chemical control, competition from other ant species, and frequency of disturbance (Urbani and Kannowski 1974; Ali *et al.* 1986; Tschinkle 1986; Stiles and Jones 1998).

Fire ant population densities and distribution within blackland prairie remnants of Mississippi and Alabama and what effects, if any, imported fire ants have had on native flora and fauna remain unknown. The objective of this study was to determine the occurrence of imported fire ants in various habitats in and surrounding the blackland prairies and if mound size and abundance vary with habitat type. This was achieved through ground surveys and analysis of remotely sensed imagery.

MATERIALS AND METHODS

Study sites. The study was conducted at two sites, each containing blackland prairie remnants and disturbed areas: Osborn Prairie (Oktibbeha Co., MS, about 33°30’45” N, 88°44’6” W, also known as 16th Section Prairie) and Crawford Prairie (Lowndes Co., MS, about 33°18’2” N, 88°36’41” W). Osborn Prairie was a mixture of open prairie, chalk outcrops, mesic oak-hickory forest, bottomland hardwood, and prairie cedar woodland. Two regularly maintained powerline rights-of-way crossed the site from southwest to northeast. These contained both native prairie vegetation and planted fescue (*Festuca* sp.). A cemetery planted in fescue bordered the prairie. Crawford Prairie was mostly prairie cedar woodland and open prairie, with relatively few chalk outcrops. A powerline crossed through the western part of the site. Additionally, roadside plots at this site were sampled. Both sites were located in an area containing populations of *S. richteri* and *S. invicta* x *richteri*. Although mounds were not sampled for queens, the low mound densities and large mound sizes found here are characteristic of monogyne populations (Macom and Porter 1996; Vogt *et al*. 2004)

Field survey. Preliminary plot sites were assigned on a modified grid sampling scheme by overlaying 100 x 100 m grids on U. S. Geological Survey digital orthophoto quarter-quadrangle (DOQQ) images. Within each grid square, 0.01 ha (5.65 m radius) plots were selected to include a single land cover type.

Plot locations were modified in the field to reflect changes since the earlier images were acquired. At the Osborn Prairie, 49 plots were configured on a modified 7 x 7 grid (Figure 6.1). At the Crawford Prairie, 45 plots were surveyed with 30 on the original grid layout and 15 representative of the land cover types present (Figure 6.2). Road construction and the growth of a large stand of mature pine prevented sampling on a 100 x 100 m grid at this site.

On 7-9 September 2004, a ground census was taken of all visible fire ant mounds within each plot. Plot centers were georeferenced using a Starlink Invicta® DGPS receiver and SoloField® software (Tripod Data Systems, Corvallis, OR). Plots were thoroughly searched for imported fire ant mounds. Each mound encountered was designated as active or abandoned by probing and looking for the emergence of workers. The length, width, and height of each active mound were recorded. Plots were assigned tentative habitat categories at the time of sampling.

Remotely sensed imagery. Digital imagery was obtained from Geodata Airborne Mapping and Measurement, Inc. (Macon, MS) with spatial resolution of 0.12 m. Flights were conducted on 4 October 2004 in a Cessna 172 equipped with a GPS antenna and 12-channel receiver, which was integrated with the data collection system. The camera system

(GeoVantage™, Inc., Swampscott, MA, USA) consisted of four monochrome digital cameras with 10 nm band-pass filters (centered at 450, 550, and 650 nm) and a 20 nm band-pass filter (centered at 850 nm). An inertial measurement unit provided acceleration and rotation rates for the cameras. Proprietary software (GeoVantage™) was used to georegister and mosaic the images. We received the images as two, 3-band composite images for each site: a red-green-blue image displayed in true color and a color infrared composite image consisting of the green band displayed as blue, red band displayed as green, and near infrared (NIR) band displayed as red.

Data analysis. Because landscape characteristics differed between the two sites, data for each site were analyzed separately. Two-by-two contingency tables were used to compare ant infestation rates among the six habitats identified at each site. Fisher's exact test (PROC FREQ; SAS Institute 1985) was used for comparisons between presence and absence data in paired habitat types because counts in some of the cells were too low to obtain valid results using the chi-square statistic. Mound volume was calculated using the formula:

$$\text{Volume} = 2/3 * \pi * a * b * c,$$

where a in is the semi-major axis, b is the semi-minor axis, and c is the height above ground level (Porter *et al.* 1992). Because mound numbers were low, only descriptive statistics were used for mound size and mound density comparisons.

Images were analyzed using Idrisi Kilimanjaro (Clark Labs, Worcester, MA) for land cover classification and ArcView 3.2 (ESRI, Redlands, CA) for all other spatial analyses. For land cover classification, six categories representative of ground covers were used: bare ground, short grass, tall grass, small trees and shrubs, mature trees, and chalk outcrop. These categories are roughly the equivalent of the habitat types described in the ground survey, but describe cover at a finer scale. For example, powerline habitats contain primarily short grass and tall grass, while prairie habitats contain primarily tall grass and shrubs. Land cover classification was conducted by supervised classification using all four image bands and the maximum likelihood classifier. Polygons containing pure ground covers were selected as training sites. Training sites were sometimes adjacent to, but not the same as, survey plots.

Prairie plots were examined for factors that may help predict imported fire ant presence. To analyze the effect of distance from disturbed habitat, habitat patches at the Osborn Prairie were selected manually, using the habitat types identified in the ground survey. Polygons of a single habitat were drawn by visual interpretation of the false color composite image. Distances of prairie plot centers to the nearest powerline, dirt road, or cemetery / lawn patch were measured. Distances to disturbance were compared for infested versus uninfested plots. Habitat patches containing the plots that were described as prairie or prairie with trees in the ground survey were selected from the classified image of Osborn Prairie. Patches were selected by outlining contiguous areas that contained primarily grasses, shrubs, and small trees. Mature trees, bare ground, and chalk outcrops were included when they were within a larger grassy area, but otherwise marked the borders between prairie and non-prairie. Prairie patches were clipped from the image of the whole site, and patch size and ground cover composition were determined from the number of pixels of each ground cover within each patch. Analysis of prairie plots was not conducted on the Crawford images because the site contained a single large prairie running north to south through its center, with no prairie plot being >75 m from a disturbed habitat.

RESULTS

Habitats. From the ground survey, 13 habitats were identified at the Osborn Prairie and 11 at the Crawford Prairie. Some of these categories fell along gradients, such as mixed prairie habitats and various mixtures of mature trees. To simplify comparison of habitats all prairie habitats were combined and all habitats containing primarily mature trees were combined. A total of six habitat categories was subsequently used for each site. Osborn Prairie plots fell within dirt road, cemetery, powerline, prairie, forest, or chalk outcrop habitats. Crawford Prairie contained dirt road, roadside, powerline, prairie, forest, and chalk outcrop habitats.

Fire ant population density. Fifteen fire ant mounds were found at Osborn Prairie and 18 at Crawford Prairie (Tables 6.1 and 6.2). Excluding forest and chalk outcrop plots where fire ants were never found, mound densities (mean ± SE) were 53.6 ± 0.12 mounds / ha at Osborn Prairie and 54.5 ± 0.12 mounds / ha at Crawford Prairie. At the Osborn Prairie, infestation rates were higher in the cemetery than in the prairie (Fisher's exact test, $P = 0.0117$) and were lower in forest than in the dirt road ($P = 0.05$), cemetery ($P = 0.0001$), powerline ($P = 0.0012$), or prairie ($P = 0.0404$). At the Crawford Prairie, infestation rates were lower in the forest than in powerline habitat ($P = 0.0147$), with the results of no other comparisons being significant.

Mound size. Mound volumes ranged from 7.0 to 285.0 L (mean ± SE = 71.7 ± 22.0) at Osborn Prairie and from 18.4 to 79.1 L (mean ± SE = 46.6 ± 9.7) at Crawford Prairie. In general, mean mound size was greater in the infrequently disturbed prairie and dirt road habitats than in more frequently disturbed powerline, cemetery, and roadside habitats.

Prairie patch analysis. At the Osborn Prairie, distances from prairie plots containing imported fire ants to the nearest disturbed habitat (powerline, lawn, or dirt road) ranged from 18.6 to 94.4 m (mean ± SE = 56.0 ± 16.4), while distances ranged from 18.4 to 156.1 m (mean ± SE = 97.3 ± 14.2) for prairie plots without imported fire ants (Figure 6.3).

Ten areas within the Osborn Prairie were identified as prairie patches (Table 6.3, Figure 6.4). Four of these patches contained active imported fire ant mounds. There was no trend in patch size of infested versus uninfested plots. Plots with imported fire ants ranged in size from

656 to 13,688 m^2 (mean ± SE = 5,601 ± 2,924) while plots with no fire ants had areas of 1,566 to 10,233 m^2 (mean ± SE = 6,261 ± 1,484). The only apparent trend in patch composition was that imported fire ants were never found in patches containing >22% mature trees. Infested plots had 6 – 22% (15 ± 4%) tree cover while uninfested plots had 14 – 45% (31 ± 5%) tree cover. At Crawford Prairie, all prairie plots were within a single large prairie or a smaller patch separated from the main prairie by a newly-constructed road (Figure 6.5).

DISCUSSION

This study provides the first description of imported fire ant distribution within blackland prairie habitats. The mean mound densities of 53.6 and 54.5 mounds / ha are lower than the 150 mounds / ha or more commonly reported for pasture areas in northeast Mississippi (Vogt *et al.* 2003). Although imported fire ant mound densities were low at both sites, trends in the types of habitats infested are similar to those found in other studies. Mound sizes were similar to those found in Mississippi pasture, where volumes range from < 1 to > 200 L, with mean volumes of 28.2 – 45.1 L (Vogt 2004). At both of our sites, mounds tended to be smaller in more disturbed habitats. Stiles and Jones (1998) found the same trend in mound sizes in South Carolina road and powerline habitats containing red imported fire ants. As in other studies (Brown 1980; Tschinkel 1986; Porter and Savignano 1990), we found imported fire ants in open areas, with the highest infestation rates in disturbed habitats. While we never found imported fire ant mounds in forest habitats or prairie containing a large proportion of mature trees, Stiles and Jones (1998) and Coulson *et al.* (1999) found *S. invicta* in both open and shaded habitats, although at lower densities in the shaded habitats. This difference may be attributed to the overall low densities at our sites and for black and hybrid imported fire ants in general, relative to red imported fire ants (Vogt *et al.* 2003). In our study, infested and unifested plots did not differ in proximity to disturbed habitats. This was not surprising because monogyne fire ants can disperse 2 km or more by nuptial flights (Markin *et al.* 1971). Distance to disturbed areas would be expected to be more important for polygyne populations, which could move in from disturbed areas by colony budding.

In addition to contributing to our knowledge of imported fire ant distribution in natural habitats, the results of this study have practical implications for habitat conservation and restoration. Very little native blackland prairie remains because of heavy agricultural pressure (Schauwecker 2001). The remaining blackland prairie remnants are in danger of being replaced by invasive species. The eastern red cedar, *Juiperus virginiana*, that is widespread in Mississippi prairies now may have historically been absent or much less common in the presence of natural disturbances such as fire or grazing by American bison (Peacock and Miller 1990; Leidolf and McDaniel 1998). Likewise, native arthropods in the prairie communities may be in danger of displacement by imported fire ants. With this new information about which habitats within blackland prairies have been invaded by imported fire ants, restoration groups can target these specific areas for fire ant control and native arthropod conservation. Given the small size of remaining prairies (0.5 – 5.0 ha) (Schauwecker 2001) and relative low mound densities, individual mound treatments are feasible in some cases. This is particularly true of the Osborn Prairie, which is currently leased to Starkville High School and is used for educational purposes (Melby *et al.* 1998). A master plan has been developed for this site and includes plans to restore prairie vegetation, remove a portion of the cedar woodland, and rehabilitate badly degraded soil (Melby *et al.* 1998). As wooded areas are removed from such sites in the restoration process, new potential imported fire ant habitats will be established, making targeted management even more important.

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Table 6.1. Imported fire ant mounds in Osborn Prairie plots, by land cover. Each plot covered an area of 0.01 ha.

Land cover	N (plots)	Plots with mounds	Plots without mounds	% plots infested	Mounds / infested plot	Mound volume (L)
Dirt road	1	1	0	100%	1	153.2
Cemetery	4	4	0	100%	1.25	41.1
Powerline	6	4	2	67%	1.25	42.96
Prairie	17	4	13	24%	1.0	103.7
Forest	19	0	19	0%	-	-
Chalk outcrop	2	0	2	0%	-	-

Table 6.2. Imported fire ant mounds in Crawford Prairie plots, by land cover. Each plot covered an area of 0.01 ha.

Land cover	N (plots)	Plots with mounds	Plots without mounds	% plots infested	Mounds / infested plot	Mound volume (L)
Dirt road	5	2	3	40%	1	77.5
Roadside	5	1	4	20%	1	18.4
Powerline	7	4	3	57%	1	32.3
Prairie	16	7	9	44%	1.6	44.2
Forest	10	0	10	0%	-	-
Chalk outcrop	2	0	2	0%	-	-

Table 6.3. Land cover composition of Osborn Prairie patches, in m^2, followed by percentage of total patch area.

	Patch #									
Land cover	1	2	3	4	5	6	7	8	9	10
Fire ants	no	no	no	yes	yes	no	no	no	yes	yes
Bare ground	383 (6%)	73 (1%)	917 (11%)	177 (3%)	203 (10%)	117 (6%)	21 (1%)	877 (9%)	1212 (9%)	122 (19%)
Short grass	46 (1%)	9 (0%)	166 (2%)	58 (1%)	38 (2%)	94 (5%)	116 7(%)	249 (2%)	336 (2%)	38 (6%)
Tall grass	1316 (20%)	990 (12%)	702 (8%)	1715 (29%)	434 (21%)	739 (36%)	426 (27%)	2555 (25%)	5976 (44%)	93 (14%)
Small trees & shrubs	2817 (42%)	3605 (42%)	3883 (46%)	2754 (46%)	906 (44%)	788 (39%)	348 (22%)	4136 (40%)	4491 (33%)	364 (55%)
Mature trees	2067 (31%)	3858 (45%)	2545 (30%)	1299 (22%)	424 (21%)	286 (14%)	654 (42%)	2194 (21%)	1389 (10%)	39 (6%)
Chalk	81 (1%)	1 (0%)	280 (3%)	4 (0%)	46 (2%)	2 (0%)	0 (0%)	222 (2%)	283 (2%)	1 (0%)
Total	6710 (100%)	8537 (100%)	8495 (100%)	6008 (100%)	2052 (100%)	2025 (100%)	1566 (100%)	10233 (100%)	13688 (100%)	656 (100%)

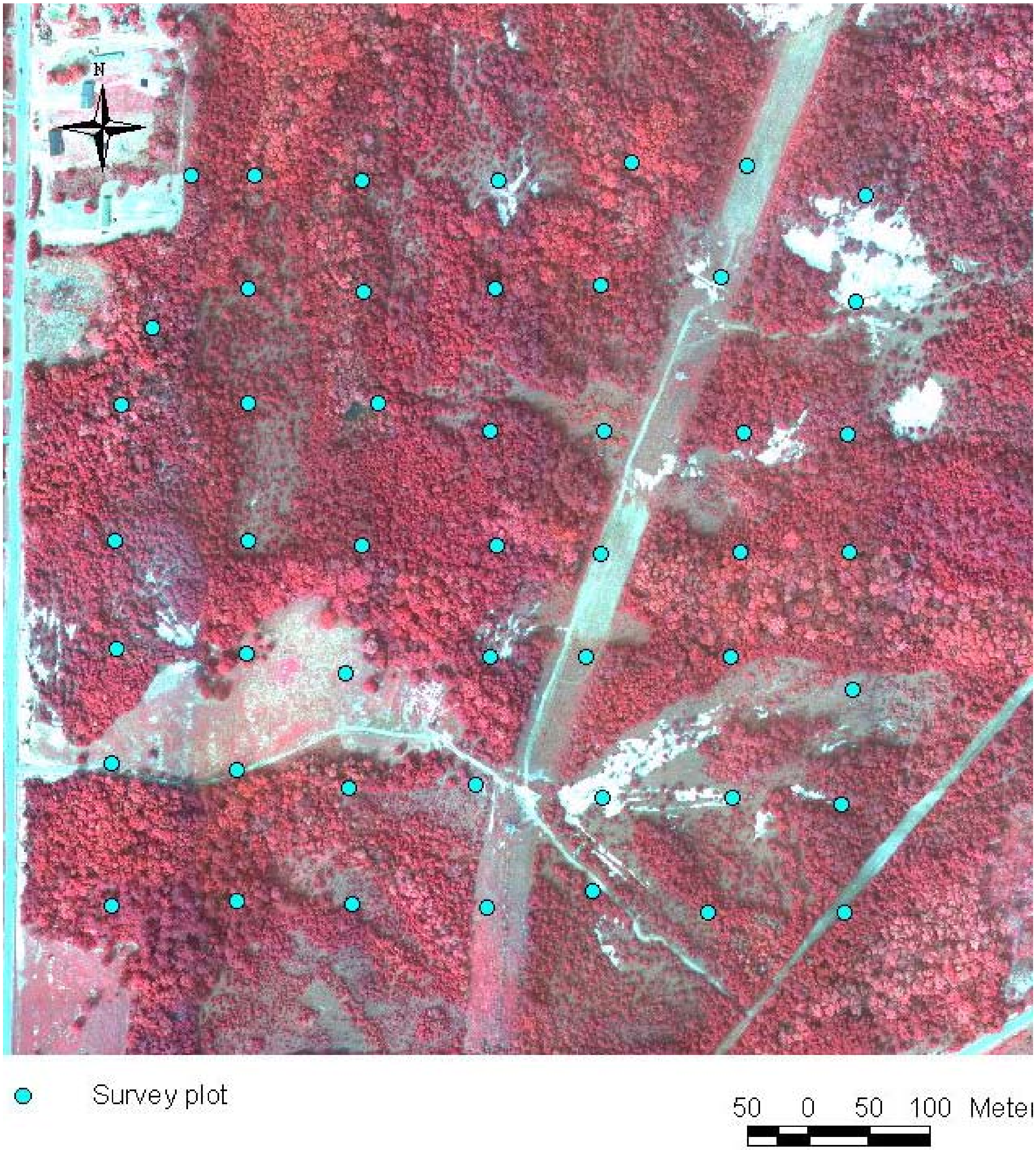

Figure 6.1. Locations of 49, 0.01-ha survey plots (5.65 m radius), overlayed on color infrared composite image of Osborn Prairie.

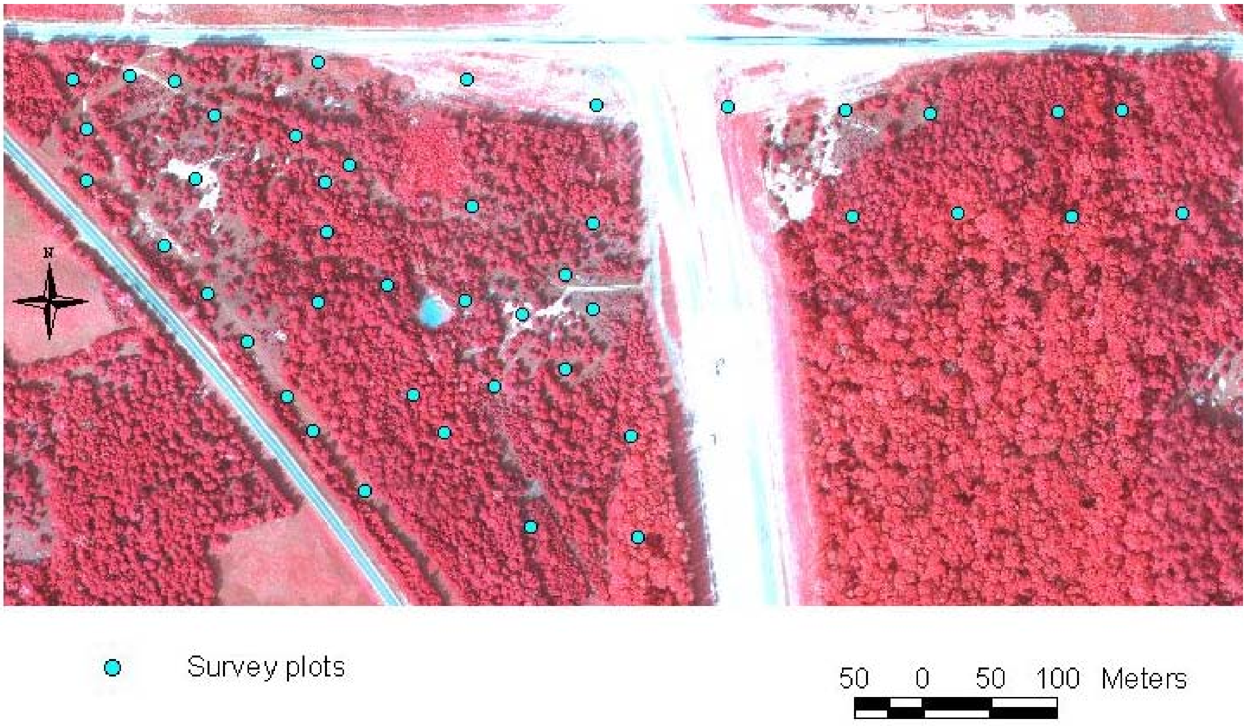

Figure 6.2. Locations of 45, 0.01-ha survey plots (5.65 m radius), overlayed on color infrared composite image of Crawford Prairie.

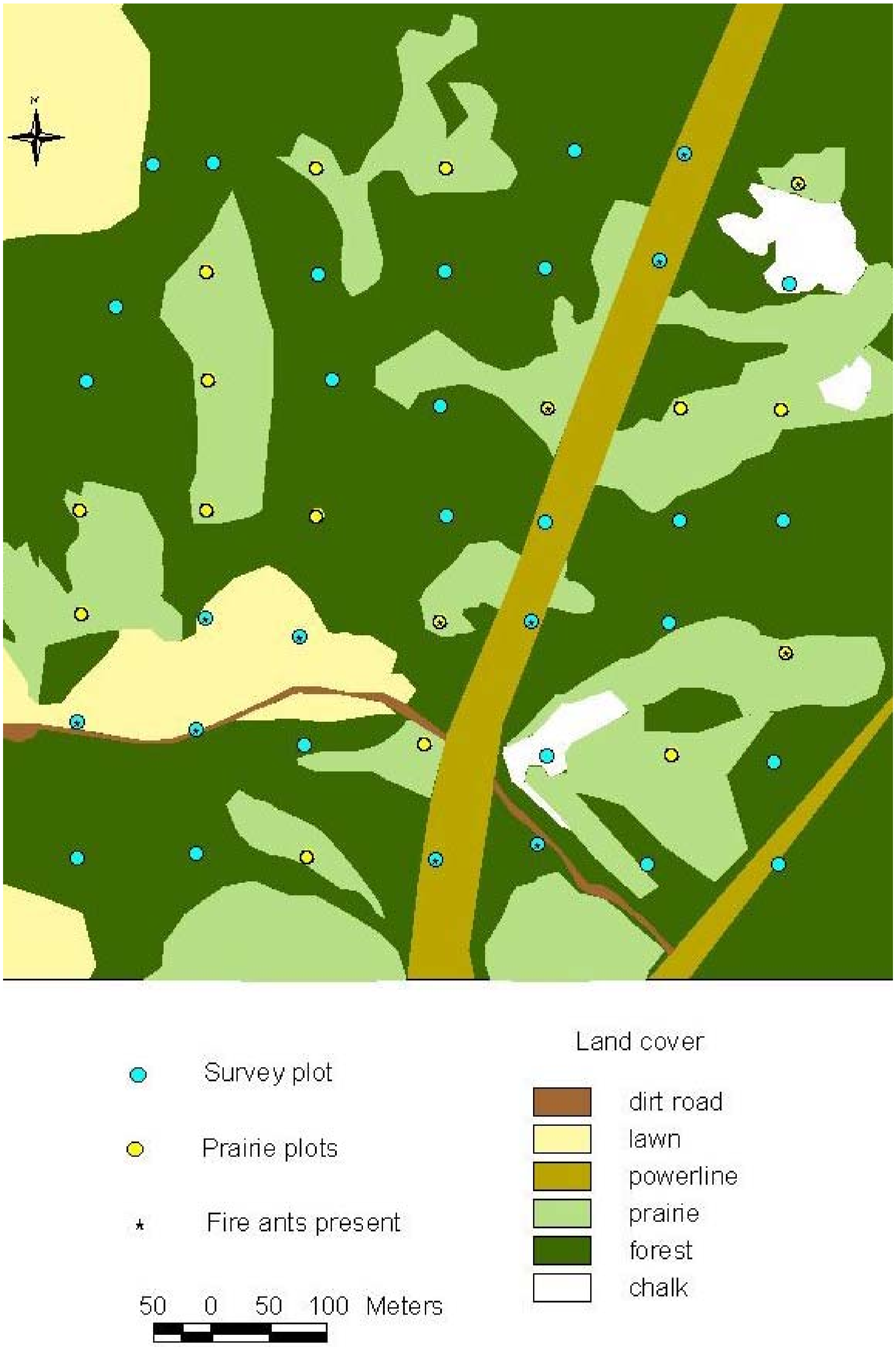

Figure 6.3. Manual classification of habitat patches at Osborn Prairie, with locations of survey plots in prairie habitat and those containing active imported fire ant mounds.

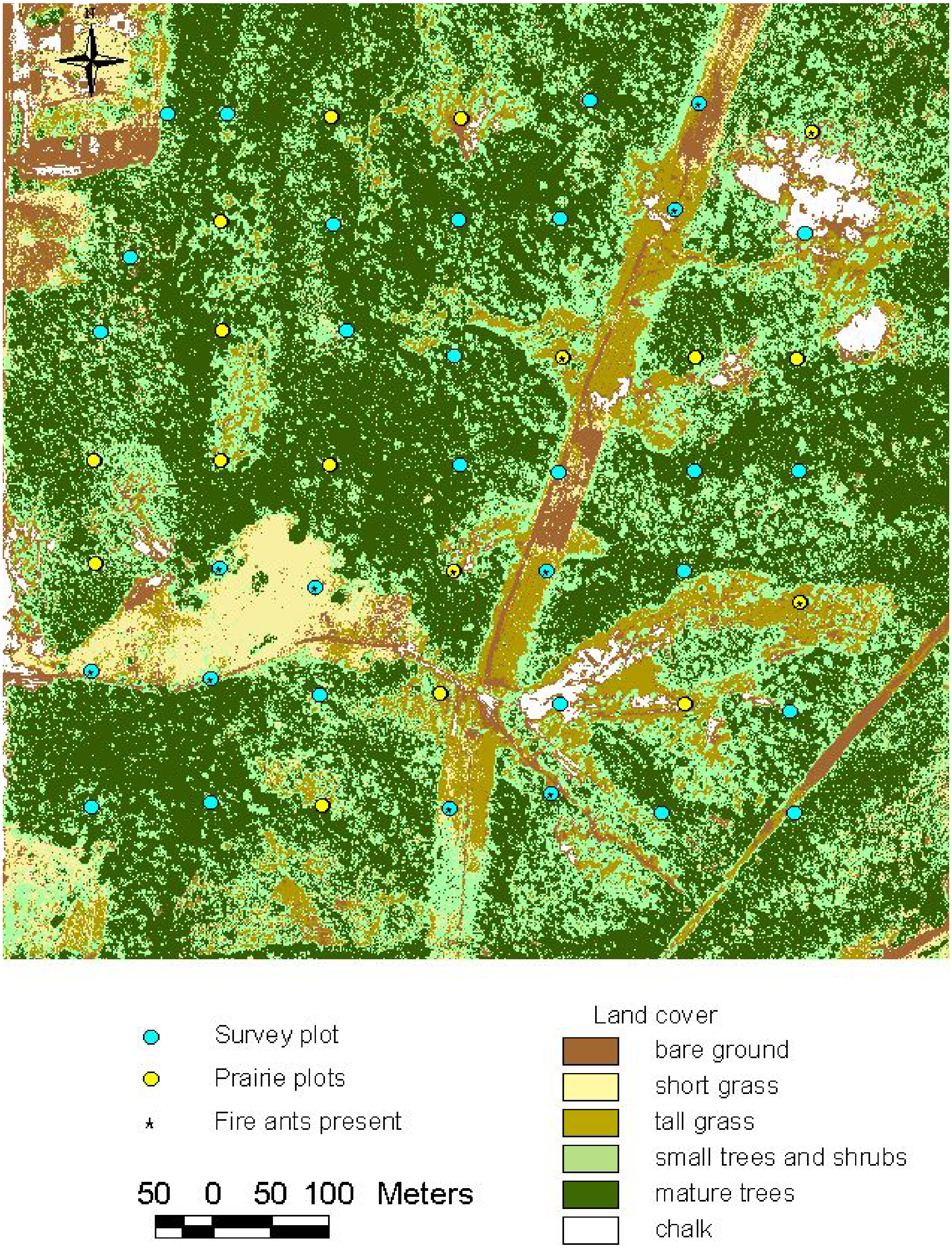

Figure 6.4. Land cover classification of Osborn Prairie and locations of plots with active fire ant mounds.

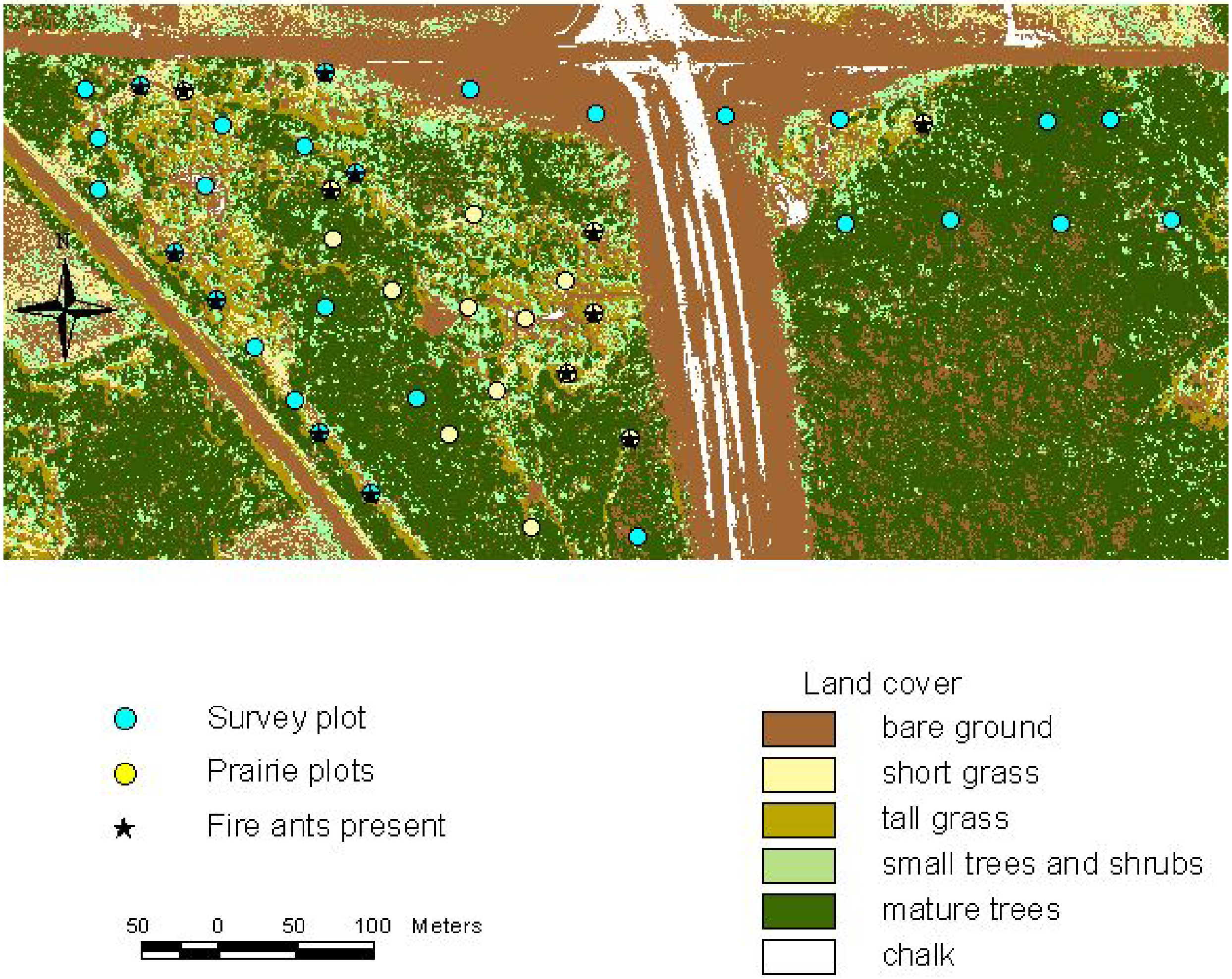

Figure 6.5. Land cover classification of Crawford Prairie and locations of plots with active fire ant mounds.

CHAPTER 7

CONCLUSIONS

The public is concerned with the overuse of pesticides, yet has little tolerance for the pests themselves. The best way to simultaneously manage urban pests and reduce pesticide usage and environmental impacts is to make alternative control methods available while implementing more targeted application methods when chemical toxicants are needed. These methods include proper placement and timing of pesticide application and the use of control methods that are more target specific.

The results contained within this dissertation represent an effort to identify ways to use the biology and ecology of imported fire ants and Argentine ants to develop more targeted methods of controlling these pests, using both traditional insecticides and alternative methods.

In an evaluation of pesticide properties against the two ant species we found that bifenthrin had properties best suited for use as barrier or mound treatments. It was the fastest acting of the toxicants tested and was the only insecticide that acted as a barrier to ant movement. Fipronil had high horizontal toxicity and delayed topical toxicity, properties that are desirable in a broadcast treatment. Chlorfenapyr and thiamethoxam were best suited to use as mound treatments, as they had low horizontal toxicity and did not impede ant movement in barrier tests.

Basil, citronella, lemon, peppermint, and tea tree essential oils deterred Argentine ants and red imported fire ants from crossing treated surfaces. In continuous exposure tests, citronella oil was toxic to both Argentine ants and fire ants, and peppermint and tea tree oils demonstrated mild toxicity to Argentine ants. These essential oils provide an alternative treatment method for situations where the use of deterrent or toxic natural products is preferred over contact insecticides. Additionally, these oils may be used in conjunction with other methods to provide additional protection.

Triolen, a component of the red imported fire ant brood recognition pheromone enhanced removal of treated granules by *S. invicta* workers. At a rate of 0.06% fipronil plus triolein, there was 90.5% mortality in laboratory colonies, versus 46.5% with the same rate of fipronil without triolein. This technique has potential to provide a more species specific ant control method and to alleviate the problem of variable food preferences that sometimes makes control of some species by baiting difficult. Areas for future studies include evaluation of various toxicants, identification of compounds that elicit handling behaviors in other species, and evaluation of species specificity.

Ground surveys conducted at two sites in northeastern MS provide the first description of imported fire ant distribution within blackland prairie habitats. We found mean fire ant mound densities of 53.6 and 54.5 mounds / ha at the two sites. Mounds tended to be smaller in more disturbed habitats. We found imported fire ants in open areas, with the highest infestation rates in disturbed habitats and no fire ant mounds being found in forest habitats or prairie containing a large proportion of mature trees. Infested and unifested plots did not differ in proximity to disturbed habitats. In addition to contributing to our knowledge of imported fire ant distribution in natural habitats, the results of this study have practical implications for habitat conservation and restoration. With this new information about which habitats within blackland prairies have been invaded by imported fire ants, restoration groups can target these specific areas for fire ant control and native arthropod conservation. Wooded areas are currently being removed from prairie sites undergoing restoration, creating potential imported fire ant habitats and increasing the importance of targeted management practices.

APPENDIX A

METHODS USED FOR GIS ANALYSIS IN IMPORTED FIRE ANT LANDSCAPE ECOLOGY STUDY

I. Plot site selection – conducted in ArcView 3.2

a. Imagery:

USGS DOQQ images (MrSid format) were obtained for each of the sites from www.geocom.com - 3388e6c.sid – West Point (Osborn Prairie) and 3388c5c.sid – Crawford East (Crawford Prairie).

Projection: Mississippi transverse Mercator (MSTM)

Scale Factor: 0.9998335

Central Meridian: -89° 45’ 00”

Central Latitude: 32° 30’ 00”

False Northing: 1,300,000 m

False Easting: 500,000 m

Projection Units: meters

Spheroid: GRS80

Datum: NAD83

b. Site selection

i. To import MrSID images into ARcView 3.2, downloaded the file AVMrSID.dll from Lizardtech.com to the ArcView/Bin32 directory. This corrects problems with georeferencing and incorrect placement of .sid and the associated .sdw file.

ii. Extensions used in ArcView 3.2: Graticules & measured grids, MrSid support, Projection utility wizard, Spatial Analyst.

iii. In new View for each site, set View Properties with custom projection from image metadata listed above.

iv. Created plot locations point themes over MrSide images. Each site had ~ 50 plots, modified from a 100 m grid to include a single land cover type

v. Unprojected the point shapefiles into geographic coordinate system (decimal degrees) using Projection Utility extension. Saved unprojected output as new shapefiles.

vi. Added unprojected point themes to empty, unprojected view.

vii. Obtained coordinates using addxycoo.ave script (sample script included with ArcView). This script returns latitude and longitude coordinates to the theme attribute tables.

viii. Added MrSid images to the views containing the unprojected plot shapefiles.

ix. Set projection based on the MrSid metadata listed above.

- c. Coordinates from the attribute tables (.dbf files) were uploaded to the GPS for navigation to the plots. Final sites were selected and logged in the field. Some plots were moved because of changes that occurred at the sites since the MrSid images were acquired.

II. Land cover classification – Conducted in Idrisi Kilimanjaro

- a. Imagery: for each site, two 3-layer .bmp images (Red-green-blue and near infrared-red-green) were obtained from the vendor (Geodata Airborne Mapping and Measurement, Macon, MS). Projection: UTM16N.
- b. Imported .bmp files using BMPIDRIS. Output: 3-band .rst files
- c. Decomposited .rst files into bands using GENERIC RASTER, resulting in 4 unique images for each site, fom the blue, green, red, and near-infrared bands. Set layer properties for raster files to UTM16N.
- d. In Idrisi Database Workshop, imported .dbf file cobntaining actual plot locations (from GPS) and created a point vector file in geographic coordinates. Projected vector files into UTM16N.
- e. Composed map from image (raster) and plot (vector) files.
- f. Supervised classification: After running several training site selection processes and supervised and unsupervised classifiers, found that the results in closest agreement with ground truth were obtained with the following methods:
  - i. Training site creation:
    1. Categories: bare ground, short grass, tall grass, small trees and shrubs, mature trees, chalk outcrop.
    2. Digitized polygons of a pure land cover type.

        3. Created signature files for each of the 6 land cover types, inputting land cover polygons and the image files (4 image files per site) into MAKESIG.
    ii. Clasification: Maximum likelihood classifier (MAXLIKE), using the signature files created above, equal prior probabilities, and classifying all pixels. Output: classified raster image.

III. Prairie plot composition analysis – conducted in ArcView 3.2

a. Adding land cover classification from Idrisi:
    i. In Idrisi, export to ArcInfo raster binary format.
    ii. In ArcView, Import data source – binary raster, add to View.

b. Prairie patch selection
    i. Installed the utility "Grid PIG Tools", downloaded from www.esri.com
    ii. Used Grid PIG Tools to truncate the classified image (Pixels in the imported classified images have real number values. Truncating converts these values to integers, thus creating six discrete values, each of which corresponds to a land cover type.)
    iii. Selected prairie patches from truncated image
        1. Drew a polygon around a patch that contained primarily grasses, small trees, and shrubs ("prairie").
        2. With graphic selected, set analysis extent to intersection of inputs and analysis cell size to current value.
        3. Used Grid PIG Tools to extract grid from graphic and truncate the extracted patch

4. Converted the patches to grids
5. Obtained pixel counts for each land cover in each prairie patch from its theme attribute table. Pixel counts were used to calculate areas and percentages of the patch occupied by each land cover type.

APPENDIX B

GEOGRAPHIC COORDINATES OF PRAIRIE SURVEY PLOTS

Site	Latitude (Decimal degrees)	Longitude (Decimal degrees)
Crawford Prairie	33.29848	-88.60896
Crawford Prairie	33.29853	-88.60981
Crawford Prairie	33.29875	-88.61111
Crawford Prairie	33.29915	-88.60903
Crawford Prairie	33.29916	-88.61154
Crawford Prairie	33.29916	-88.61050
Crawford Prairie	33.29938	-88.61175
Crawford Prairie	33.29939	-88.61074
Crawford Prairie	33.29947	-88.61011
Crawford Prairie	33.29960	-88.60954
Crawford Prairie	33.29973	-88.61206
Crawford Prairie	33.29995	-88.60990
Crawford Prairie	33.30001	-88.61151
Crawford Prairie	33.30001	-88.60934
Crawford Prairie	33.30003	-88.61035
Crawford Prairie	33.30006	-88.61238
Crawford Prairie	33.30013	-88.61096
Crawford Prairie	33.30022	-88.60957

Site	Latitude (Decimal degrees)	Longitude (Decimal degrees)
Crawford Prairie	33.30038	-88.61273
Crawford Prairie	33.30048	-88.61145
Crawford Prairie	33.30056	-88.60935
Crawford Prairie	33.30064	-88.60731
Crawford Prairie	33.30065	-88.60557
Crawford Prairie	33.30066	-88.61031
Crawford Prairie	33.30067	-88.60648
Crawford Prairie	33.30070	-88.60469
Crawford Prairie	33.30079	-88.61335
Crawford Prairie	33.30082	-88.61249
Crawford Prairie	33.30082	-88.61147
Crawford Prairie	33.30083	-88.61250
Crawford Prairie	33.30094	-88.61128
Crawford Prairie	33.30111	-88.61170
Crawford Prairie	33.30114	-88.61336
Crawford Prairie	33.30125	-88.61239
Crawford Prairie	33.30133	-88.60671
Crawford Prairie	33.30134	-88.60737
Crawford Prairie	33.30135	-88.60830
Crawford Prairie	33.30135	-88.60570
Crawford Prairie	33.30136	-88.60934

Site	Latitude (Decimal degrees)	Longitude (Decimal degrees)
Crawford Prairie	33.30137	-88.60519
Crawford Prairie	33.30146	-88.61345
Crawford Prairie	33.30148	-88.61267
Crawford Prairie	33.30151	-88.61302
Crawford Prairie	33.30151	-88.61037
Crawford Prairie	33.30161	-88.61154
Osborn Prairie	33.50929	-88.74082
Osborn Prairie	33.50932	-88.73554
Osborn Prairie	33.50933	-88.73750
Osborn Prairie	33.50933	-88.73432
Osborn Prairie	33.50934	-88.73868
Osborn Prairie	33.50935	-88.73971
Osborn Prairie	33.50946	-88.73656
Osborn Prairie	33.51012	-88.73438
Osborn Prairie	33.51015	-88.73649
Osborn Prairie	33.51017	-88.73533
Osborn Prairie	33.51019	-88.73873
Osborn Prairie	33.51022	-88.73761
Osborn Prairie	33.51030	-88.73973
Osborn Prairie	33.51034	-88.74084
Osborn Prairie	33.51097	-88.73429
Osborn Prairie	33.51103	-88.73878

Site	Latitude (Decimal degrees)	Longitude (Decimal degrees)
Osborn Prairie	33.51116	-88.73967
Osborn Prairie	33.51117	-88.73750
Osborn Prairie	33.51118	-88.74081
Osborn Prairie	33.51118	-88.73665
Osborn Prairie	33.51119	-88.73537
Osborn Prairie	33.51195	-88.73654
Osborn Prairie	33.51197	-88.73866
Osborn Prairie	33.51197	-88.73530
Osborn Prairie	33.51198	-88.74084
Osborn Prairie	33.51199	-88.73745
Osborn Prairie	33.51199	-88.73433
Osborn Prairie	33.51200	-88.73967
Osborn Prairie	33.51283	-88.73753
Osborn Prairie	33.51284	-88.73652
Osborn Prairie	33.51285	-88.73529
Osborn Prairie	33.51285	-88.73436
Osborn Prairie	33.51297	-88.74080
Osborn Prairie	33.51300	-88.73968
Osborn Prairie	33.51302	-88.73853
Osborn Prairie	33.51355	-88.74054
Osborn Prairie	33.51382	-88.73432
Osborn Prairie	33.51384	-88.73970

Site	Latitude (Decimal degrees)	Longitude (Decimal degrees)
Osborn Prairie	33.51384	-88.73867
Osborn Prairie	33.51388	-88.73751
Osborn Prairie	33.51391	-88.73657
Osborn Prairie	33.51399	-88.73552
Osborn Prairie	33.51460	-88.73425
Osborn Prairie	33.51466	-88.73870
Osborn Prairie	33.51467	-88.74022
Osborn Prairie	33.51467	-88.73750
Osborn Prairie	33.51468	-88.73966
Osborn Prairie	33.51481	-88.73531
Osborn Prairie	33.51482	-88.73632