Investigating Termite Behavior and Application Methods of Non-repellent Termiticides for the Control of Eastern Subterranean Termites (Isoptera: Rhinotermitidae)

by

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Abstract

The effects of three non-repellent termiticides were evaluated in the laboratory against the eastern subterranean termites, *Reticulitermes flavipes* Kollar, to determine their efficiency in controlling this species. Treating above-ground tunnels and soil treatment were used to evaluate the termiticides. The three termiticides that were used in this study include dry ready-to-use (RTU) Termidor (active ingredient: fipronil 0.5%), Altriset (active ingredient: chlorantraniliprole 18.4%), and Termidor H.E. Termiticide Copack (active ingredient: fipronil 9.1%).

The non-repellent termiticide (Dry RTU Termidor) caused a decreased in termite population movement and 100% mortality at day 5 and 7 for the 0.30 and 0.15 mg dose treatments, respectively. Termites constructed significantly fewer tunnels post-treatment compared to control termites; this provided strong evidence that locally treating a single tunnel with dry RTU fipronil near feeding sites was effective for the control of termite group population.

Altriset caused 100% termite mortality in 19 days post-treatment at 100 and 50 µg/g and 27% termite mortality at 25 µg/g when treating the soil contiguously to established foraging tunnels at a fixed 1m distance. When testing the distance effect of the soil treatment (2m and 4m) to satellite termite populations at a fixed 50 µg/g concentration, Altriset caused 100% termite mortality in 22 days post-treatment at both 2m and 4m. Finally when assessing the effect of differing application methods using 12.5 and 25 µg/g prior to the establishment of foraging tunnels at a fixed 1m distance, Altriset caused 100% mortality in 9 days post-treatment at 25 µg/g and 12 days post-treatment at 12.5 µg/g. Results amend label information on treatment concentration, distance and application methods of Altriset.

Termidor H.E. was utilized to evaluate the interaction between termite-fipronil and termite-termite at the colony level using localized soil application. One ppm a.i. of the product caused control in of all the tested colonies of eastern subterranean termite in 50 days. Accordingly, exposed termites perished at the nest site where they received intensive grooming from active colony mates rather than in perish in the treated area or nearby adjacent tunnels. The presence of exposed termites neither repelled nor deterred the surrounding active colony mates. Similar results have been noted.

The current work has provided a detailed understanding of the effectiveness of treating mud tunnels and soil using powdered and liquid formulations against eastern subterranean termites. The approach of localized treatment is an important step in reducing termiticide exposure to humans and the environment. It is critical for termite control methods to be continuously researched and to reflect newer, more advanced technologies.

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CHAPTER 1

GENERAL INTRODUCTION

Termites are eusocial insects belonging to the isopteran order. Globally there are over 2,600 described termite species within seven families and approximately 280 genera ((Kambhampati and Eggleton 2000, Engel and Krishna 2004). However, only 183 species known worldwide to attack buildings, with 83 of these species causing significant damage (Su and Scheffrahn 2000). Termites are a major structural pest capable of forming large colonies and constructing an intricate network of tunnels when foraging for wood in search for cellulose, which is their main food source. In the United States, economic loss due to termites is estimated at $11 billion (Su 2002). Rhinotermidae, the most economically important family, has an economic value of $3 billion per annum (Su 2002).

Subterranean termites have adapted to live in many different geographic regions which include tropical, sub-tropical, and temperate regions of the world (Von Hagen 1942, Hickin 1971). The most important termite species in the United States include: *Coptotermes formosanus* Shiraki, *Reticulitermes flavipes* Kollar, *Reticulitermes hesperus* (Banks), *Reticulitermes virginicus* (Banks), and *Reticulitermes tibialis* (Banks) (Kofoid 1934, Weesner 1965, Edwards and Mill 1986). *Reticulitermes* species are distributed widely in North America and can be found throughout the southeastern United States, north along the Atlantic coast into Maine, and west along the southern shores of the Great Lakes (Weesner 1970). The eastern subterranean termite, *R. flavipes*, and the Formosan subterranean termite, *C. formosanus* cause the most damage in the

United States (Su and Scheffrahn 1990). The wider geographical distribution of the eastern subterranean termite, however, makes it the single most economically important termite species in the United States (Su 1996).

Evolution of Termites

The termites (order: Isoptera), along with mantids (order: Mantodea), and cockroaches (order: Blatteria) form the most basal group of winged insects referred to as the Dictyoptera (Deitz et al 2003). The isopterans which are members of the orthopteroid group (Hennig 1981), are thought to share a common ancestor with the Blattaria. This is based on the proventriculus and female genitalia of Blattaria that are morphologically similar to the isopteran family, Mastotermidae (McKittick 1965). The most primitive living termite, *Mastotermes darwinensis* (Froggatt), has a similar wing structure to cockroaches and females of this termite species lay their eggs in an ootheca (Snyder 1948). The primitive cockroach, *Cryptocerus punctulatus* (Scudder), which burrows into and consumes decaying wood utilizing gut protozoa similar to those in termites (Guthrie and Tindall 1968). These similarities suggest that the order Isoptera diverged from the ancient ancestral cockroach lineage ~200 million years ago (Nalepa and Bandi 2000).

The families of termites are further phylogenetically classified into either higher or lower termites (Krishna and Weesner 1970). The lower termite families (Mastotermitidae, Termopsidae, Hodotermitidae, Kalotermitidae, Serritermitidae, and Rhinotermitidae) (Kambhampati and Eggleton 2000) are distinguished from the higher termites by a presence of symbiotic protozoa in their hindgut for cellulose digestion (Bignell 2000); whereas the higher termite families (Termitidae) (Kambhampati and Eggleton 2000) have developed other mechanisms for digesting cellulose without the help of protozoa (e.g. symbiotic fungi and higher endogenous cellulase production levels) (Breznak and Brune 1994). Evidences from phylogenetic analyses (Austin et al 2004, Inward et al 2007, Lo et al 2004) suggests that the parent group of two genera, *Reticulitermes* and *Coptotermes*, is possibly a sister group of Termitidae, the higher termite. Members of these two genera exhibited features that are intermediate between higher and lower termites. These features include characteristics of lower termite feeding habits and nesting habits which are between lower termites single site nesting (in wood) and higher termites central nesting site in order to access multiple sources of food (Shellman-Reeve 1997).

Termites are eusocial insects which is characterized by groups who possess the following main characteristics: (1) a complex, usually easily defended, nest, (2) communication of some kind among colony-mates (i.e. alarm and colony defense and recruitment to food), (3) cooperation in caring for brood, (4) reproductive division of labor, (5) overlap of generations, and (6) decentralized decision making (Gullan and Cranston 2005). The evolution of eusocial behavior theoretically followed a path from solitary individuals, to one or more different levels of pre-social groups (e.g sub-social, para-social) to finally eusocial colonies (Wilson 1971). Hamilton’s (1963, 1964, 1972) kin selection theory predicted that eusociality develops as the relatedness between altruist and beneficiary becomes higher than the ratio of costs to benefits. Hymenoptera have a haplo-diploid system for sex determination while termites are diplo-diploid thus Hymenopteran females are more related to their siblings than to their own offspring (Crozier and Pamilo 1996). This type of relatedness leads to an increase in their inclusive fitness when raising female siblings and not their own offspring (Hartl and Brown 1970). Diploid termites lack this characteristic. The fact that termites are not haplo-diploid and have still developed eusociality has puzzled scientists, indicating that some additional factors may be significant in the evolution of eusociality in termites (Thorne 1997).

Termite Life Cycle and Biology

The establishment of a new colony usually occurs when winged reproductive termites disperse, often at the start of the rainy season (Snyder 1915). Both male and female winged adults typically disperse simultaneously and pair off. After pairing with a member of the opposite sex, the royal pair sheds their wings, locates a suitable nesting location, excavates a nuptial chamber, mates, and begins brood rearing. The newly mated queen lays eggs which develop into workers that tend the colony, build nest structures, and forage for food. As the colony develops, soldiers are produced to defend the colony. When the colony reaches maturity alates are produced and the cycle continues.

The life cycle of a termite is complex. Upon hatching, immatures develop through several undifferentiated instars (Thorne 1998). These white immatures are nutritionally dependent on their older nest-mates. The immatures then develop into individuals that belong to one of several distinct morphological or developmental lines (soldier, worker, and reproductive) (Thorne 1998). Each individual (worker, soldier, and reproductive) is determined during post-embryonic development (Laine and Wright 2003) depending on the variety of pheromones present in the colony (Suiter et al 2002) and each individual has its own task.

In rhinotermitidae, the immature develops into another type of morphotype named “pseudergates” that includes non-reproductive individuals that revert from the reproductive into worker-like individuals (Thorne 1996). Pseudergates retain the brain and reproductive structures of reproductives yet lack the wing-buds of their counterparts (Thorne 1996). Another distinct developmental characteristic of rhinotermitidae colonies is the existence of at least three different types of reproductives (Thorne 1998). These include the primary reproductives usually the original founding king and queen and the two additional forms of secondary or neotenic reproductives (Thorne 1998). The two forms include the “brachypterous” and “apterous” neotenics (Thorne 1998). Brachypterous neotenics originate from the reproductive line but they do not fully develop into alates. Instead they only develop wing-buds before becoming reproductively active within their own natal colony. The apterous neotenics are derived from the worker line with no wing-buds and they remain in the natal colony. Since both types remain in the original colony the nymphs are sometimes referred to as “supplemental reproductives” in the case of the death of a primary reproductive (Thorne 1998).

Each caste can be visually differentiated by several key traits. Workers are typically unpigmented, white, lack eyes, and show no signs of wing development. Soldiers have an enlarged sclerotized head capsule and mandibles to aid in colony defense. Reproductive caste: the primary reproductives (king and queen) are alate derived with evidence of wings, have fully developed eyes, and are fully pigmented. Neotenic reproductive's lack wing development, compound eyes, and are not uniformly pigmented. Nymphoid reproductives are developed from nymphs, and are therefore characterized by the presence of small wing buds. Ergatoid reproductive’s are developed from workers and lack wing buds (Suiter et al 2002, Thorne 1996).

Subterranean termites are most commonly found within the soil, thriving in an environment of high humidity and darkness. Like most termites, subterranean termites survive primarily on cellulosic materials such as wood, roots, and grasses (Waller and LaFage 1987, Tayasu et al 1997). Worker termites transfer nutrients to immatures, soldiers, and reproductives via stomodeal and proctodeal trophallaxis. Subterranean termites typically live in large numbers that can range from 50,000 to several million individuals in a colony (Su et al 1993). Eastern subterranean termites consume numerous species of wood including slash pine, loblolly pine, and sugar maple (Smythe and Carter 1970).

Termite Social Organization

Subterranean termite members within the colony are divided into castes, each of which has a specialized function within the colony. The reproductive caste, consisting of primary reproductives and secondary reproductives, carry out tasks of reproduction and species distribution. The soldier caste is responsible for nest and colony defense. Termites in the worker caste carry out the majority of tasks, such as building and repairing the nest and tending the termite larvae and reproductives. The needs of the colony determine what individuals will become workers, soldiers, or reproductives. When there is a suitable balance of these three basic castes, a healthy, productive, efficient colony can result (Thorne 1999).

Newly hatched larvae are able to develop into any caste in Rhinotermitid termites, but the persistence of this developmental plasticity varies between different species of termite (Krishna 1969). The earliest instar termites are often referred to as a larvae. These larvae, also known as white immatures, are defined as having no significant cuticular sclerotization (Thorne 1996) and they are dependent on a liquid diet provided by the workers (McMahan 1969). As the termite larva molts and matures, the termite’s exoskeleton changes from white to a light tan. This change is most evident in the head capsule. Third instar workers are referred to as ‘true workers’ if there is no divergence to a soldier or reproductive developmental line (Noirot and Pasteels 1988). Soldiers comprise a low percentage of individuals in the colony, approximately 1-2% in

R. flavipes (Howard and Haverty 1980, 1981, Haverty and Howard 1981, Grace 1986, Thorne et al 1997). Therefore, the majority of the termites in the colony are workers (Kofoid 1934).

The division of labor within the colony, in which castes perform specialized tasks, is unique to social insects and allows the colony to function efficiently to ensure its survival and growth. Workers are responsible for tending the brood, maintaining and repairing the nest, foraging for food (Krishna 1969), grooming all castes, and feeding other castes through trophallaxis. Termites are either groomed by other termites (allogrooming) or do so themselves (autogrooming). Allogrooming involves rhythmic lateral movements of the mandibles while the head capsule remains in contact with various body parts of another termite (Whitman and Forschler 2007). Trophallaxis in termites involves the exchange of food and information either by mouth to mouth (stomodeal) or mouth to anus (proctodeal) contact (Wheeler 1918, Wilson 1975, Sleigh 2002). Termites either feed on food donated via the mouth or anus of nestmates (allofeeding) or directly on the food source (autofeeding). In autofeeding, termites feed on clear and viscous regurgitated materials that result from allogrooming activities (Whitman and Forschler 2007). Soldiers and larvae are almost entirely dependent on workers for hydration and nutrients. Workers also reduce the chances of bacterial and fungal growth within the colony and construct tunnels within the soil and mud tubes above the soil.

The reproductive caste consists of primary and secondary reproductives (Lee and Wood 1971, Thorne 1999). Primary reproductives play a major role in dispersal as alates and founding colonies, excavation of the first galleries, and feeding and care of the first young (Light 1934). The primary reproductives consist of males (kings) and females (queens), which are highly sclerotized, pigmented, have compound eyes, and develop from winged adults (Krishna 1969). Colony size and maturity are central to determining the production of winged primary reproductives, or alates (Nutting 1969). The secondary reproductives act as substitutes for the king or queen if one or both should die, or supplement the egg production of the queen after the subterranean termite colony is established (Lee and Wood 1971, Potter 2004). In *Reticulitermes* spp., secondary reproductives also help to expand the foraging territory of the colony (Forschler 1999). All of the offspring in the colony are produced by either primary or secondary reproductives.

Soldier termites are more highly specialized than are the workers. The soldier caste is traditionally considered the defensive caste (Wheeler 1928, Kofoid 1934). *Reticulitermes* spp. soldiers have a distinctively modified head with elongated mandibles. Because of their mandibles, soldiers cannot chew wood and are entirely dependent on the worker termites for food (Traniello et al 1985, Su and LaFage 1987). Soldier termites develop from a pre-soldier stage that develops from a larva or worker (Lee and Wood 1971). Soldier termites may act aggressively toward competitors, predatory ants, and even other termites. Methods of defense range from mechanical methods to the complete reliance on chemical methods. Mechanical defense include phragmosis, mandibular biting, mandibular snapping, autothysis, and abdominal dehiscence. Termites that use phragmosis typically have cylindrically-shaped heads to use as stoppers to plug exits and thus prevent entry of ants and other intruders into the termite nest. Mandibular defense of a termite colony is achieved either by snapping or biting intruders. Autothysis defense involves explosive release of fluid from the labial gland reservoir through weaknesses in abdominal or thoracic walls. Abdominal dehiscence defense involves explosive defecation through an abdominal rupture occurring under extreme pressure during defecation by soldiers or workers (Mill 1982). Chemical defense involves the release of chemicals such as greases, irritants, contact poisons or glues from frontal, salivary or cibarial glands (Prestwich 1984).

Ecology

Termite feeding habits have shown that the food gathered by a worker is the basic energy source of the colony (Lee and Wood 1971). It consists of living or dead plant material that is either partially or almost entirely decomposed (Lee and Wood 1971). Subterranean termites may feed on a wide variety of food including sound wood, decaying wood, parts of living trees and shrubs, plants, books, cardboard, and paper. The major nutritional ingredient in all of these food sources is cellulose (Noirot and Noirot-Timothee 1969). Cellulose is a carbohydrate continuously produced by plants and one of the most commonly encountered organic compounds on earth (Light 1934). Subterranean termites may also chew through non-nutritive materials such as foam insulation (Gyuette 1994, Smith and Zungoli 1995, Ogg 1997), plastic, rubber products (Sternlicht 1977), plaster, and stucco (Potter 2004).

Termite castes (soldiers, nymphs, and reproductives) are unable to feed themselves and are fed via trophallaxis from the workers. Trophallaxis is the process in which termites within the colony mutually exchange food and nutrients either by regurgitating or anally excreting it in order to replace the symbiotic microbiota in the hindgut of termites that have recently molted (Snyder 1948). Lower termites depend on protozoans for cellulose digestion, a function performed by bacteria in higher termites (LaFage and Nutting 1978).

Subterranean termites are xylophagous and forage for cellulose containing materials, mainly wood. Termites have endogenous enzymes, a characteristic that has been identified in both higher and lower termites (Upadhyaya et al 2012, Watanabe et al 1998). Many of these enzymes perform essential functions for the termite. In return, the termite gut environment provides the enzymes with an appropriate environment and a steady supply of nutrients.

Termites depend on bacteria (Proteobacteria, Spirochaetes, Bacteroidetes, Firmicutes, Actinobacteria, and Endomicrobia) and protozoans (the Parabasalia which includes hypermastigid and trichomonad flagellates and the Oxymonadida) (Fisher et al 2007, Ohtoko et al 2000, Yamin 1979) for cellulose and hemicellulose digestion (Smith and Koehler 2007) and reduction of these compounds to simple sugars that can be used in energy production. Termites attack the softer springwood, usually leaving the less digestible summerwood along the grain, intact. Excavated galleries usually contain brownish specs of fecal material (Potter 2004). Unlike other wood infesting insects, subterranean termites do not eject pellets, powder, or sawdust from their feeding galleries (Potter 2004).

Subterranean termites usually require some kind of contact with the ground to build nests and tunnels. Subterranean termites construct both underground tunnels and above ground mud-tubes in search for food. In moist sand, subterranean termites construct the tunnel network by pushing their heads forward through the moist sand, then pressing the sand grains from side to side with their head, body, or mandibles (Ebeling and Pence 1957). The smaller grains of sand are taken into the buccal cavity (Ebeling and Pence 1957) to be combined with saliva and feces (Noirot 1970) and cemented to the wall of the tunnel to make a smooth and hard surface. Above ground mud-tubes are constructed during the search for food and after an adequate food source has been located. Termites carry sand grains to the surface and construct above ground mud-tubes. Above ground foraging begins with the movement of termites on the surface of the soil, trees, buildings, or other structures. As the termites search, they may find nearly any type of cellulosic material to be an adequate food source, including wood structures (Ebeling 1975). In the process of searching above ground, the termites leave a faint pheromone trail (Stuart 1969, Runcie 1986). Once a food source has been located, the chemical trail is reinforced causing other termites to be recruited to the food source (Thorne 1996). The completed mud-tube protects the termites from predators and desiccation (Potter 2004). As the colony grows and searches for food, the tunnel network increases in size.

History of Termite Control

The subterranean termite's cryptic life cycle makes control difficult (Su and Scheffrahn 1998).The history of control strategies for subterranean termites includes the use of wood preservatives, physical barriers, application of liquid termiticide, baits, and the application of dust and foam termiticide formulation. Wood preservatives include Copper naphthenate and borates, which was first used as a wood preservative in Germany in 1889, with commercial use starting in 1911. Borates are inorganic minerals mined from naturally formed deposits in the earth and are toxic to many species of wood destroying insects and fungi. These compounds maintain their preservative properties for extended periods when they are not rewetted constantly (Potter 2004).

Physical barriers include stainless steel wire mesh and particulate materials such as sand, granite and basalt. Stainless-steel wire meshes such as Termi-Mesh® was developed and patented in Australia and is a flexible, corrosion-resistant, stainless steel mesh that has performed creditably in field trials (Lenz and Runko 1994, Grace et al 1996, Kard 1999). It has an aperture size of 0.66 x 0.45 mm that is small enough to prevent entry of all economically important species of subterranean termites (Potter 2004). Installation is done pre-construction beneath entire concrete slabs but usually limited to utility penetrations, expansion joints, and sometimes the perimeters of slabs due to cost considerations. The product is popular in Australia and increasingly so in Hawaii and Texas (Potter 2004). Particulate material such as sand, granite, and basalt used to protect structures is based on the observed the inability of termites to tunnel through sand of a certain particle size (Ebeling and Pence, 1957). To be good physical barrier, sand particles must be small enough to prevent termites from wiggling through, but larger than the dimensions of the mandibles and other mouthparts involved in soil/sand excavation. Su and Scheffrahn (1992) reported that soil particles within the 2.0 to 2.8 mm size range meet these requirements and are thus effective physical barriers to termite penetration of structures.

The use of chemical applications as a means of eliminating subterranean termites from structures was suggested as early as the late 1800s, but the actual evaluation of potential chemicals did not get underway until the 1940s (Aventis Environmental Science 2003). Cyclodienes, a class of chemical compounds identified as highly effective termiticides, became commercially available (Ware 2000). Soil treatments with cyclodienes became the standard method of subterranean termite prevention from the late 1940s until 1988 (Lewis 1980, Su and Scheffrahn 1990). The cyclodienes, particularly chlordane, were extremely efficacious and stable in soil, often protecting structures from subterranean termite infestation for several decades (Grace et al 1993, Lenz et al 1990, Su and Scheffrahn 1990). For instance, the chlorinated hydrocarbons (i.e. chlordane, heptachlor, endrin, aldrin, and dieldrin) function by repelling worker termites from tunneling toward the foundation of structures and were very effective with proper application and a stable environment (Su and Scheffrahn 1990). Because of their residual longevity, questions were raised about the environmental impact of these chemicals (Lewis 1980, Su and Scheffrahn 1990, Wood and Pierce 1991). Environmental persistence and public health concerns led to their withdrawal from the market in 1988, an action that necessitated a shift to organophosphates (e.g., chlorpyrifos) and pyrethroids (e.g., permethrin, cypermethrin, bifenthrin, and fenvalerate).

Organophosphates are less persistent in the environment and were widely accepted, although they were more toxic to vertebrates than chlorinated hydrocarbons which led to their ban by the EPA in 2000. With the loss of organophosphates, pyrethroids were the primary termiticide available for subterranean termite prevention. Pyrethroid termiticides have a relatively long residual life, effective at low concentrations, and a low acute mammalian toxicity (Potter 1998). Pyrethroids are repellent compounds and are used to deter foraging workers away from a treated structure (Su et al 1982). Remedial control with repellent compounds was difficult due to the ability of termites to detect, seal off, or otherwise avoid the treated sections of the colony (Su et al 1982). Furthermore, the environmental toxicity and harmful effects of repellent compounds on non-target organisms are apparent (Silver and Soderlund 2005, McCann et al 2001), therefore, alternative compounds that were effective at low use rates were needed. After 1990, several new non-repellent soil termiticides appeared on the market: imidacloprid (Premise), fipronil (Termidor), chlorfenapyr (Phantom), indoxacarb (Aperion), and chlorantraniliprole (Altriset). Non-repellent termiticides are an improvement over the pyrethroids because subterranean termites cannot detect gaps in the treatment and use them to gain access to structures (Potter and Hillery 2001). While there is some evidence of colony suppression or elimination following perimeter treatments with non-repellent termiticide (Parman and Vargo, 2010), other studies have shown that a reduction in activity occurs over only a small portion of a colony’s foraging range, making it unlikely that soil treatments affect the overall termite population (Osbrink et al 2005, Rust and Saran 2006, Su 2005).

Non-repellent termiticides are toxic but usually slow-acting compounds that can be applied as liquid treatments. Application of liquid termiticides involves trenching around the perimeter of a structure and/or drilling holes at regular intervals into the foundation block and slabs (Rambo 1985). As termites tunnel into treated soil, they come in contact with the termiticide and pick up the lethal dose and become intoxicated which leads to death. Even though liquid termiticides have been shown to suppress termite pest populations, they have several limitations. For example, liquid applications are limited by the type of soil found near the structure. Sandy soils accept termiticide solutions readily, but depending on the particle size of the sand, the termiticide may not spread evenly beneath the soil to give a continuous barrier (Rambo 1985). The behavior of liquid termiticides in clay soils also varies with its consistency. Many clay soils are too compacted to receive termiticides because organic matter interferes with the distribution of the termiticide in the soil and reduces the chances of achieving a continuous barrier in the soil (Rambo 1985). Finally, the active ingredients in liquid termiticides degrade in the soil over time and eventually have to be reapplied (Mauldin et al 1987).

Although liquid applications are considered the primary method of termite control in the pest management industry, bait systems are widely used today as well (Kistner and Sbragia 2001). The bait is installed in the ground surrounding the structure. Its technology consists of a toxicant formulated into a cellulose matrix substrate that targets termites directly without releasing large quantities of termiticide into the environment, as is the case with liquid termiticides. The bait system, Sentricon® Termite Elimination System was developed by Dow AgroSciences in 1990 with hexaflumuron, a chitin synthase inhibitor as the active ingredient. Noviflumuron replaced hexaflumuron in later versions of the bait, including the latest, Recruit IV termite bait. Since the introduction of the Sentricon® System, other bait systems have been designed and marketed. Diflubenzuron, another chitin synthase inhibitor, is the active ingredient in Exterra®. A third widely used bait system was created by FMC in 1996, the Firstline® Termite Bait System. This system contains the slow acting stomach toxicant sulfluramid. Unlike the hexaflumuron, noviflumuron, and diflubenzuron, sulfluramid works by disrupting energy metabolism in termites (Valles and Koehler 1997). Bait systems are a useful method of subterranean termite control, but they also have limitations. The performance of baits can be compromised by the presence of competing food sources. In a natural environment, other food sources may compete with the baits, and foraging termites may be divided among a number of food sources near the bait.

Insecticidal dusts have been used against termites for decades (Madden et al 2000). Currently, there is an interest in the use of dust toxicants to suppress/eliminate termite colonies. While the chemical termite barrier is effective in isolating termite activity from structures, the termite colony remains active outside the treated zone (Su and Scheffrahn 1988). The utilization of dust toxicants makes use of termite social behavior, and therefore, does not require determining the location of the nest as termites will distribute the toxicant to other colony members through grooming, contact, cannibalism, and trophallaxis (Ahmed 2000). The use of dusts also allows for reduced amounts of insecticide required to treat a termite colony, and therefore reduces the environmental hazard when compared with some other treatment methods (Ahmed 2000).

Foam formulation is another termiticide that reduces the risk of contaminating underground water and causing environmental hazards. The foam formulation works by injecting the formulation above ground into the walls of a structure infested with termites or other undesirable pests. The inner spaces of the affected walls are flooded at and above ground level to ensure that the foam formulation reaches all passages open to the soil under the structure and establishes an above-ground layer of pest control chemicals. The foam penetrates all porous material exposed to the injection and impregnates it with active ingredients that remain embedded in the material long after the foam disappears; thereby retaining the properties of the active agents for a long time without exposure to normal degrading forces (Magnuson-Hawkins 1998). Foam is good for small spaces where liquid can't cover all infested areas. It is especially ideal for houses that have settled and left gaps where termites can accumulate.

Factors Effecting Termite Control

The control of structural infestations of subterranean termites depends upon the consideration of a variety of factors including soil properties such as moisture, mobility, clay content, pH, and organic matter content. Environmental factors include temperature, application rate and application methods, termiticide selection, and distance.

Mobility is one of the most important factors in determining the efficacy of a soil treatment. If a termiticide is too mobile, it fails to protect the structure while increasing risk of groundwater contamination. However, if the chemical is too tightly bound to soil particles, bioavailability is limited. Mobility is affected by the termiticide's sorption, water solubility, and external influences that include soil properties. Sorption describes the attraction between a chemical and soil, vegetation, or other surfaces (Alley 1993). Soil texture has a strong impact on termiticide performance as well. In assays conducted with bifenthrin, chlorfenapyr, and fipronil, *C. formosanus*, mortality was generally highest when clay content was low (Wiltz 2010). Clay content of soil was significantly related to termite mortality across all termiticides, rates, and exposure times. Likewise, Osbrink and Lax (2002) found that *C. formosanus* workers experienced greater mortality in fipronil-treated sand than in treated potting soil or a mixture of soil and clay. The pH of soil also effects termiticide performance. Low pH soils increase the adsorption of weakly acidic termiticide (Boivin et al 2005, Carrizosa et al 2000, Halfon et al 1996) compared to soil with neutral pH (6-8) (Kumar and Philip 2006). The partitioning of termiticides between organic matter and soil solution also affects termite control (Felsot and Lew 1989). Mulrooney et al (2007) applied fipronil and found that *R. flavipes* mortality decreased with increasing organic carbon. Henderson et al (1998) applied pyrethroids at low rates and found insecticide was less available in soils with high organic content.

Both biotic and abiotic pathways have also been found to be important for termiticide performance (Racke et al 1996). Moisture along with soil temperature affects termite control. Extreme moisture and temperatures may affect the chemical degradation of termiticides, resulting in a shorter residual effect, mainly in tropical climates (Reid et al 2002). Khan et al (1996) found that lindane adsorption to silty loam and silty clay loam soils increased with temperature. Higher temperature and moisture also intensify termite behaviors such as tunneling, foraging and feeding. This increases the chances of greater toxicant uptake and transfer. Furthermore, it might have a detrimental effect on the survival of termites (Spomer et al 2008).

Variations in application rate and methods can influence the availability, persistence, and penetrability of toxins. Within certain ranges of application rates, availability increases with rate. However, the opposite is true at other rates. Application rate affects both initial availability and degradation rate. Saran and Kamble (2008) reported the higher the concentration the greater the bioavailability. Finally, the application method also has an influence on the soil-termiticide interaction. This is done by trenching the soil and drenching the exposed section with termiticide or drilling into structural foundations and injecting the termiticide. These application methods create a termiticide barrier around structural perimeters and under foundations. This full-barrier treatment is disruptive to properties, labor-intensive, invasive to occupants, and requires the use of considerable amounts of termiticide. A possible alternative is the method of localized soil treatment using non-repellent termiticide. The benefits of using this method include a reduction of labor and the quantity of chemical application. A laboratory study investigated the efficacy of a dry, ready-to-use (RTU) fipronil against *Reticulitermes flavipes*' foraging activity and survival using localized treatment into foraging tunnels (Barwary et al 2013). The authors reported 100 % mortality and complete reduction in termite movement between day 5 and day 7. It also was reported that termites in the treated units constructed significantly fewer tunnels post-treatment compared to control termites. They concluded their results provided strong evidence for the efficacy of the dry RTU fipronil formulation against *R. flavipes* activity at the group level when a single tunnel was treated. Hickman and Forschler (2012) conducted a study to evaluate the efficacy of three ready-to-use (RTU) formulations: imidacloprid foam, a dry fipronil formulation, and an experimental fipronil formulation to treat cypress lumber naturally infested by the drywood termite *Incisitermes snyderi* (Light) using localized treatment. They reported that the dry RTU fipronil formulation and foam imidacloprid performed significantly better and provided evidence of elimination of infestation without removal of every board that was treated. The authors proposed that less than 100% elimination was due to the difficulty in treating all termite galleries and the short duration of the trial, which may not have allowed enough time for unexposed termites to contact treated galleries. However, for subterranean termites, the above-ground tunnels are obvious and can be accurately treated with termiticide. Under laboratory conditions localized treatments with commercial dust and liquid formulations of fipronil against Formosan subterranean termites resulted in mortality over 91% mortality within the 9 days. The authors concluded that localized (or spot) treatment with either commercially available dust or liquid formulations of fipronil can be a viable option for control of termite infestation where complete soil drenching is not desirable (Gautam et al 2013). Termiticide properties (i.e. solubility, degradation, and microbial degradation) also impact the ability of a compound to control termites and protect structure from infestation (Narahashi 2001). Different termiticides have different attributes and conditions for application (Pitts-Singer and Forschler 2000). Therefore, more research is needed to evaluate new and existing products under a larger range of conditions.

In addition to the above properties, subterranean termite foraging also affects the performance of termiticide. All foraging insects follow a hierarchy of behaviors when searching for food. Initially, an insect starts a broad search for a food. Once a likely foraging area is located, insects search within it for potential resources. When a nutritional resource is located, it must be examined and recognized as potentially edible. Finally, the food must be accepted and consumed (Matthews and Matthews 1978). For the Isoptera, the search for a food is truncated. First, the primary reproductives choose the initial nesting site at the peak of the nuptial flight. The foraging area for the colony is established based on the nest location, although the search for food may extend out many meters from that center (King and Spink 1969, Su and Scheffrahn 1988, Lys and Leuthhold 1991, Su et al 1993). Second, the search for food within the area consists of exploratory tunneling around the nest complex (Robson et al 1995, Reinhard et al 1997) and once the food is located and if accepted will be consumed. (Lys and Leuthhold 1991, Hedlund and Henderson 1999, Campora and Grace 2001). The forager will then lay a pheromone trail back to the nest. A primary gallery is then constructed around this recruitment trail (King and Spink 1969, Lys and Leuthhold 1991, Reinhard et al 1997). The search for food within the area is characterized as a non-random activity (Robson et al 1995, Reinhard et al 1997). Rhinotermitidae employ a strategy that minimizes redundant foraging (Robson et al 1995, Reinhard et al 1997, Hedlund and Henderson 1999). It has been shown in the laboratory setting that subterranean termites begin with a division of the foraging area around the starting point into equal units. Workers build long straight tunnels that radiate out in a star-like pattern from the point of origin, (where termites were introduced into the arena). The search radius continues to expand outward until food is found or a barrier is encountered. If food is located an expanded gallery or primary tunnel is constructed along the recruitment trail (Becker 1972, Robson et al 1995, Campora and Grace 2001, Cornelius and Osbrink 2001, Puche and Su 2001). After the food is depleted, exploration of the environment begins again with the current food resource as the new center of activity (Reinhard et al 1997, Campora and Grace 2001, Puche and Su 2001). By initiating a new search remote from the original starting location, the probability of searching the same area twice is reduced. While organized foraging by subterranean termites has now been well documented, research is needed to illustrate the efficacy of treating the area where subterranean termites are likely to forage.

Su (2005) investigated the distance effects of two non-repellent termiticides, fipronil and thiamethoxam, and the termite bait noviflumuron against laboratory populations of the Formosan subterranean termite *C. formosanus* in a laboratory arena with a linear foraging distance of greater than 50 m. The results showed that during the 10-wk test period, all termites were killed by noviflumuron baits within the 50 m arena. The non-repellent termiticides fipronil and thiamethoxam, however, divided the laboratory populations into two groups after causing 25-35% worker mortality. The horizontal transfer of lethal effects of fipronil and thiamethoxam was short-lived because of their dose-dependent lethal time and application method. Ripa et al (2007) evaluated two perimeter soil applications with cypermethrin and fipronil and two bait systems with hexaflumuron and sulfluramid for their efficacy and potential to control the eastern subterranean termite in Chile. Monitoring stations were installed at all sites to measure the overall termite activity next to and at distances up to 30 m away from infested structures. The results of the four strategies used in this study demonstrated that only the hexaflumuron baits produced a measurable effect on *R. flavipes* activity beyond several meters from the treated structures. The faster acting bait sulfluramid failed to reduce foraging activity around the baited structures, and swarms continued activity within structures. Fipronil provided limited distance effects while cypermethrin did not show any distance effects. Potter and Hillary (2002) assessed whether subterranean termites could be eliminated by applying non-repellent liquid termiticide (Termidor® (fipronil) or Premise® (imidacloprid)) around the exterior of infested buildings and if such treatment would reduce/eliminate foraging activity around the structure. Results showed that infestations could eliminate by applying non-repellent liquid termiticides solely around the exterior perimeter of buildings. Reduction of foraging activity was also observed, particularly with Termidor. Termite activity within monitoring stations was less affected by exterior Premise applications, although visible activity involving structural components eventually ceased. Potter and Hillary (2002) further suggested that the effect of treating the exterior only extends inward and well beyond the exterior site of application. Accordingly, it is possible to eliminate a colony whose foraging range is within 5 m of a treatment using non-repellent termiticides, but the treated soil must contain the ideal (active ingredient) concentration(s) that is both lethal and slow-acting coupled with the appropriate application method.

Termite Behavioral Responses to Termiticide

Non-repellent termiticides are designed to exploit the predictability of termite behavior in order to improve control techniques. Through the use of non-repellent slow acting termiticides, termites are susceptible to transporting toxicants on or inside their bodies and to other parts of the colony, thereby contaminating nest mates. The transportation and transfer of these toxicants to nest mates are affected by the behavior of termites under both normal and intoxicated conditions. Therefore, termiticide induced behavioral response is a factor pertaining to the insect, the termiticide, and the environment. Available literature shows a number of studies have been conducted using various termiticides and reveal numerous aspects of termite-termiticide interactions. This work offers insight into the ecological, physiological, and biochemical factors relevant to the behavioral response of termites to specific termiticide. Available literature also establishes a number of insecticide transfer and behavioral studies conducted using both social and non-social insects.

Gautam and Henderson (2011) investigated the uptake and potential transfer of chlorantraniliprole and fipronil by the Formosan subterranean termite *Coptotermes formosanus* Shiraki in the laboratory using donor-recipient model bioassays. Their data showed that chlorantraniliprole was more effective in controlling *C. formosanus* in a treated sand substrate, whereas fipronil was more effective in a soil treated substrate. This was due to the high organic substrate content in the soil in addition to soil texture, as well as pH playing an important role in binding the toxicant in the soil. Therefore the effectiveness of chlorantraniliprole can be high when applying it to sandy areas. The current work will show that fipronil is effective in a soil treated substrate, lending credibility to the use of a sand and organic matter mixture.

Bagnères et al (2009) compared contact and feeding methods through toxicity, uptake, and horizontal transmission of fipronil among American and French populations of *R. flavipes*.

Maximal uptake and a significant horizontal transfer were observed in the contact method. In addition to the contact method causing 60% mortality after 55 and 64 h in the French and American populations, respectively, the feeding assay caused 60% mortality observed after 7 d in both populations. They concluded that social behavior, such as contact and grooming, were components of the horizontal transfer process that might have contributed to the efficacy of fipronil in the study.

Ibrahim et al (2003) evaluated the relative susceptibility of workers and soldiers of *C. formosanus* to fipronil, compared horizontal transmission of fipronil within and between soldiers and workers, and studied the repellent action of fipronil using three concentrations (0.01, 0.0625, and 0.125%). This work showed that fipronil was highly effective against both workers and soldiers at very low doses. Based on our studies we have also found that fipronil is highly effective against both workers and soldiers at low doses. The horizontal transmission of fipronil from soldier to worker was more effective than that from worker to soldier. According to the authors this is due to the relative slow speed of fipronil acting on soldiers which allows them more time to interact with workers before dying. The symptoms of poisoning appear faster in workers making it possible for soldiers to identify sick and dead workers and avoid contact with them. Lastly, fipronil did not show repellency to termite workers.

Buczkowski et al (2012) evaluated the toxicity and horizontal transmission of chlorantraniliprole against field collected eastern subterranean termites, by investigating the effect of exposing termites to only treated substrate, only treated food, or both (topical, oral, or both). Their results indicated that the exposure route has no significant effect on chlorantraniliprole toxicity, demonstrating that chlorantraniliprole is highly active through feeding and contact routes. Chlorantraniliprole is transferred efficiently among the termites, however the rate and level of secondary mortality in the recipient termites depended on both the concentration of chlorantraniliprole and the duration of exposure in the donors. Buczkowski et al (2012) demonstrated that chlorantraniliprole has dose-independent toxicity, delayed toxicity, and is readily transferred in eastern subterranean termites. This premise was illustrated in our second study through variable dosage treatments and high mortality counts.

Behavioral response to termiticide intoxication is an important factor when deciding upon an appropriate insecticide for applicable use. Depending not only on repellency, the physiological effects of certain active ingredients can have a wide range of behavioral effects. Some of these resultant effects such as increased locomotion can be detrimental when dispersal or a large lethal dose is desirable. Quarcoo et al (2010) conducted research to describe and establish abnormal behavioral endpoints in termites treated with fipronil, indoxacarb, and chlorantraniliprole to aid in the assessment of their toxicity against eastern subterranean termites. The results showed abnormal behaviors of fipronil and indoxicarb treated termites were similar and inclusive of incipient intoxication (disorientation), followed by ataxia (uncoordinated movements), and morbundity (lack of ability to walk) leading to death. Whereas abnormal behavior of chlorantraniliprole treated termites was a reduction in walking speed followed by morbundity, and finally death. The authors concluded that the behavioral endpoints can complement mortality-based methods in predicting the performance of non-repellent termiticides, with these endpoints capable of providing information on potential for horizontal transfer of toxicants. Behavioral endpoints can also be used in identifying the active ingredients responsible for intoxication in termites. This could be particularly useful in cases where treatment failures necessitate retreatment by different pest control companies using different active ingredients.

Quarcoo et al (2012) first described the tunneling behavior of eastern subterranean termites treated with slow acting non-repellent indoxacarb and fipronil and also determined the effects of indoxacarb (50, 100, and 200 ppm) and fipronil (1, 10, and 50 ppm) concentration post-exposure time (10 min) on termite mobility. The authors concluded that exposure of *R. flavipes* to fipronil or indoxacarb significantly reduced termite walking and tunneling as well as the number of tunnel branches compared to the control termites. Quarcoo et al (2010) determined the effects of indoxacarb concentration (45, 90, 135, and 180 ppm) and exposure time (5, 10, 20, 40, 80, and 160 min) on onset and duration of induced behaviors between individuals and groups of eastern subterranean termites. The results illustrated that abnormal behaviors, morbundity, and death occurred in a predictable sequence: disorientation, ataxia, then morbundity followed by death. The authors concluded that higher concentrations and longer exposure periods resulted in faster onset of abnormal behaviors, morbundity, and death. The average onset time of abnormal behaviors, morbundity, and death was faster for groups of termites compared with individuals exposed to similar concentrations and periods of contact.

Henderson (2003) determined the difference in the speed of behavioral modification between fipronil and imidacloprid treated termites. The author hypothesized that slower acting non-repellent termiticides will increase transmission, thus having a greater impact on the targeted colony. The results showed that fipronil treated termites are not affected as quickly as imidacloprid treated termites. The author concluded that this delayed behavioral affect may allow healthy nest-mates to come in contact with fipronil treated termites more often which will increase the horizontal transmission of fipronil, increasing colony mortality. This is a basic principle embedded into the paradigm of the current research, as well as many of the non-repellent studies that hypothesize the efficacious use of this new technology.

Acquisition of physical evidence which records active termite behavior is important for the foundation of understanding termite biological interaction. Whitman and Forschler (2007) conducted an experiment videotaping *R. flavipes* and describing several behavioral observations in detail that include mating between kings and queens, ecdysis in the worker caste, tail-chasing, feeding, excavation, and oscillatory movements. The results demonstrated that primary reproductive pairs were observed to mate on average once every 3 days. Ecdysis lasted 43 min and involved multiple allogrooming attendants. Excavating termites manipulated the substrate with their mouthparts and showed fidelity to the site of excavation but not the site of deposition. Oscillatory movements were always ending in defecation. Worker feeding behaviors involve swallowing materials and evoke use of the super organism concept. The author concluded the relevance of these observations can be used to better understand different topics including biology, management, and evolution of these economic pests. Forschler (1994) evaluated survivorship and tunneling response of the eastern subterranean termite in a larger bioassay arena using (0.5, 5, and 50 ppm) of commercially formulated termiticides (isofenphos, chlorpyrifos, fenvalerate, chlordane, cypermethrin, and permethrin) as a soil barrier with and without gaps of untreated soil. The results illustrated cypermethrin did not affect termite survivorship but did reduce termite tunneling activity after contact with treated soil. Permethrin and fenvalerate did not affect termite survivorship or tunneling activity in untreated soil. Chlordane, chlorpyrifos, and isofenphos affected termite survivorship and reduced tunneling activity. The author concluded that termite location and exploitation of untreated gaps within a termiticide soil barrier appeared to be the result of random termite foraging behavior. Thus, soil barriers should provide adequate protection from termite invasion as long as application provides a complete (unbroken) barrier.

Insecticide tests conducted on cockroaches are especially relevant to studies on termites because of the similarities in morphology and ecology. As mentioned earlier, the order Isoptera derived originally from orthopteran ancestors and shares many similarities with Blattidae. Buczkowski and Schal (2001) evaluated the horizontal transmission of fipronil among German cockroaches, *Blattella germanica* (L.) by using three different insecticide delivery method, topical, residual, and oral. Their results revealed that the oral method was most effective in causing high mortality of untreated adults and nymphs and had a greater secondary kill. Kaakeh et al (1997) evaluated the toxicity of fipronil compared to chlorpyrifos against German *Blattella germanica* (L.) and American cockroaches *Periplaneta americana* (L.), using topical and oral application methods. Their results demonstrated that fipronil was significantly more toxic than topically applied chlorpyrifos against both species. Oral toxicity also showed that fipronil is an effective toxicant against both *B. germanica* and *P. americana*.

The group dynamics associated with the life of social insects makes their behavioral responses much more relevant in a bid to understand insecticide-induced behaviors of termites. Studies on social insects such as ants and bees provide information pertaining to horizontal transfer of toxicants between insects as well as their behavioral response to treated individuals and zones. Soeprono and Rust (2004) tested three insecticides bifenthrin, β-cyfluthrin, and fipronil as barrier sprays for Argentine ant control in laboratory colonies. Exposed donor ants were placed with unexposed ant colonies and mortality was monitored to compare the ability of donors to transfer lethal doses to untreated individuals. Fipronil was readily transferable between individuals resulting in high mortality rates, whereas Bifenthrin and β-cyfluthrin were less transferable, exhibiting mortality rates similar to the controls. Soeprono and Rust (2004) concluded the closer the barriers and treatments are to the nest, the greater the likelihood of toxicants being transferred into the nest and resulting in dramatically control the colony. Hassani et al (2005) evaluated the effect of sub-lethal doses of fipronil on the behavior of the honeybee (*Apis mellifera* (L.)) under controlled laboratory conditions using oral and topical application methods. Their results showed a significant effect to the olfactory learning of the honeybee and sucrose reduction when a sub-lethal dose of fipronil is applied topically compared to the oral treatment.

Beetles (Coleoptera), constitute the largest group of insect pests, have not been ignored in behavioral response studies. Grosman and Upton (2006) evaluated the efficacy of the systemic insecticides dinotefuran, emamectin benzoate, fipronil, and imidacloprid in order to prevent attacks and brood production of southern pine engraver beetles (Coleoptera: Curculionidae: Scolytinae) and wood borers (Coleoptera: Cerambycidae). These authors examined standing, stressed trees, and bolt sections of loblolly pine, *Pinus taeda* L., in eastern Texas using the Arborjet Tree IV system. Results illustrated that emamectin benzoate significantly reduced the colonization success of engraver beetles and associated wood borers in both stressed trees and pine bolt sections. Fipronil was nearly as effective as emamectin benzoate in reducing insect colonization in bolt sections. Imidacloprid and dinotefuran were ineffective in preventing bark beetle and wood borer colonization of bolts, standing, and stressed trees. It is important to consider this work on another eusocial insect, however different, to further understand eusocial interactions and their dynamics.

The research cited above have described various aspects of insect-insecticide interactions and offer insight why the application methods, ecological, physiological, and biochemical factors are important to termite management. However, available literature lacks a descriptive study on the above ground tunnel treatment and the use of the localized treatment method. This information, generated in the current study, will increase the effectiveness of the eastern subterranean termite. The over arching goal of my study was to investigate the effect of behavior and insecticide application protocol on termite management, specifically, eastern subterranean termite. Accordingly, the first objective of this work was to determine the effect of foraging tunnel treatments with Termidor DRY on this species. The second objective was to study the effects of concentration, distance, and application methods of Altriset. The third objective was to investigate the performance of Termidor H.E. against functional colonies. of Reticulitermes flavipes (Isoptera: Rhinotermitidae). The fourth objective was to assess the population effect of treating above-ground tunnels with RTU Dry Termidor.

CHAPTER 2

THE EFFECT OF FORAGING TUNNEL TREATMENT WITH TERMIDOR® DRY ON *RETICULITERMES FLAVIPES* (ISOPTESRA: RHINOTERMITIDAE)

Abstract

The efficacy of a dry, ready-to-use (RTU) termiticide formulation of fipronil was evaluated against termite foraging activity and survival by injection into foraging tunnels of *Reticulitermes flavipes* Kollar (Isoptera: Rhinotermitidae) in a laboratory study. Groups of workers (older than 3rd instars) were placed in bioassay units consisting of a group site and a feeding site connected by termite tunnel(s) within a soil-filled Tygon tube. Two treatments (0.15 and 0.30 mg a.i. fipronil per treatment) were conducted by injecting the doses into a foraging tunnel near the feeding site. Bioassay units were monitored for termite movement utilizing Termatrac T3i until no movement was detected. Termite movement ceased at day 5 and 7 for the 0.30 and 0.15 mg dose treatments, respectively. Dissection of the bioassay units confirmed 100% mortality at the days when both visual observations and Termatrac T3i indicated no termite movement. Termites in the treated units constructed significantly fewer tunnels post-treatment compared to control termites. Our results provided strong evidence for the efficacy of the dry RTU fipronil formulation against *R. flavipes* activity at the group level when a single tunnel was treated.

Introduction

Subterranean termites (Isoptera: Rhinotermitidae) forage for cellulosic food sources and frequently cause significant damage to structural wood and wood products. In the United States, Rhinotermitidae, the most economically important family, has an economic value of $3 billion per year (Su 2002), $1 billion of which is attributable to the eastern subterranean termite (Potter 2004) that has a wide geographical distribution (Wang et al 2009).

Subterranean termites live underground. Their cryptobiotic or ‘‘hidden’’ lifestyle makes their presence and damage difficult to detect. Termites often build tube-like extensions not only underground but also aboveground to connect colonies to food and water sources. These conduits, commonly called mud tubes, become obvious when they extend over concrete foundations and other exposed surfaces and become a valuable sign for detection.

The control of structural infestations of subterranean termites currently depends on the use of soil-applied termiticides for prevention and remedial treatment (Hu 2011). Soil-applied termiticides are used to treat soil around and beneath the foundation of structures to establish a chemical barrier against termites (Su and Scheffrahn 1990).

Fipronil is one of the most commonly used non-repellent compounds for termite management in the United States (Hu 2011). Fipronil was identified in 1987 by the French company Rhone-Poulenc and registered in the United States in 1996 as a broad spectrum insecticide in the phenyl pyrazole class (Rhone-Poulenc 1995). Fipronil acts on the insect’s central nervous system by blocking the GABA (l-aminobutyric acid) receptor and regulating chloride channels, resulting in excess neuronal stimulation and eventual death of the exposed insect (Cole et al 1993). Numerous studies have evaluated the efficacy fipronil as a liquid soil termiticide. The effect of fipronil in reducing the foraging range and activity of subterranean termites has been investigated in both laboratory (Saran and Rust 2007, Quarcoo et al 2010,

2012) and field studies (Potter and Hillery 2002, Kard 2003, Hu and Hickman 2006). The non-repellency and horizontal transmission from worker to worker, and worker to soldier, have been studied using groups of termites (Hu 2005, Hu et al 2006, Saran and Rust 2007). Hu et al (2006) further demonstrated vertical transmission from foraging workers to reproductives as well as horizontal transmission to other caste members in laboratory colonies. The influences of liquid fipronil concentration and dose, duration of exposure, and the ratio of donor to recipient termites have been examined for various termite species (Ibrahim et al 2003, Shelton and Grace 2003, Remmen and Su 2005). The impact of soil type, compaction, temperature gradients, pH value, and the presence of various woods on the penetration and action of fipronil in the soil has also been examined (Mulrooney et al 2007, Gautam and Henderson 2011).

Recently, a new formulation of dry ready-to-use (RTU) fipronil has been developed. This product can be placed directly into aboveground foraging tubes or other matrices where termites are active. The purpose of this study was to determine the efficacy of tunnel treatments at various doses of this formulation against the foraging and survival of eastern subterranean termites, *Reticulitermes flavipes*.

Materials and Methods

Termites

Eastern subterranean termites were collected from field colonies in the city of Auburn, Alabama (Lee County) using underground traps as described by Hu and Appel (2004). Traps consisted of open-bottom plastic buckets (18 cm high, 13 cm in diameter) provisioned with corrugated cardboard rolls (15 cm high and 11 cm in diameter) that were set in the ground. Closed-bottom plastic buckets (18 cm high, 13 cm internal diameter) were used to transfer corrugated cardboard rolls with termites to the laboratory. Termites were extracted by gently tapping the cardboard to allow termites to drop onto a moist paper towel. Workers and soldiers were used within two days of collection.

Soil

Soil was obtained from an area immediately surrounding a termite colony in a field in Auburn. The soil was sieved to remove debris and sterilized in an autoclave (Thermo Fisher Scientific, Waltham, MA) at 60°C for 24 h, two days prior to the experiment. Distilled water was added to the soil (8 ml H_2O/100 g of soil). Moistened soil was then used to fill Tygon® (VWR Corp., Radnor, PA) tubes (0.8 cm inside diameter) and plastic cylinders.

Chemical

Termidor® DRY, a RTU dry formulation containing 0.5% active ingredient fipronil [5-amino-1-(2,6-dichloro-4-(trifluoromethyl) phenyl)-4- ((1,R,S)-(trifluoromethyl)sulfinyl)-1-H-pyrazole-3-carbonitrile] was provided by BASF Corporation (BASF Pest Control Solutions, St. Louis, MO). Blank formulation containing no active ingredient but only the micro-crystalline cellulose carrier, Microllose, was also provided by BASF Corporation (Research Triangle, NC).

Experimental Design

Each bioassay unit was composed of two plastic cylinders (8.1 cm high and 8.5 cm in diameter) connected by a soil-filled Tygon tube (91.4 cm in length). One cylinder was the feeding site, which contained one block (1.8 x 1.8 x 2.1 cm^3) of damp southern yellow pine as a food source. The other cylinder was the group site with a yellow pine block on the bottom of the cylinder covered with a 3-cm deep layer of moistened soil. A small hole (1 cm in diameter) was burned into the side at the base of each cylinder using a soldering iron. The two cylinders were connected at the holes with Tygon tubing using hot glue. Groups of 500 termites (2 soldiers per

100 workers, the ratio found in field) were introduced into the group site cylinder. All cylinders were covered with lids and sealed with Parafilm to maintain moisture. Fifteen bioassay units were maintained in the dark at room temperature to allow termites to tunnel from the group sites through the connecting tubes into the feeding sites.

Treatment

The dry RTU 0.5% fipronil formulation was injected into a termite tunnel within the Tygon tube two days after the tunnels were established. Two doses (0.15 and 0.30 mg) of the active ingredient were tested. The blank formulation was used as a control and applied at a volume corresponding to the low rate of the active formulation. Using a soldering iron, a small hole (0.6 mm) was punched in the Tygon tubes 30 cm away from the feeding site and directly into a termite tunnel. The designated doses of RTU fipronil formulation and blank formulation were injected into the holes in the Tygon tubes using a customized injecting device. The injection device consisted of an Eppendorf-style pipet tip (200 mL in volume and 4.8 cm in length) (Fisher Health Care Corp., Houston, TX) attached to a 10-ml syringe tip. The syringe plunger forced air and the dry formulation into the termite tunnel, with the tip of the applicator aimed at the group site at a 45 degree angle, so the treatment could be spread a distance of 8 to 12 cm inside the tunnel without blocking the tunnel at the treatment point. Each treatment was replicated five times.

Data collection

The distances termites tunneled through the moist soil in the Tygon tube from the group site towards the feeding site was visually observed and recorded daily before the treatment. This was to determine the time taken for termites introduced into the group site to tunnel through Tygon tube into feeding site. New tunnel construction within the feeding site arena and termite movement within the bioassay units were recorded every other day after the treatment until no termite movement was detected. New tunnel construction inside the feeding site was noted by visual observations. Termite movement within the bioassay units was recorded using two methods: 1) Visual observations of termites' movement at the bottom of the transparent cylinders and the tunnels at portions of the Tygon tube where no soil/mud had been deposited during tunnel formation; and 2) Termatrac T3i recording (Termatrac Australia Pty Ltd. Queensland, Australia). The Termatrac T3i was used to corroborate the visual observations. After both visual observation and Termatrac T3i corroboration indicated no movement, the cylinders were disassembled to count living termites. Termites were recorded as dead when they could not move any appendages.

The Termatrac is a 3-in-1 device that comes with a remote thermal sensor with a laser guide, a built-in moisture sensor, and patented termite detection radar. It emits a fixed frequency microwave beam and measures the intensity and frequency of signals reflected back to a collocated receiver (Tirkel et al 1997, Protec USA 2002). A signal processor computes the intensity of the reflected energy and the difference between emitted and reflected frequencies, and displays the result on a Bluetooth® (Bluetooth Special Interest Group, https://www.bluetooth.org/en-us) wireless PDA (personal digital assistant). Termatrac can detect motion or vibration by any object in the field of view whose dielectric constant (a relative measure of the capability to store electric charge) differs from that of the substrate (Edde 1993, Protec USA 2002). Any moving objects not of interest were excluded from the field of view. Termatrac T3i was positioned under each cylinder (i.e., feeding site and group site) and above the Tygon tube. Termatrac T3i detection radar signals were calibrated to identify termite location and intensity of activity which was measured in gain (y-axis) over time (x-axis), positively correlating gain with movement intensity. The signal graphs were sent to a computer. The peaks below 2 gain, between 2 and 4 gain, and above 4 gain from each graph were counted, and the average of the peaks for each graph was used as responding value.

Statistical analyses

Pre-treatment termite tunneling data and post-treatment termite mortality were analyzed using one-way analysis of variance (ANOVA) utilizing Minitab 13 software (Proc GLM, Minitab Institute, State College, PA) (Minitab Institute 2003).

Results

Behavioral observations pre-treatment

Introduction of termites into the group site cylinders resulted in an initial burst of tunneling activity. Termites radiated out through the moist soil to the base of the cylinders, then tunneled into the Tygon tubes toward the feeding site cylinders. Termites tunneled through the 91.4 cm length of Tygon tube and reached the feeding site in three days. After reaching the feeding site, termites constructed mud tubes to reach the wood blocks (Figure 2.1). These tubes were constructed by transporting soil particles from walls of the earlier portions of the tunnel. Termites tunneled significantly longer distances during the first day (36.7 cm ± 3.4) than during the second day (20.9 cm ± 2.4) or the third day (20.9 cm ± 2.2) (F = 11.15, P = 0.0001, df = 2,44). Termite movement consisted mainly of walking inside the constructed tunnels between the two sites, in addition to local movements in the group and feeding arenas.

Behavioral observations and mortality post-treatment

A significant reduction in termite activity, as indicated by Termatrac readings and visual observations, occurred in the two fipronil treatments compared to the control, where little or no change in activity occurred. Post-treatment, Termatrac reading data were collected from examining the graphs generated from the Termatrac device for each treatment to determine whether there was termite movement. Most termites showing fipronil intoxication (Quarcoo et al 2010) ultimately ended up dying in the group site rather than dying inside the treated tunnels or feeding sites. Therefore, Termatrac readings from group sites were used to analyze the efficacy results of the experiment.

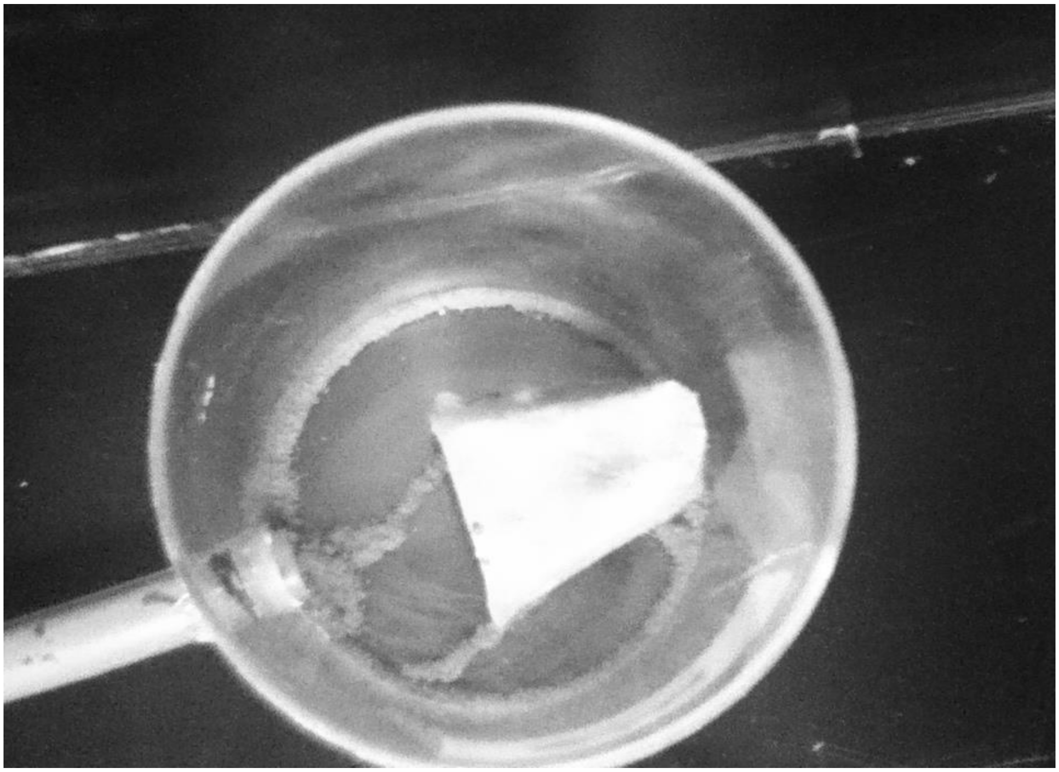


Figure 2.1. Termites constructing mud tubes pre-treatment three days after reaching wood blocks in the feeding site cylinder.

Visual observations of termite activity in the groups, tubes, and feeding sites were corroborated by Termatrac T3i readings from the group sites (Figure 2.2). The decrease in termite movement was considerably faster in the feeding sites and inside Tygon tubes compared to the group site. Termites in Tygon tubes and at feeding sites showed little or no behavioral change before a complete absence in these two locations. On the other hand, termites at the group site appeared to aggregate and display the general signs of fipronil intoxication (Hu et al 2006) within 24 h of treatment in response to the 0.30 mg dose and at 48 h to the 0.15 mg dose, until no movement was reached as indicated by Termatrac readings. The Termatrac T3i is capable of detecting termite movement and not the size of termite populations. The generated readings showed decreasing peaks and wave heights over time, indicating the decrease in termite movement intensity (Figures 2.2 and 2.3). Fipronil exposure also resulted in cessation of tunneling activity. Termites exhibiting signs of fipronil intoxication appeared to be less mobile and unable to construct tunnels compared to unaffected termites.

Dose effect

The abnormal behaviors and cessation of activity/movement were observed earlier at the higher dose (0.30 mg/group) than with the lower dose (0.15 mg/group). Tunneling construction deceased in the high dose (0.30 mg) within 24 h after treatment, whereas in the low dose (0.15 mg) tunneling

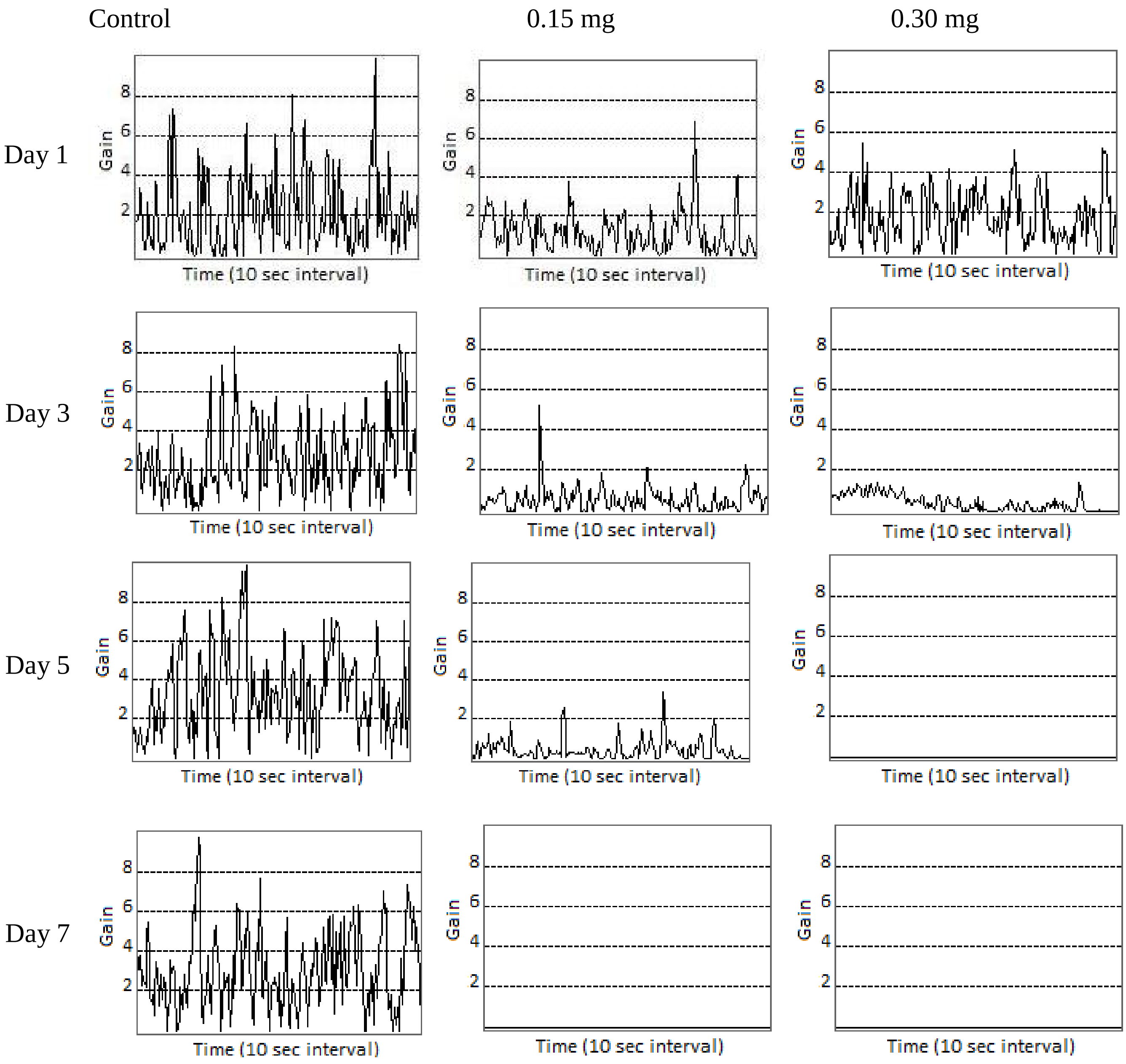
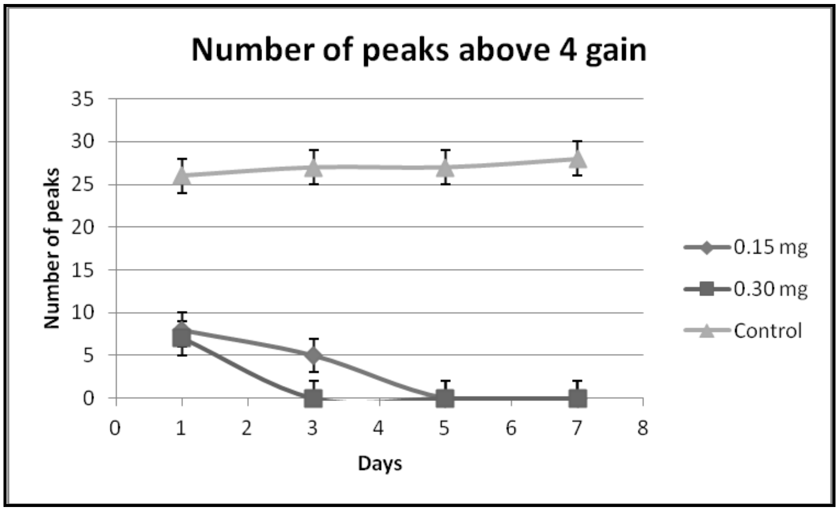


Figure 2.2. Generated graphs by Termatrac T3i post-treatment to record eastern subterranean termite movement intensity. The graphs are represented by gain versus time in 10 sec intervals measured in the group site cylinder.

construction decreased within 48 h after treatment. Visual observations and Termatrac reading showed that the higher dose resulted in no movement at five days post treatment; whereas the lower dose resulted in a longer display of disorientation and no movement at seven days post treatment (F = 141.66, P < 0.001, df = 3). Dismantling of the experimental units revealed only one out of ten units had a few live workers hiding in a feeding gallery inside a wood block, indicating a 90% accuracy of Termatrac readings.

Discussion

This study tested a newly developed dry RTU formulation of fipronil against eastern subterranean termites using a localized treatment technique. It demonstrated for the first time the effect of locally treating a single tunnel with



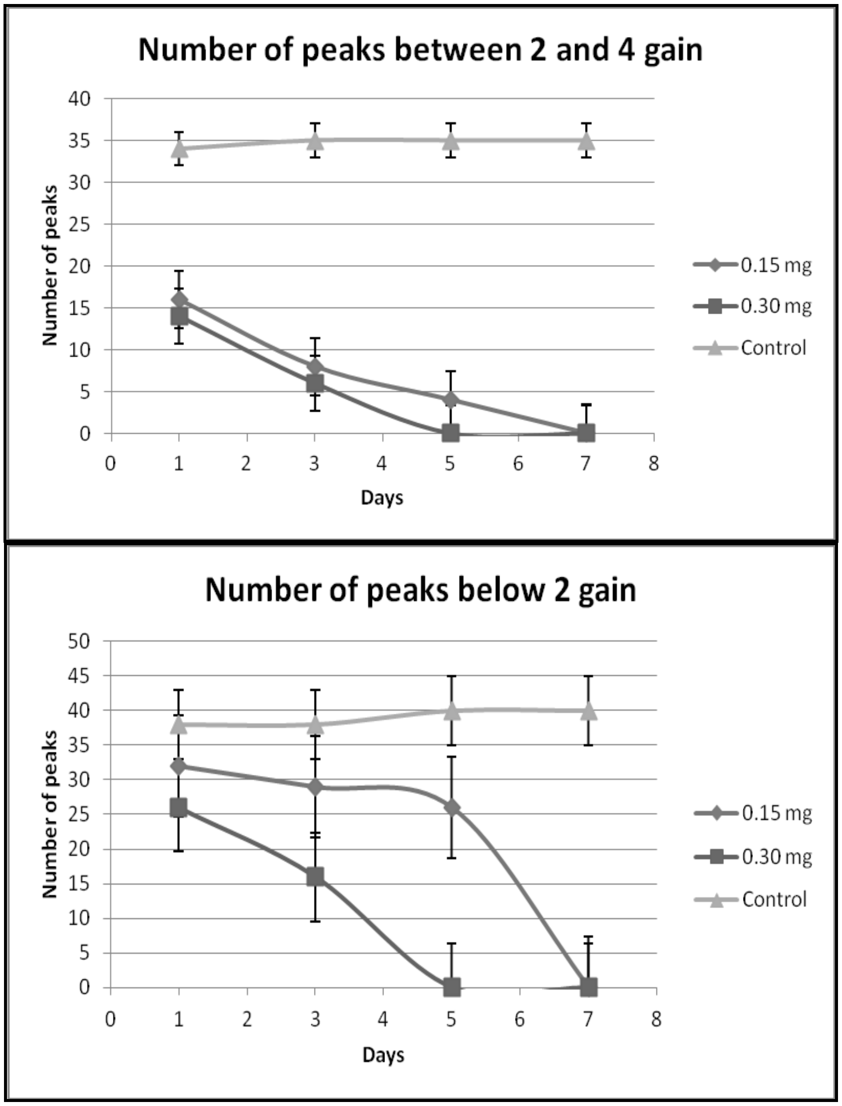


Figure 2.3. Number (mean ± SE) of peak readings below 2 gain, between 2 and 4 gain, and above 4 gain from each Termatrac T3i graph of the group site cylinders post-treatment.

dry RTU fipronil formulation against eastern subterranean termites. The results from this study demonstrated that locally treating a single tunnel with dry RTU fipronil near feeding sites was effective for control of termite groups. Currently, liquid fipronil soil treatments are commonly used to control eastern subterranean termite population, which involves trenching around the perimeter of a structure and/or drilling holes at regular intervals into the foundation block and slabs (Rambo 1985). The success of soil treatment largely relies on a uniformly treated soil barrier, which can be affected by soil pH, clay and organic matter content, and soil moisture (Rambo 1985). Eastern subterranean termites are widely dispersed in the soil, making accurate and effective soil treatment difficult to achieve (Myles 1996). It is tactically and economically advantageous to treat above-ground tunnels with dry RTU fipronil formulation. The present study provides evidence that termites at both group sites and feeding sites suffered 100% mortality in 5-7 d post-treatment of a single tube.

Caution should be taken in applying these laboratory findings to termite control in the field. Potential limitations of treating above-ground tunnels with a dry RTU formulation include the blockage of tunnels by dead termites or clotting of dry RTU formulation, which may reduce the residential efficacy and control effectiveness. In addition, this study was conducted with a termite group population of 500 individuals, in contrast to the millions of termites in a typical colony in the field. Furthermore, the experiment was conducted in a laboratory setting in which workers were in confined locations, whereas within the field, termite colonies have multiple feeding sites, broad foraging areas, and multiple above-ground tunnels at various locations.

The time period we used to evaluate the treatments (7 d) was considerably shorter than for some other studies that employed a 30-d interval (Lewis and Rust 2009). Therefore, we hypothesize that all of our treatments would have shown 100% mortality given more time. According to Ibrahim et al (2003), Song and Hu (2006), and Saran and Rust (2007) lower doses of termiticide require a longer period of time to manifest symptoms of poisoning.

Many previous studies have investigated the influence of horizontal transfer of fipronil between termites. Saran and Rust (2007) reported that the maximum transfer of fipronil from donors to recipients in western subterranean termites, *Reticulitermes hesperus* Banks, occurred within the first 24 h. Quarcoo et al (2012) reported that higher fipronil concentrations resulted in faster onset of abnormal behaviors, morbundity, and death. Hu (2005) demonstrated the efficacy and non-repellency of fipronil-treated soil and various concentrations against field-collected eastern subterranean termite. Song and Hu (2006) reported that the time required for full expression of transferable lethal effects of fipronil on untreated termites increased as the dose on treated termites decreased at given donor-recipient ratios, and the efficacy of fipronil soil treatment was positively correlated to applied concentration and soil-barrier thickness.

To date, the only study that investigated the effect of a dry RTU fipronil formulation was tested on the drywood termite, *Incisitermes snyderi* (Kalotermitidae), in naturally infested cypress lumber using localized treatment techniques (Hickman and Forschler 2012). They reported that the dry RTU fipronil formulation performed as effectively as foam imidacloprid, and they provided evidence of elimination of infestation without removal of every board that was treated. They proposed that less than 100% elimination was due to the difficulty in treating all termite galleries and the short duration of the trial, which may not have allowed enough time for unexposed termites to contact treated galleries. For subterranean termites, the above-ground tunnels are obvious and can be accurately treated with dry RTU formulation. Our study showed that treatment with a dry RTU formulation of fipronil to a single active tube connected to two locations where termite workers and soldiers aggregate eliminated all termites to a point where no population recovery could occur. Future study will test this treatment concept on termite colonies in laboratory and then in the field.

In conclusion, this study helped us gain a good understanding of the effectiveness of treating mud tunnels using powdered formulations against eastern subterranean termites. The approach of localized treatment is an important step in reducing termiticide exposure to humans and the environment. It is critical for termite control methods to be continuously researched and to reflect newer, more advanced technologies.

CHAPTER 3

EFFECTS OF CONCENTRATION, DISTANCE, AND APPLICATION METHODS OF ALTRISET (CHLORANTRANILIPROLE) ON EASTERN SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE)

Abstract

The effects of various concentrations, distance, and application methods of Altriset (chlorantraniliprole) were investigated against one of the most destructive termites, the eastern subterranean termite, *Reticulitermes flavipes* Kollar. Three laboratory experiments were conducted. First, we examined the concentration effect of treating the soil contiguously to established foraging tunnels at a fixed 1m distance. The results demonstrated 100% termite control in 19 days post-treatment at 100 and 50 µg/g and 27% termite mortality at 25 µg/g. Second, we tested the distance effect of the soil treatment (2m and 4m) on the efficacy of Altriset to the satellite termite populations at a fixed 50 µg/g concentration. This resulted in 100% termite control in 22 days post-treatment at both 2m and 4m. Third, we examined the effect of differing application methods using 12.5 and 25 µg/g prior to the establishment of foraging tunnels at a fixed 1m distance. This illustrated 100% termite control in 9 days post-treatment at 25 µg/g and 12 days post-treatment at 12.5 µg/g. The third experiment demonstrated soil treatments that were applied prior to termite tunnel establishment had greater efficacy than applications made post tunnel construction. Our results provide a comprehensive understanding about the efficacy of Altriset treatments on eastern subterranean termites.

Introduction

Control methods of subterranean termites have always been an important concern to homeowners and the pest control industry. Subterranean termites live underground and maintain a cryptic or “hidden” lifestyle, making their presence and damage difficult to detect. When searching for food and water, termite workers often build tunnels underground and tube-like extensions aboveground to expand the foraging potential of a colony. The tubes are composed of a mixture of saliva, soil, and fecal matter (Eggleton 2011). These aboveground tubes are termed mud tubes, which become easily visible when they extend over concrete foundations and other exposed surfaces. These susceptible mud tubes are considered an ideal target for using the recently developed method of localized treatment.

Currently the control of structural infestations of subterranean termites primarily depends upon the use of liquid termiticides for prevention and remedial treatment (Hu 2011). The treatment involves trenching the soil and drenching the exposed section with termiticide, or drilling into structural foundations and injecting the termiticide. These application methods create a termiticide barrier around structural perimeters and under foundations. These applications are labor-intensive and require large amounts of termiticide to achieve the label rate concentration levels in the soil. A possible alternative is the Perimeter-Plus-Localized-Treatment using non-repellent termiticide. The benefits of using this method include a reduction of labor and the quantity of chemical application.

Altriset (Syngenta, Greensboro, NC) is a non-repellent termiticide, with chlorantraniliprole as the active ingredient (18.4% by weight). Chlorantraniliprole is from the anthranilic diamide class of insecticides with a novel mode of action that targets and activates ryanodine receptors, causing the release of internally stored calcium. The release of stored calcium causes loss of muscle control leading to rapid feeding cessation, lethargy, partial paralysis, cardiac muscle failure, and regurgitation (Cordova et al 2006). Altriset's non-repellency and delayed action has been demonstrated in laboratory studies (Quarcoo et al 2010), and the potential of toxic transfer has been investigated (Gautam and Henderson 2011). The bioavailability in various soils has been examined (Spomer et al 2009), as well as the toxicity and horizontal transfer (Buczkowski et al 2012). All of these studies were conducted by directly exposing termites in or on treated substrates, with the termite mortality data directly recorded from the exposed termites. There is no information detailing the effects of localized soil treatment on termite mud tubes leading to satellite foraging sites.

This study investigated the effects of localized soil treatment using varying application methods, concentrations, and distances when Altriset is tested for its control efficacy against the eastern subterranean termites, *Reticulitermes flavipes* Kollar. Experiment one examined the effect of concentration on termite mortality when treating the soil adjacent to the established foraging mud tubes leading to the satellite foraging site at a fixed distance. The second experiment examined the effect of treating various established mud tube lengths at a fixed concentration on termite mortality. The third experiment investigated the effect of different application methods at various concentrations for fixed distances on termite mortality.

Materials and Methods

Termites

Eastern subterranean termites (*Reticulitermes flavipes*) were collected from field colonies in the city of Auburn, Alabama (Lee County) using underground traps (Hu and Appel 2004). Traps consisted of open bottom plastic buckets (18 cm high, 13 cm in diameter) provisioned with corrugated cardboard rolls (15 cm high and 11 cm in diameter). Closed bottom plastic buckets

(18 cm high, 13 cm internal diameter) were used to transfer corrugated cardboard rolls with termites to the laboratory. Termites were extracted by gently tapping the cardboard to allow termites to drop onto a moist paper towel. Workers and soldiers were placed in the bioassay within two days of collection.

Soil

Soil, sandy clay, was obtained from the area immediately surrounding the termite's collected location. The soil used in this study was not known to have received any prior pesticide treatment. The soil was sieved to remove debris and sterilized in an autoclave (Thermo Fisher Scientific, Waltham, MA) at 60°C for 24 h, two days prior to the experiment. Distilled water was added to the soil (10 ml H_2O/100 g of soil), moistened soil was then used to fill Tygon® (VWR Corp., Radnor, PA) tubes (0.8 cm inside diameter) and plastic bioassay units.

Wood

Southern yellow pine was obtained from local hardware store. The wood was sterilized in an autoclave (Thermo Fisher Scientific, Waltham, MA) at 60°C for 24 h, and weighed using an analytical balance (Denver Instrument Company, Bohemia, NY) before the experiment. Distilled water was added to the wood to achieve saturation two days before the experiment.

Chemical

Altriset® contains 18.4% of the active ingredient chlorantraniliprole: (3-Bromo-N-[4-chloro-2-methyl-6-[(methylamino) carbonyl] phenyl]-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide) (liquid termiticide) and was provided by the DuPont Corp. (Wilmington, DE. Altriset now marketed by Syngenta). A stock solution of Altriset was serially diluted to obtain

0.0515% (100 µg/g), 0.0258% (50 µg/g), 0.0129% (25 µg/g) and 0.00645% (12.5 µg/g) active ingredient solutions.

Experimental units

Each unit was composed of two plastic cylinders (8.1 cm high and 8.5 cm in diameter) connected by a soil-filled Tygon tube (Figure 3.1). One cylinder held the feeding site, which contained two blocks of damp southern yellow pine (4 x 4 cm) as a food source positioned opposite of the Tygon tube connection. The other cylinder held the group site, filled with a 2 cm thick layer of moist soil and one piece of damp southern yellow pine block embedded at the bottom. A small hole (1 cm in diameter) was burned into the side wall near the bottom of each cylinder using a soldering iron. The two cylinders were connected at the holes with Tygon tubing (0.8 cm inner diameter) using hot glue. Groups of termites (workers: soldiers = 99:1) were introduced into the group site cylinders. All the cylinders were covered with lids and sealed with Parafilm to maintain moisture and humidity. The experimental units were maintained in a dark enclosure to allow termites to tunnel freely from the group site, through the connected tube, into the feeding site. Termite groups were observed every 24 h until they reached the feeding site. Treatments were applied two days after a network of tunnels were established in the feeding site for experiment one and two. In experiment three, the treatment was applied prior to termites tunneling from the group site, through the connected Tygon tube, and into the feeding site.

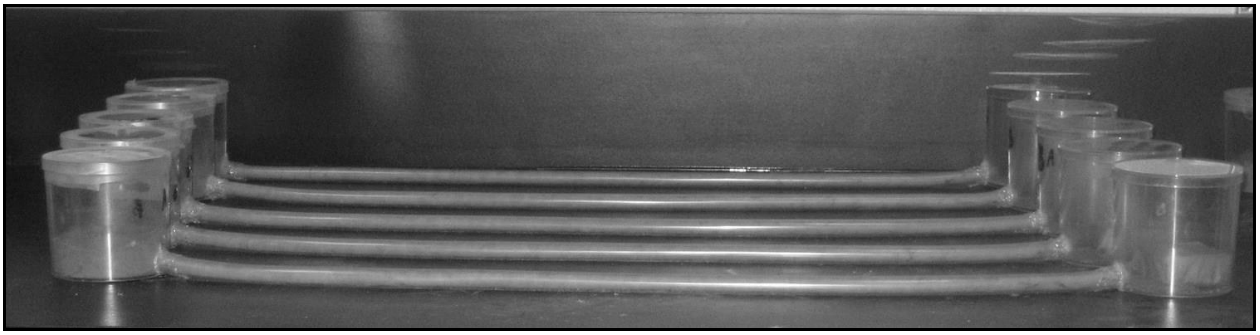


Figure 3.1. General experimental design for investigating the concentration, distance, and application method of Altriset.

Experiments

Groups of 200 termites (experiment one and three) and 2000 termites (experiment two) were introduced into the group site cylinders. The cylinders were connected to the feeding site using 1m (experiment one and three), 2m and 4m (experiment two) soil filled Tygon tubes. Using a 3 ml syringe, a small amount (1.51 ml) of the prepared solution was injected at 1 cm intervals into a 6 cm section of the Tygon tube to achieve 25, 50 and 100 µg/g soil treatment (a.i. w/w) for experiment one, 50 µg/g soil treatment (a.i. w/w) for experiment two and 12.5 and 25 µg/g soil treatment (a.i. w/w) for experiment three. The treatment injection was applied at 1 cm intervals into a 6 cm section of the Tygon tube to achieve uniform distribution. The 50 µg/g treatment was selected based on the label rate being 0.05%, the 100 µg/g was a representative for the above label rate, and the 25 µg/g and 12.5 µg/g represented soil concentration after a period of natural termiticide degradation. The injection was carefully applied to the soil and not directly to any established foraging tunnels for experiment one and two. For experiment three, the injection was carefully applied to the soil near the feeding site before the established foraging tunnels were constructed. This was performed to compare the efficacy of soil treatment prior to tunnel construction against soil treatment after tunnel construction. The location of the treatment section began from the feeding site. Experiment one and three had five replicates per treatment, and experiment two had three replicates per treatment.

Data collection

Estimated termite mortality and movement intensity at the group site for each of the replicates were recorded every 24 h until 100% mortality. Two methods were used: 1) Visual observation and 2) Termatrac® T3i recording (Termatrac Australia Pty Ltd. Queensland, Australia). The Termatrac T3i was used to corroborate the visual observations.

Termatrac is a 3-in-1 device that comes with a remote thermal sensor with a laser guide, a built-in moisture sensor, and patented termite detection radar. It emits a fixed frequency microwave beam and measures the intensity and frequency of signals reflected back to a collocated receiver (Tirkel 1997). A signal processor computes the intensity of the reflected energy and the difference between emitted and reflected frequencies, and displays the result on a Bluetooth ® (Bluetooth Special Interest Group, https://www.bluetooth.org/en-us) wireless PDA (personal digital assistant). Termatrac can detect motion by any object in the field of view whose dielectric constant differs from that of the substrate (Edde 1993). Any moving objects not of interest were excluded from the field of view in this study.

Termatrac T3i was positioned under each container (i.e., feeding site and group site) and above the Tygon tube. Termatrac T3i detection radar signals were calibrated to identify termite location and intensity of activity, which was measured in gain (y-axis) over time (x-axis), positively correlating gain with movement intensity. The signal graphs were then sent to a computer. After both visual observation and the indication of 100% mortality from the Termatrac T3i, cylinders were disassembled to count living termites. Termites were recorded as dead when they could not move any appendage. In addition, wood consumption was recorded. This was done by subtracting post-treatment weight from pre-treatment weight of oven-dry wood blocks in experimental units.

Data analyses

Estimated mortality at the group site cylinders were averaged, arc-sin transformed and analyzed using one-way analysis of variance (ANOVA) utilizing SAS 9.1 software (SAS

Institute, Cary, NC) (SAS Institute 2008). Tukey's honestly significant difference (HSD) was performed following ANOVA to determine significant differences between concentrations and distance. The statistical analysis was conducted at the $P < 0.05$ level of significance. The difference between pre-treatment and post-treatment for wood consumption was calculated and analyzed using one-way analysis of variance (ANOVA) utilizing SAS 9.1 software (SAS Institute, Cary, NC). Tukey's honestly significant difference (HSD) was performed following ANOVA to determine significant differences between pre and post treatment wood consumption. The statistical analysis was conducted at the $P < 0.05$ level of significance.

Results

Most termites exhibiting Altriset intoxication died in the group site cylinders rather than inside the treated tunnels or feeding site cylinders. This suggests the horizontal transfer of the active ingredient occurred through social interaction such as grooming, trophallaxis, and regurgitation. Therefore, data from the group site cylinders were used to analyze the efficacy results of the study.

Experiment one: concentration effect at a fixed 1m distance

Five days after exposure termite movement was affected and observed visually. By day 19 post-treatment there was 100% reduction in termite movement within groups that were exposed to 50 and 100 µg/g treatments, compared to the reduction in termite movement exposed to 25 µg/g treatment and control (Figure 3.2). Termites exposed to both 50 and 100 µg/g soil treatments discontinued feeding on the damp southern yellow pine food source (0g consumed), in comparison to contiguous feeding of the termites exposed to 25 µg/g (0.72g consumed) soil treatment and control (0.83g consumed).

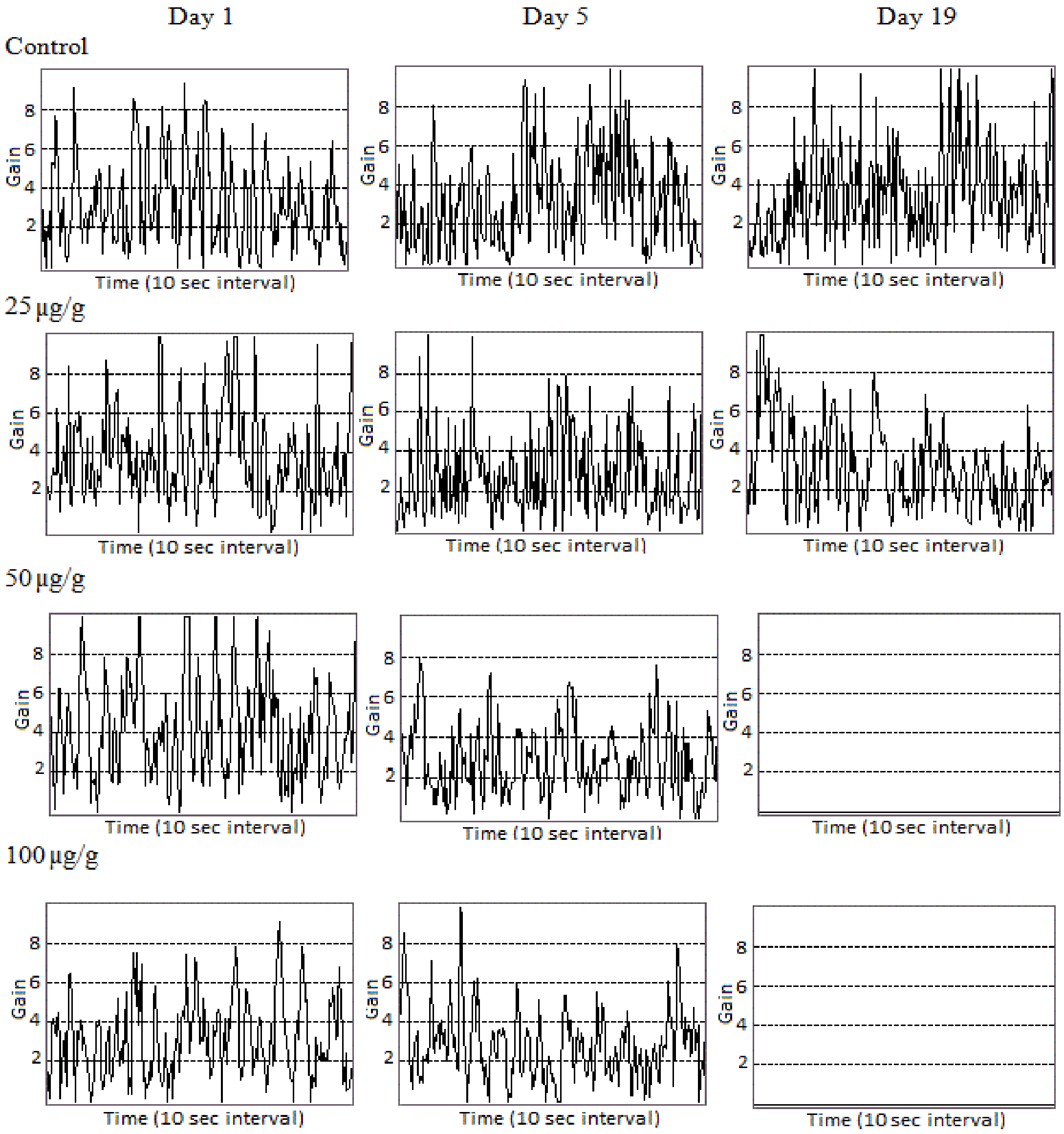


Figure 3.2. Graphs of the concentration effect at a fixed 1m distance generated by Termatrac T3i post-treatment to record eastern subterranean termite movement intensity. The graphs are represented by gain versus time in 10 sec intervals measured in the group site cylinder. Most termites showed Altriset intoxication in the group site rather than inside the treated tunnels or feeding sites.

Termite groups for both 50 and 100 µg/g soil treatments reached 100% mortality 19 days post-treatment. This was a significantly greater than that for 25 µg/g treatment (27%) and control (4%) ($F_{3.84} = 33.35$, $P < 0.0001$) (Figure 3.3). Dismantling of the experimental units for both 50 µg/g and 100 µg/g bioassays revealed only one out of ten cylinders contained a few paralyzed workers near death.

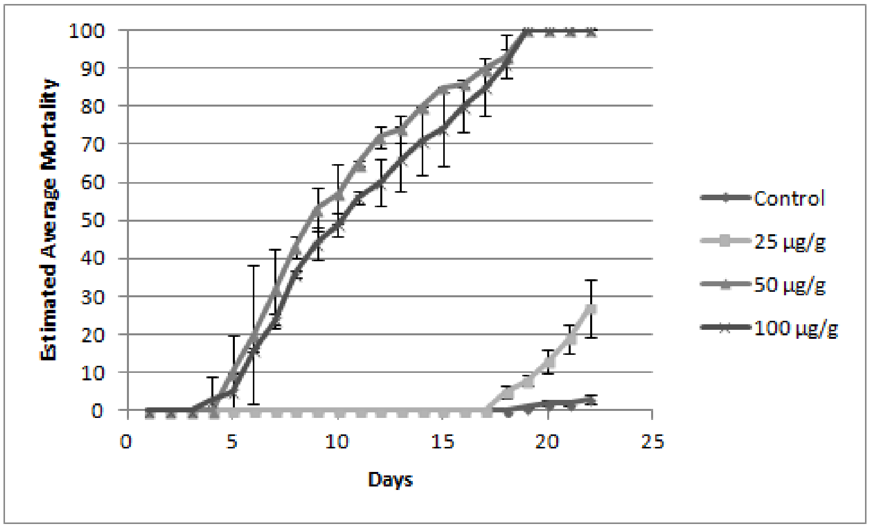


Figure 3.3. Estimated average termite mortality (mean ± SE) post-treatment for the concentration effect at a fixed 1m distance.

Experiment two: distance effect at a fixed concentration of 50 µg/g

A decrease in termite movement was first observed five days post-treatment in both lengths of 2m and 4m Tygon tube treatments. By day 22 no termite movement was detected by Termatrac in all bioassay units (Figure 3.4). Termites in the 2m and 4m long Tygon tube bioassay units discontinued feeding on the damp southern yellow pine food source (0g consumed) in comparison to the control (4m) (0.54g consumed).

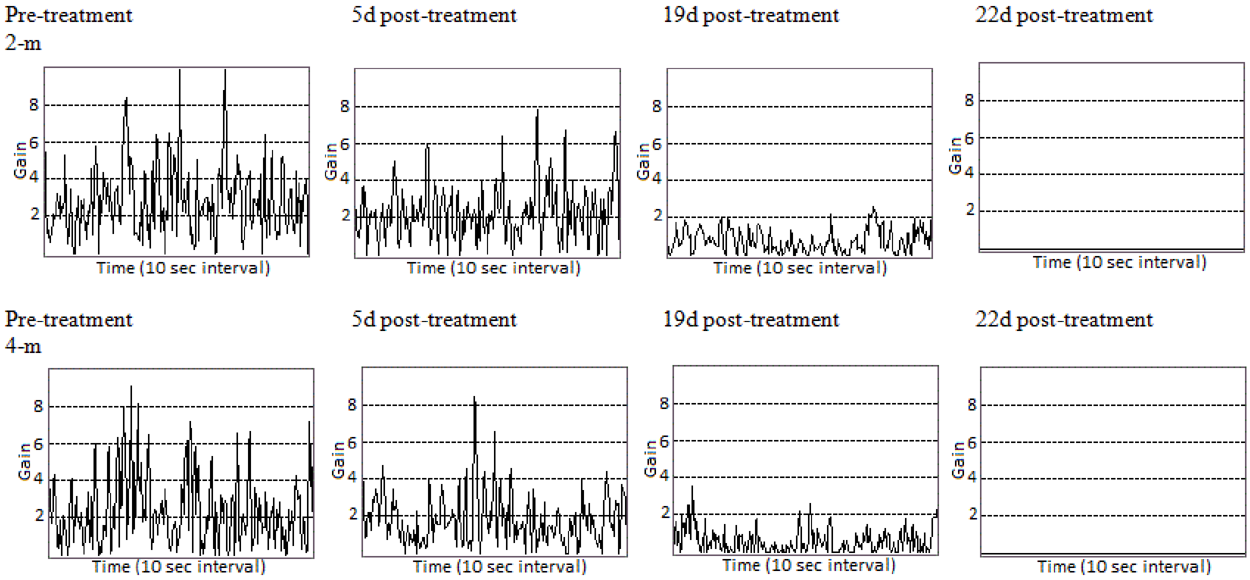


Figure 3.4. Graphs of the distance effect at a fixed concentration of 50 µg/g generated by Termatrac T3i Pre and post-treatment to record eastern subterranean termite movement intensity. The graphs are represented by gain versus time in 10 sec intervals measured in the group site cylinder. Most termites showed Altriset intoxication in the group site rather than inside the treated tunnels or feeding sites.

Complete mortality (100%) in termite populations was achieved in 22 days for both 2m and 4m length treatment in comparison to the control with less than 5% mortality. There was no significant difference between the two distances ($F_{2.63}$ = 27.03, *P* < 0.0001) (Figure 3.5). Dismantling of the experimental units revealed only two out of six cylinders had a few paralyzed workers near death.

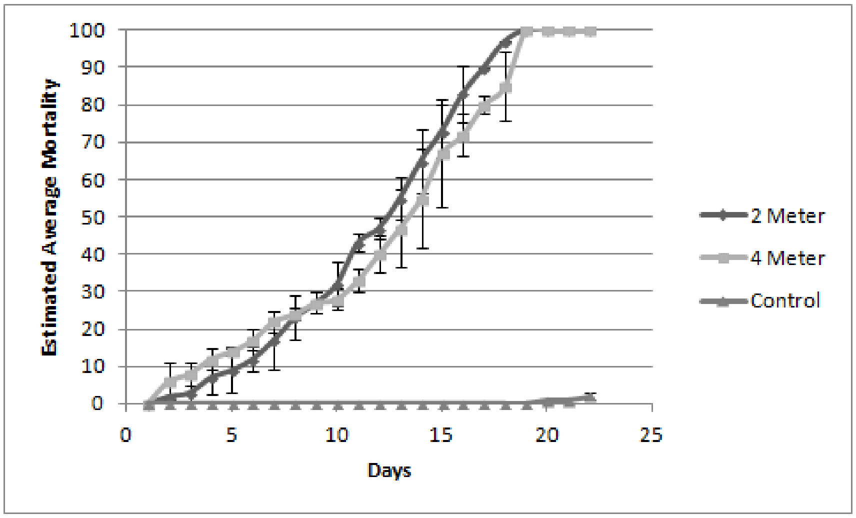


Figure 3.5. Estimated average termite mortality (mean ± SE) post-treatment for the distance effect at a fixed concentration of 50 µg/g.

In the 2m Tygon tube bioassay units, no tunnels were blocked by dying termites. However in the 4m long Tygon tube, one bioassay unit contained a blocked tunnel constructed by the dying termites. This incident was considered an outlier and was not counted as part of the experiment.

Experiment three: effect of soil application pre-tunneling

Termites reached the feeding site three days post-treatment and termite movement was affected due to exposure to Altriset while tunneling through the treated soil, compared to the unencumbered termites in the control unit. There was 100% reduction in termite movement 9 days post-treatment for the 25 µg/g soil concentration, and 12 days post-treatment for the 12.5 µg/g soil concentration (Figure 3.6). Termites exposed to both 25 and 12.5 µg/g soil treatments discontinued feeding on the damp southern yellow pine food source (0g consumed) in contrast to the uninhibited control (0.79g consumed).

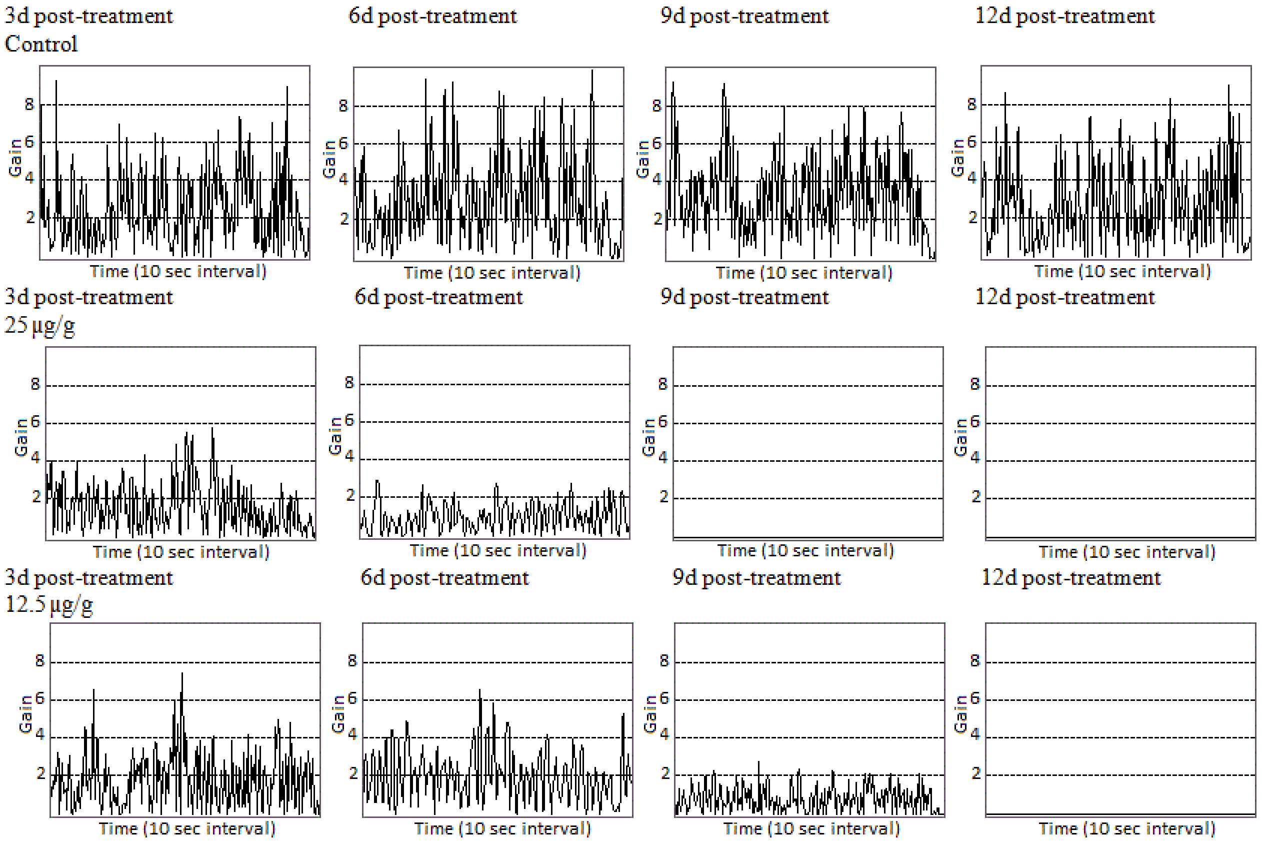


Figure 3.6. Application method graphs generated by Termatrac T3i post-treatment to record eastern subterranean termite movement intensity. The graphs are represented by gain versus time in 10 sec intervals measured in the group site cylinder. Most termites showed Altriset intoxication in the group site rather than inside the treated tunnels or feeding sites.

Complete mortality (100%) in termite populations was achieved in 9 days for 25 µg/g soil concentration, and 12 days post treatment for 12.5 µg/g soil concentration. The control group of termites did not experience group mortality during the experiment. There was no significant difference between the two Altriset concentrations, but both were significant relative to the control ($F_{2.33} = 29.75$, $P < 0.0001$) (Figure 3.7). Dismantling of the experimental units revealed only one out of ten had a few paralyzed workers near death.

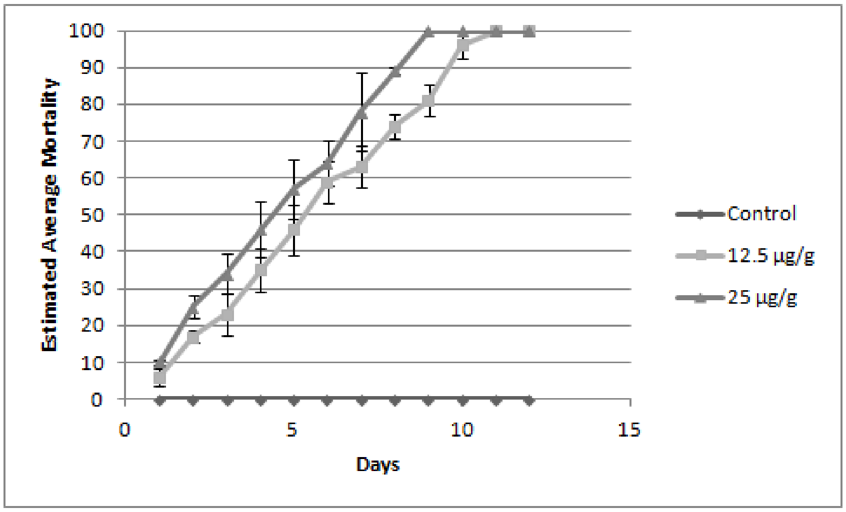


Figure 3.7. Estimated average termite mortality (mean ± SE) post-treatment for the effect of application method.

Discussion

This study tested a commercial insecticide of a new chemical class, the anthranilic diamides in the laboratory. The results demonstrated the effects of concentration, distance, and application methods for Altriset on eastern subterranean termites. During the first three days of the trial, termite groups were not visibly weakened. Termites readily tunneled through the treated soil as a result of Altriset's delayed action. Complete termite control was achieved by 19 days post-treatment at 100 and 50 µg/g, respectively. The treatment of 25 µg/g resulted in 27% control in 19 days, indicating that termites were more susceptible to the label rate and higher concentrations of Altriset when treating the soil and not directly to any established foraging mud tubes. The mud tubes are used primarily to protect termites from predators while maintaining a hospitable environment (Potter 2004). Consequently, the properties of the mud tube's protective barrier were somewhat successful in keeping lower concentrations of the Altriset treatment confined during this study. Gautam and Henderson (2011) have also noted that higher organic matter and clay content in the soil might play a role in hindering the bioavailability of the termiticide. Thus, the label rate and higher concentrations penetrated the mud tubes more proficiently in comparison to lower concentrations. When chlorantraniliprole is applied at 50 µg/g or greater, the active ingredient may precipitates within the soil resulting in increased bioavailability to termites (Spomer et al 2009).

In the second experiment, termite control was attained 22 days post-treatment at both 2m and 4m, indicating that distance was not significant at the same treatment concentration. It is possible the foraging distance was too short and the bioavailability of Altriset was efficacious, resulting in the termiticide being more readily taken up from the soil. Studies have shown that the active range of non-repellent termiticides is limited when treating a colony whose foraging range is ≥ 5 m (Osbrink et al 2005, Su 2005, Rust and Saran 2006, Saran and Rust 2007). Laboratory studies have also found that non-repellent termiticide effectiveness diminishes over time on soils that pose bioavailability problems (Gold et al 1996, Su et al 1993, Tamashiro et al 1987). To manage populations of eastern subterranean termites in a large area, a number of factors must be taken into consideration including the application method, the chemical class, and the concentration applied to the soil.

In the third experiment, termite control was achieved by 9 days post-treatment for 25 µg/g and 12 days post-treatment for 12.5 µg/g. These results indicate that termites were more susceptible to treatment concentrations below the label rate when treating the soil prior to the establishment of the mud tubes, in contrast to the 27% termite control of the 25 µg/g treatment when treating the soil and not directly to any established foraging mud tubes. Termites quickly acquired a lethal dose of termiticide when treating the soil prior to the establishment of the mud tubes by carrying the contaminated soil particles to construct foraging tubes. Oral route is thought to be the primary route of exposure for soil applied termiticides (Forschler 2009).

Currently soil treatment with non-repellent liquid termiticides are widely used as a control method for structural infestations of subterranean termites (Potter and Hillery 2002, Reid et al 2002, Wagner 2003, Ibrahim et al 2003, Remmen and Su 2005, Saran and Rust 2007, Mao et al 2011). The standard method incorporates drilling through the structure's foundation and then injecting termiticide into the soil, in addition to trenching and termiticide drenching the perimeter of the structure. This application method can be laborious, expensive, disruptive to properties, and require extensive amounts of termiticide while having a higher risk of water contamination. Therefore, it is optimal both economically and environmentally to perform localized soil treatments with liquid formulation Altriset. This study demonstrated that 100% mortality of termites was attained between 9-22 days post-treatment using this method.

Potential limitations of this study include blockage of the mud tube by dying termites, eliminating further contact from the rest of the colony with the treated soil**.** This was the case for the 4m long Tygon tube in experiment two, which led to the survival of the group population. These three experiments were conducted with variable termite populations of 200-2000 individuals, in contrast to the millions of termites in a typical colony found in the field (Grace et al 1989, Su et al 1993) that dead termites are usually removed by live termites (Hu personal communication). Furthermore, this study was conducted in a laboratory setting offering termites only one passage to one food source in a confined location, whereas termite colonies in the field can have multiple feeding sites, broad foraging areas, and several above-ground tunnels. The time frame we used to evaluate the treatment was 22 days which could be considered somewhat brief. This suggests that all of our treatments would have shown 100% mortality given more time, since lower doses of termiticide require a longer period of time to manifest symptoms of poisoning (Ibrahim et al 2003, Song and Hu 2006, Saran and Rust 2007). The correlation between laboratory results and actual field application data on termite colonies has yet to be investigated.

Many previous studies have investigated the influence of Altriset using topical and exposure bioassays, as well as transmission between termites. A laboratory study was conducted utilizing the method of topical application and exposure to treated soil to determine the toxicity of Altriset to eastern subterranean termites, *Reticulitermes flavipes* Kollar (Spomer et al 2009). Their reported lethal concentration dosages were comparatively high for Altriset at 48 hrs but more typical at 144 hrs. The uptake and potential transfer of Altriset by Formosan Subterranean Termite, *Coptotermes formosanus* Shiraki, was investigated in the laboratory using donor-recipient model bioassays (Gautam and Henderson 2011). They reported Altriset caused 100% mortality to donor and recipient termites 10 d post-treatment in a sand substrate using concentrations of 25 and 50 µg/g when compared with the control. The toxicity and the horizontal transfer of chlorantraniliprole was examined against eastern subterranean termites, *Reticulitermes flavipes* (Buczkowski et al 2012). They reported that chlorantraniliprole is readily transferred in termite colonies causing high secondary mortality. The efficacy of Altriset on Formosan Subterranean Termite *Coptotermes formosanus* Shiraki feeding rates, lateral transfer among nestmates, and to control infestation in a field trial was conducted (Puckett et al 2012). The study reported termites that were exposed to Altriset consumed significantly less food source and the mean percent mortality was significantly greater than unexposed termites. The bioavailability of Altriset in different soil types and the feeding activity of termite workers were assessed (Neoh et al 2011). The study reported the topical bioassay using Altriset caused 50%

mortality of *C. gestroi* at 7 d post-treatment. Exposure to Altriset resulted in >90% mortality to donor and recipient termites by day 14, and termite workers ceased to feed after 1 h of exposure. Currently there is no precedent on how localized soil treatment can affect termite populations of satellite locations. This study investigated this missing information through treatment concentration and distance using label rate and higher concentrations of Altriset, and the effect of an application method using lower than the label rate of Altriset. Future study will test this treatment concept on termite colonies in the field.

In conclusion, this study demonstrated the efficacy of Altriset against *R. flavipes* termite populations. The liquid formulation of Altriset can be used to bolster efforts toward better control. This study demonstrated the high efficiency of an Altriset treatment when applied properly, and can be considered a useful IPM tool which could potentially reduce termiticide exposure to humans and the environment.

CHAPTER 4

PERFORMANCE OF TERMIDOR H.E. AGAINST FUNCTIONAL COLONIES OF *RETICULITERMES FLAVIPES* (ISOPTERA: RHINOTERMITIDAE)

Abstract

A laboratory study was conducted to evaluate the effects between termite-fipronil and termite-termite at the colony level under simulated natural habitat conditions using infrared videotaping technology. Localized soil application of fipronil (1 ppm a.i.) of the product Termidor H.E. resulted in control of the tested colonies of eastern subterranean termite, *Reticulitermes flavipes* Kollar, in 50 days. We observed intensive grooming and contact between exposed and unexposed termites within the colony. The results showed exposed termites decomposed at the nest site where they received intensive grooming from active colony mates rather than die in the treated area or nearby adjacent tunnels. The presence of exposed termites neither repelled nor deterred the surrounding active colony mates. The results also demonstrated that the new formulation (Termidor H.E.) is effective against termite colonies.

Introduction

Subterranean termites *Reticulitermes flavipes* Kollar are one of the most destructive pests in the United States, with economic losses estimated at $3 billion per year (Su 2002). Termites' cryptic soil-dwelling nature makes their presence and damage difficult to detect until the evidence of a reproductive swarm (Thorne 1999). Their foraging network of multiple feeding sites makes termite control a great challenge. Currently the control of subterranean termites depends primarily on the use of liquid non-repellent termiticides for prevention and remedial treatment of structural infestations (Hu 2011). Non-repellent termiticides allow termites to forage through the treated soil acquiring exposure to the toxicant and affect other colony members through toxicity transmission (Hu et al 2006). Examples of currently registered termiticides include fipronil (Termidor, BASF Corporation, Florham Park, NJ), imidacloprid (Premise, Bayer Environmental Science, Research Triangle Park, NC), chlorantraniliprole (Altriset, Syngenta, Greensboro, NC), and chlorfenapyr (Phantom, BASF Corporation, Ludwigshafen, Germany).

Fipronil is one of the most commonly used compounds for termite management in the United States (Hu 2011). Fipronil acts on the insect's central nervous system by blocking the GABA (λ-aminobutyric acid) receptor and regulating chloride channels, resulting in excess neuronal stimulation and eventual death of the exposed insect (Cole et al 1993). The non-repellency and transferability of fipronil have been demonstrated in laboratory studies in many insect pests including German cockroach, *Blattella germanica* (L.) (Buczkowski and Schal 2001); Argentine ant, *Linepithema humile* (Mayr) (Soeprono and Rust 2004); and termite species (Ibrahim et al 2003, Shelton and Grace 2003, Song and Hu 2006, Gautam and Henderson 2011). Previous studies evaluating the formulation of fipronil suspension concentrate (SC) or water dispersible granules (WG) used donor-recipient model involving termite groups, except for Hu et al 2006 who utilized termite recipient colonies to elucidate the complex horizontal (among castes of the same colony generations) and vertical (foragers to castes of different generations within the colony) lethal transmission on control efficacy. These previous studies reveal various aspects of insect-insecticide interactions and offer insightful factors that may be useful to improve application methods of specific insecticides given their mode of action.

Most recently, a new fipronil formulation, Termidor H.E. Copack (9.1% fipronil), is developed by BASF (St. Louis, MO). Termidor H.E. Copack utilizes a new high efficient (H.E.) technology, a BASF-proprietary additive that "releases" the active ingredient into the soil with more precision and even distribution, with the advantage of utilizing 50% less water and 33% less labor than Termidor SC and Termidor WG application (Nagro 2012). This study investigated the non-repellency, toxic effect, and lethal transfer of fipronil formulation H.E. against laboratory recipient colonies of the eastern subterranean termite. The hypothesis was Termidor H.E. performs as effective as Termidor SC, if not better, against recipient colonies. This concept was tested using videotaping technology by examining 1) behavioral responses and outcome of the directly exposed termites and 2) survival of the recipient colonies.

Materials and Methods

Chemical

Liquid Termidor High-Efficiency Termiticide Copack containing Termidor H.E. Technology and 9.1% fipronil: 5-amino-1-(2,6-dichloro-4-(trifluoromethyl) phenyl)-4-((1,R,S)-(trifluoromethyl)sulfinyl)-1-H-pyrazole-3-carbonitrile was provided by BASF Corp. (BASF Pest Control Solution, St. Louis, MO).

Termites

Eastern subterranean termites were collected from field colonies in Auburn (Lee County, Alabama) using underground traps as described by (Hu and Appel 2004). Traps consisted of open bottom plastic buckets (18 cm high, 13 cm in diameter) provisioned with corrugated cardboard rolls (15 cm high, 11 cm in diameter). Closed bottom plastic buckets were used to transfer corrugated cardboard rolls with termites to the laboratory. Termites were extracted by gently tapping the cardboard to allow termites to drop onto a moist paper towel.

Soil

Soil was obtained from an area immediately surrounding a field termite colony in Auburn (Lee County, AL) that had not received any pesticide treatments previously. The soil was sieved to remove debris and sterilized in an autoclave (Thermo Fisher Scientific, Waltham, MA) at 60°C for 24 h. Prior to the experiment, distilled water was added to the soil (10 ml H_2O/100 g of soil), and moistened soil was used in experimental units.

Wood

Southern yellow pine was available at the laboratory. The wood was sterilized in an autoclave (Thermo Fisher Scientific, Waltham, MA) at 60°C for 24 h. Distilled water was added to fully saturate the wood two days before placing it in experimental units.

Experimental units

Each unit was composed of a glass box ($30 \times 20 \times 10$ cm^3) provisioned with fifteen blocks ($17.3 \times 2.5 \times 2.5$ cm^3) of moistened southern yellow pine located at one side covering 1/3 of the glass box unit. Approximately six thousand workers and soldiers (in natural ratio at collection) were then introduced, and the unit was covered with a glass lid. Visual inspections were conducted by viewing the wood-blocks from the bottom of the boxes. The appearance of 1st and 2nd instar larvae was used as an indication that a functional recipient colony was established. After the establishment of a colony, a layer of moistened soil (1 cm thick) was placed into the unit to cover the remaining 2/3 unoccupied area. Termites began excavating into the soil to establish a network of tunnels.

Treatment

A section of soil (8 × 3 × 1 cm^3) was then carefully removed from the middle of the opposite end of the wood blocks (nest site) and replaced with the same volume of treated soil containing 1 ppm fipronil that was stained with Nile Blue A (0.1%). The treated soil was prepared using 36 ml of water, 3 ml of 1% Nile Blue A, 0.0088g of Termidor H.E., and 400 g of dry soil. The dry soil was placed in a clean zip lock bag; water and Nile Blue A were added to the dry soil. The moist soil was kneaded to mix the soil and water-Nile Blue A solution well. Termidor H.E. was then added to the soil and kneaded again. There were three treatment colonies and three control colonies. The experimental units were positioned on the upper glass shelves in an incubator preset at 24 ± 1°C and the interior of the incubator was lined with black flannel to reduce reflections and ambient light from two red Sylvania bulbs (25 watt) using a dimmer control (APC Back-Up ES 550. Kingston, RI).

Videotaping technology

Termite colony activities within the units were videotaped 24 h/d during the entire study period. Inside the incubator, experimental units were positioned on the top of the glass shelf and three surveillance cameras (zmodo 8-channel dvr surveillance system) along with a camera (Sony DCR TRV800 with super night shot) were located on the lower glass shelf below the experimental units. This arrangement allowed the cameras to record termite activities from various angles and magnification levels. Video signals from the four cameras were split and captured at various time lapse rates by a VCR (Panasonic time lapse recorder) and a computer via an ATI video card (Figure 4.1). The tested colonies were dissected to count the number of surviving individuals after termite activity was no longer visible.

Figure. 4.1. Experimental design of the colony being videotaped 24 h/d during the entire study period in an incubator set at 24 ± 1°C. The incubator interior was lined with black flannel to reduce reflections and ambient light from the two red Sylvania bulbs (25 watt) under a dimmer control. The arrangement allowed the camera to record termite activities and send the captured videos from the camera to a VCR (Panasonic time lapse recorder) and a computer via an ATI video card.

Data collection

All six colonies (three replicates of the treatment and the control) were dissected at 50 days post-treatment and recorded by counting the number of surviving individuals.

Data analyses

Number of surviving termites was analyzed using one-way analysis of variance (ANOVA). Tukey's honestly significant difference (HSD) was performed following ANOVA to determine significant differences between treatment and control (SAS Institute 2008). The statistical analysis was conducted at the $P < 0.05$ level of significance.

Results

Viewing from the bottom of the unit illustrated that, before treatment, multiple tunnels were built in the glass boxes within 48h (Video 1). However, the tunnels along the edges of the glass boxes were constructed more rapidly and were completed within 24h. Reproductives and young instars existed in the colony nest but only the young instars were visible during inspections of the glass box. The common social interactions between termites included physical contact and grooming (cleaning).

Behavioral responses and outcome of termites after treatment

Workers tunneled into the stained treated soil immediately following introduction of treated soil (Video 2a, 2b). Exposed termites remained active for at least 14h prior to showing any symptom of poisoning. During the 14h post-treatment period, the colonies remained active with termites frequently traveling between the treated zone and the nest site while carrying out social interactions such as contacting and grooming (Video 3a, 3b). Between 14-24 hr period termites' movement decreased in the colony site. The abnormal behaviors of exposed termites became obvious approximately 24 hours post-treatment at the nest site and nearby tunnels, an indication that exposed termites did not lose mobility quickly and were not confined to the treated area. Directly exposed termites were identified by their acquisition of Nile Blue A, a liquid dye that was mixed with the treated soil which served as a prominent visual marker. Simultaneously, other termites in and outside the nest were still active.

Poisoned termites exhibited impaired mobility that resulted in twitching, convulsions, and death (Video 4). As the number of poisoned and dead workers and soldiers increased at the nest site, the number of termites traveling in treated soil and tunnels decreased (Video 5). After 30 days post-treatment, the nest site contained a greater amount of dead termites than the few found within the tunnels adjacent to the nesting site. Forty days post-treatment, no live termites could be viewed from outside of the glass unit.

Video footage clearly depicted the presence of dying termites did not affect the normal activities of surrounding colony members, whether they were unexposed or had not yet exhibited poison symptoms. Instead, dying termites had a tendency to attract active colony members to provide them with intensive grooming and care (Video 7). Active colony members were attempting to remove the toxicant, unsuccessfully minimizing the resulting lethal effect on the dying termites caused by fipronil. Poisoned termites were more likely to be cleaned by active workers, suggesting that grooming was one of the mechanisms for the transfer of fipronil.

Survival of the recipient colonies

All of the treatment colonies had 100% mortality of the first and second instar and an absence of eggs. Treatment colonies had a few survivors resting inside wood blocks. However, none of these surviving termites were functional, as further observation detailed that termites were covered with fungus, had impaired mobility, and were close to dying. In contrast to the treatment colonies, the three control colonies remained active and productive, with their populations increasing by the end of the experiment ($F_{1,4} = 736.7$, $P < 0.0001$).

Discussion

The functional colony model in this study provided the advantages of observing termite activities under undisturbed conditions that closely simulates termite natural habitat. Therefore, the results of this study should be more representative of what can be expected in the field.

Video footage revealed that termite workers that directly acquired fipronil remained active for at least 14 h post-treatment compared to termite workers that did not directly expose themselves to treated soil but indirectly acquired fipronil from contaminated colony members. The above statement explains why active termite workers were observed traveling between the treated site and the nest site carrying out social interactions. Another possible explanation is the division of labor among workers. Older workers are better at tunneling, foraging, and consuming many times the amount of food than younger workers (Crosland et al 1998). It can be suggested in this study; some older workers (i.e. larger) were responsible for constructing the tunnels and were the first ones to exhibit the abnormal behaviors leading to death. Other older workers were designated foragers that did not construct the tunnels, but only traveled within them searching for food after the tunnels were built. Young workers are fragile and carry out less work while specializing in few or no tasks. Young workers (medium to small) in this study provided care to nest members and remained inside the colony nest. Due to relative proximity to the treatment, it took a longer period of time for younger workers in comparison to the older workers (the first ones to exhibit the abnormal behaviors leading to death) to acquire the lethal dose of fipronil resulting in death.

This study also observed one that contaminated termites preferred a specific location to die, confirming the observation by Hu et al 2006, who reported that the exposed termites manage to return to the nest site and died there, rather than dying inside the treated zone and two dying termites received intensive grooming and care from colony members rather than being isolated. Buczkowski and Schal (2001) also showed that first instar German cockroaches were highly attracted to moribund cockroaches. A potential explanation is that active colony members were trying to remove the toxicant to minimize the lethal effect caused by fipronil, but when the contaminant is a non-repellent termiticide (i.e. fipronil), these social interactions create a chain reaction, promoting the spread of the termiticide throughout the colony. Soil application of fipronil takes advantage of the vulnerability termites have from being social.

The colony was dissected 50d post treatment as opposed to an earlier time in order to ensure 100% mortality was achieved. Based on our previous and current work when using the Termatrac T3i or when our own observations show there is no more termite activity, it does not guarantee all of the termites are dead, but only not producing detectable activity. They could be immobile, paralyzed, or sedentary. We chose to perform the dissection 50d post treatment which gave plenty of time for the termites to perish. Even using this precaution, there were some still alive that were twitching, paralyzed, etc.

In conclusion, this study demonstrated that localized soil application of 1 ppm a.i. can suppress and eventually kill termite colonies. Control efficiency is achieved through the complex process within the colony. This is likely resulted from the interactions between contaminated termites (those directly and indirectly exposed to fipronil) and unexposed termites at the colony site. The observations of this study were consistent with those reported by Hu et al 2006 thus; Termidor H.E. performed as effective ,if not better, than Termidor SC against recipient colonies.

It is important to note that in field conditions, colonies can reach populations ranging from thousands to millions of individuals. Termites create large and complex tunnel systems with multiple satellite locations over many years, and also develop sub-colonies with multiple food sources that are distant from the treatment sites. These factors along with biotic and abiotic variables can affect the acquisition of a lethal dose. Therefore, extrapolating data from laboratory experiments to field conditions should be done with precaution.

CHAPTER 5

THE POPULATION EFFECT OF TREATING ABOVE-GROUND TUNNEL WITH RTU DRY TERMIDOR ON EASTERN SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE)

Abstract

The efficacy of a dry, ready-to-use (RTU) Termidor was evaluated against eastern subterranean termites, *Reticulitermes flavipes* Kollar, in a laboratory study when aboveground foraging tubes are directly treated. Termite populations were placed in glass bioassay units consisting of yellow pine wood blocks as a food source and moist soil. A mixture of RTU Termidor and fluorescent blaze orange pigment powder was randomly injected one (treatment 1) and two (treatment 2) established above-ground tunnels. The fluorescent blaze orange pigment powder alone was the treatment for the control bioassay and was injected into one established above-ground tunnel. Bioassay units were monitored for termiticide transfer and termite movement by observation and implementation of a Termatrac T3i until no movement was detected. In treatment 2, the transfer of dry RTU Termidor ceased within 10 hrs compared to treatment 1 where it discontinued 2 d post-treatment. Termite movement ceased after 5 d for both treatments, respectively. Dissection of the bioassay units revealed 100% mortality within treatment 2 while treatment 1 had a few live/paralyzed workers and soldiers that were hiding in a feeding gallery inside the wood blocks. Our results provided strong evidence for the efficacy of dry RTU fipronil formulation against *R. flavipes* populations when above tunnels were treated.

Introduction

Termite management programs are focused on the development of more effective, environmentally friendly, and less expensive methods. Consequently, new chemicals with novel modes of action are being introduced into the market, but also changes in formulations of already marketed pesticides are being tested (Gautam et al., 2012). Various termiticide formulations have been used for termite control including liquid, foam, and granule. However termite control has largely depended on the use of non-repellent and delayed action properties of liquid formulations that are applied around structures for the prevention and treatment of infestations (Gahlhoff and Koehler, 2001). These termiticides include imidacloprid (Premise), fipronil (Termidor), chlorfenapyr (Phantom), indoxacarb (Aperion), and chlorantraniliprole (Altriset) (Potter and Hillary, 2002; Wagner et al., 2002; Remmen and Su, 2005; Hu and Hickman, 2006; Spomer et al., 2009; Mao et al., 2011). The treatment involves trenching the soil and drenching the exposed section with termiticide, or drilling into structural foundations and injecting the termiticide. The application methods create a termiticide barrier around structural perimeters and under foundations. These applications can be laborious, expensive, disruptive to properties, and require extensive amounts of termiticide while having a higher risk of water contamination. A possible alternative is the use of localized treatment with dust formulation.

The current study evaluated the efficacy of treating an above-ground termite tunnel with the new Ready-To-Use dust formulation of fipronil (RTU Dry Termidor). Fipronil was identified in 1987 by the French company Rhône-Poulenc and registered in the United States in 1996 as a broad spectrum insecticide in the phenyl pyrazole class (Rhône-Poulenc, 1995). Fipronil interferes with the function of the central nervous system by blocking the GABA (λ-aminobutyric acid) receptor and regulating chloride channels, resulting in excess neuronal stimulation and eventual death of the exposed insect (Cole et al., 1993). Due to its mode of action, termites are able to tunnel through the treated soil to acquire a lethal dose, transfer it to the unexposed termites through social interaction, and cause secondary mortality within the termite colony (Thorne and Berisch, 2001). Compared to soil application of liquid formulations, the benefits of using localized treatment method include an overall volume reduction of chemical product and labor cost.

The use of dust formulations against termites has been established as a reliable treatment method to suppress and eliminate termite populations (French, 1994; Madden et al., 2000; Green et al., 2008). Grace (1991) reported topical applications of borate dust in a glass Petri dish caused 100% mortality to *C. formosanus* (Shiraki) and *R. flavipes* (Kollar) workers. Gautam et al. (2012) demonstrated that 0.5% fipronil dust effectively killed *C. formosanus* and effectively transferred from treated to non-treated individuals. Barwary et al. (2013) reported evidence for the efficacy of 0.5% fipronil dust against *R. flavipes* activity at the group level when a single aboveground tunnel is treated. The dust formulation has been recently implemented, thus it is important to know its efficacy on eastern subterranean termite populations when aboveground foraging tubes are directly treated. The objectives were to determine if (1) RTU Dry Termidor will be transferred within termite populations at the tested dose, (2) the efficacy of treating above-ground tunnels by observing behavioural changes and population dynamics using Termatrac T3i, and (3) to assess the fitness of the termite population.

Materials and Methods

Termites

Reticulitermes flavipes were collected from field colonies between June to August 2013 in the city of Auburn, Alabama (Lee County) using underground traps described by Hu and Appel (2004). Traps consisted of open-bottom plastic buckets (18 cm high, 13 cm in diameter) provisioned with corrugated cardboard rolls (15 cm high and 11 cm in diameter) that were placed in the ground. Closed-bottom plastic buckets (18 cm high, 13 cm internal diameter) were used to transfer corrugated cardboard rolls with termites to the laboratory. Approximately ten thousand termites were extracted by gently tapping the cardboard to allow termites to drop onto a moist paper towel and then placed inside glass bioassay units.

Soil

Soil was obtained from the area immediately surrounding the location where the termites were collected. The soil used in this study was not known to have received any prior pesticide treatment. The soil was sieved to remove debris and sterilized in an autoclave (Thermo Fisher Scientific, Waltham, MA) at 60°C for 24 h, two days prior to the experiment. Distilled water was added to the soil (10 ml H_2O/100 g of soil), moistened soil was then used to fill glass bioassay units.

Chemical

Termidor® Dry, a dry RTU formulation containing 0.5% active ingredient fipronil [5-amino-1-(2,6-dichloro-4-(trifluoromethyl) phenyl)-4-((1,R,S)-(trifluoromethyl)sulfinyl)-1-H-pyrazole-3-carbonitrile] was provided by BASF Corporation (BASF Pest Control Solutions, St. Louis, MO). RTU Termidor (50 mg) was mixed with fluorescent blaze orange pigment powder (2.5 mg) (Day-Glo Color Corp. Cleveland, OH) to verify that termites have tunnelled through the treated area and the termiticide has transferred to the untreated tunnels. This verification was confirmed by visual inspection and black light photography.

Bioassay units

Bioassay units were composed of a glass fish tank (30.5 x 15 x 20 cm^3) containing three moistened southern yellow pine blocks (17.3 x 2.5 x 2.5 cm^3) located at one side covering 1/3 of the unit. Termites were then introduced, and the unit was covered and sealed with a plexiglass lid and black flannel to maintain moisture. Bioassay units were kept inside an incubator at 24 ± 1°C, except at the time of weekly inspections. After the establishment of a population, a layer of moistened soil (1 cm thick) was placed into the unit to cover the unoccupied area and a plexiglass divider was placed inside the unit at 45 degree angle for establishment of tunnels. Termites began excavating into the soil to establish below ground tunnels and build aboveground shelter tunnels onto the divider. There were three population replicates of each treatment and control.

Treatment

Aboveground tunnel injection with dry RTU Termidor was applied after establishment of below and above ground tunnel networks. One dose (50 mg) of dry RTU Termidor was tested. The dosage was selected on the basis of a previous study by Barwary et al. (2013) and to reflect the relative toxicity of the termiticide against termite colonies. The fluorescent blaze orange pigment powder was used for the control. The mixture of the designated dose of dry RTU Termidor and fluorescent blaze orange pigment powder were injected into the tunnels using half compression from a proprietary compression bulb applicator. This application resulted in approximately 50 mg of formulation applied to each tunnel. The applicator forced air and the dry formulation into the termite tunnel, with the tip of the applicator aimed at the group site at a 45 degree angle. This allowed the treatment to be spread uniformly inside the tunnel without blocking the tunnel at the treatment point. For treatment 1, one above ground tunnel was randomly selected and injected with the mixture containing 50 mg RTU Termidor. For treatment 2, two aboveground tunnels were randomly selected and injected with the mixture containing 25 mg RTU Termidor per tunnel, totaling 50 mg. For the control, one aboveground tunnel was injected with only fluorescent blaze orange pigment powder. The rest of the above ground tunnels were left untreated. The bioassay units were positioned on the upper glass shelves in an incubator preset at 24 ± 1°C and the interior lined with black flannel to reduce reflections.

Data collection

The transfer of dry RTU Termidor by termites in below ground tunnels was visually observed using a black light (Inova Microlight. Nite Ize, Boulder, Colorado). Data was collected by counting the spots within below ground tunnels and chambers at intervals of 3hrs, 5hrs, and 10hrs post-treatment on the first day. Subsequent data was collected 1d, 2d, 4d, and 8d post-treatment. The designated range was 0 to greater than 100 spots.

The population dynamics were recorded using Termatrac T3i (Termatrac Australia Pty Ltd. Queensland, Australia) during the time interval mentioned above. The Termatrac is a 3-in-1 device that comes with a remote thermal sensor with a laser guide, a built-in moisture sensor, and patented termite detection radar. It emits a fixed frequency microwave beam and measures the intensity and frequency of signals reflected back to a collocated receiver (Tirkel et al., 1997; Protec USA, 2002). A signal processor computes the intensity of the reflected energy and the difference between emitted and reflected frequencies, and displays the result on a Bluetooth (Bluetooth Special Interest Group, https:// www.bluetooth.org/en-us) wireless PDA (personal digital assistant). Termatrac can detect motion or vibration by any object in the field of view whose dielectric constant (a relative measure of the capability to store electric charge) differs from that of the substrate (Edde, 1993; Protec USA, 2002). Any moving objects not of interest were excluded from the field of view. Termatrac T3i was positioned under each bioassay unit, and the detection radar signals were calibrated to identify termite location and intensity of activity which was measured in gain (y-axis) over time (x-axis), positively correlating gain with movement intensity. The signal graphs were sent to a computer and used as responding value.

The fitness of the termite population was recorded at the end of the project by dissecting the tested populations and counting the number of surviving individuals after termite activity was no longer visible.

Data analyses

The average number of spots of dry RTU Termidor transferred by termites in underground tunnels for each replicate per each time interval was analyzed. The fitness of the termite population was analyzed as well using one-way analysis of variance (ANOVA). Tukey's honestly significant difference (HSD) was performed following ANOVA to determine significant differences between different treatments (SAS Institute, 2008). The statistical analysis was conducted at the $P < 0.05$ level of significance.

Results

The transfer of dry RTU Termidor by termites was observed in the below ground tunnels of the bioassay units within 3 hours post treatment. This observation was evaluated by the presence of exposed fluorescent termites and their trails present at the bottom of the units. In treatment 2 (total of 50 mg per two tunnels injection, 25 mg per tunnel) the transfer of dry RTU Termidor ceased after 10 hours post treatment compared to treatment 1 (total of 50 mg per one tunnel injection) where transfer of dry RTU Termidor discontinued 2 days post treatment. While the control containing only fluorescent blaze orange pigment powder demonstrated distribution greater than 100 spots throughout the entire in ground territory of the bioassay units within 3 hours post treatment. Treatment 2 resulted in greater transfer in comparison to treatment 1. Both treatments 1 and 2 were significant from the control and significantly different from each other ($F_{2.18} = 56.19$, $P < .0001$) (Figure 5.1).

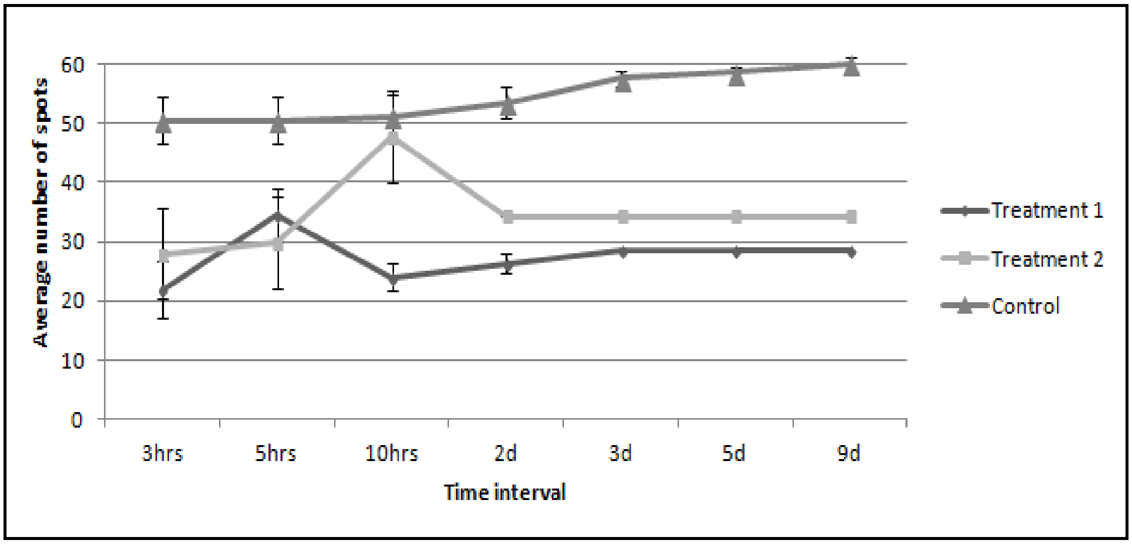
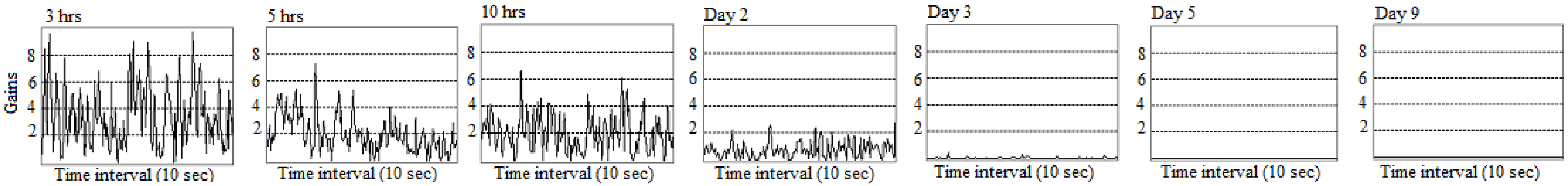


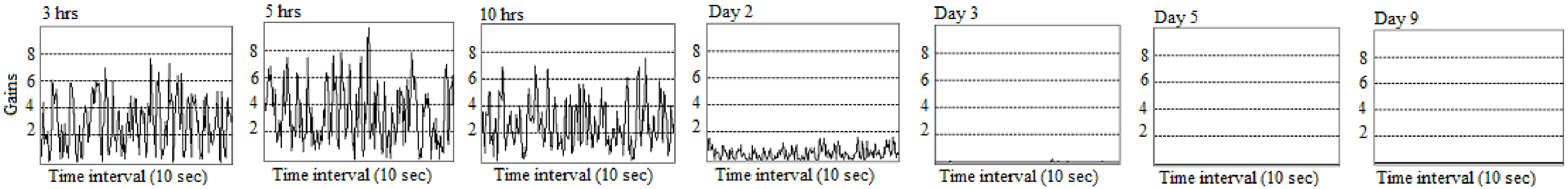
Figure 5.1. Mean ± SE of the number of spots of dry RTU Termidor transferred by termites in below ground tunnels for each replicate per each time interval.

The cessation of activity/movement was observed after 10 hours post treatment for the 2 tunnel injection and the 1 tunnel injection treatment. Visual observations and Termatrac T3i reading showed both treatments resulted in no movement at five days post treatment in comparison to the control (Figure 5.2). Termatrac T3i readings in this paper represent underneath the food source measurements. This reason we chose this is that it would be a representative of using it against a wall in a home or against a structure in the field. Visual observations of termite activity/movement were corroborated by Termatrac T3i readings.

Treatment 1 (1 tunnel injection)



Treatment 2 (2 tunnels injection)



Control

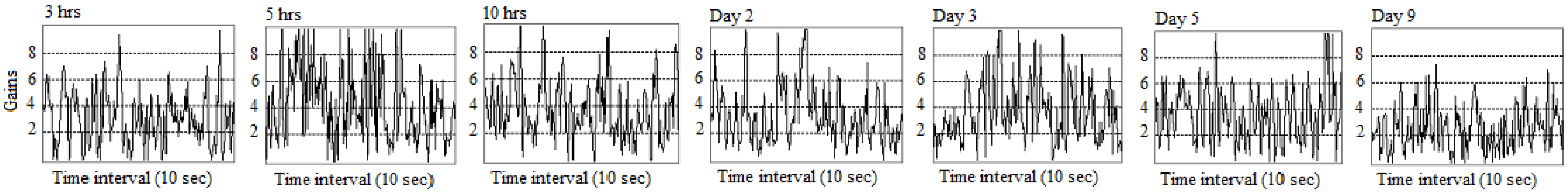


Figure 5.2. Measurement of the termite population generated by Termatrac T3i post treatment recording activity/movement intensity. The graphs are represented by gain versus time in 10 sec intervals measured in the bioassay units underneath the food source. This would be a representative of using it against a wall in a home or against a structure in the field.

After termite activity/movement was no longer visible using Termatrac T3i (9 days post treatment), the bioassay units were dissected and the number of surviving individuals were counted. Dismantling of the bioassay units revealed 100% mortality in treatment 2 while treatment 1 had a few live/paralyzed workers and soldiers that were hiding in a feeding gallery inside the wood blocks. The control showed 100% live workers and soldiers by the end of the project. Both treatment 1 and 2 were significant from the control ($F_{2.6} = 15.97$, $P < 0.05$).

Discussion

This study tested dry RTU formulation of fipronil against *R. flavipes* populations using a localized treatment technique. The results from this study demonstrated that localized treatment of two tunnels with dry RTU Termidor near feeding sites was more effective for the control of termite populations. In treatment 2, the transfer of dry RTU Termidor by termites discontinued after 10 hours post treatment compared to treatment 1 where it ceased 2 days post treatment. Termites were able to transfer a greater amount of dry RTU Termidor in a shorter period of time in treatment 2 compared to treatment 1, as shown by the presence of fluorescent powders. This indicated that the 2 tunnel injection increased the bioavailability of the termiticide in comparison to the 1 tunnel injection. This resulted in cessation of activity/movement after 10 hours post treatment for the 2 tunnel injection and 2 days post treatment for the 1 tunnel injection.

Termite population control was achieved by 9 days in both treatments. Nonetheless, when the bioassay units were dissected it revealed that treatment 1 had a few live/paralyzed workers and soldiers that were hiding in a feeding gallery inside the wood blocks. These results indicate that termites were more susceptible to the 2 tunnel injection compared to the 1 tunnel injection, in contrast to the 0% termite control in the control bioassay. Termites more readily acquired a lethal dose of termiticide when treating 2 above ground tunnels compared to treating 1 tunnel. Therefore, transfer rate of dry RTU Termidor by exposed fluorescent termites was increased for the 2 tunnel injection. This could have potentially occurred through common social interactions between termites that included physical contact and grooming (cleaning).

Many previous studies have investigated the efficacy and the influence of localized treatment utilizing dry RTU Termidor against termites. Barwary et al. (2013) studied the efficacy of a dry, ready-to-use (RTU) fipronil against *R. flavipes*' foraging activity and survival using localized treatment on foraging tunnels. The authors reported 100 % mortality and complete reduction in termite movement between day 5 and day 7. It was also reported that termites in the treated units constructed significantly fewer tunnels post-treatment compared to control termites. They concluded their results provided strong evidence for the efficacy of the dry RTU fipronil formulation against *R. flavipes* activity at the group level when a single tunnel was treated.

Hickman and Forschler (2012) evaluated the efficacy of three ready-to-use (RTU) formulations: imidacloprid foam, a dry fipronil formulation, and an experimental fipronil formulation by using the localized treatment method on cypress lumber naturally infested by the drywood termite *Incisitermes snyderi* (Light). They reported that the dry RTU fipronil formulation and foam imidacloprid performed significantly better and provided evidence of elimination of infestation without removal of every board that was treated. The authors proposed that less than 100% elimination was due to the difficulty in treating all termite galleries and the short duration of the trial, which may not have allowed enough time for unexposed termites to contact treated galleries. However, for subterranean termites, the above-ground tunnels are obvious and can be accurately treated with termiticide.

Gautam et al. (2012) assessed the effect of localized treatments with commercial dust and liquid formulations of fipronil against *C. formosanus*. They reported >91% of the termites were dead within 9 days. The authors concluded that localized (or spot) treatment with either commercially available dust or liquid formulations of fipronil can be a viable option for control of termite infestations where complete soil drenching is not desirable. In addition to the above studies, this experiment provided supplemental evidence that localized treatment with a dry RTU formulation of fipronil to two established aboveground active tunnels disrupted termites to a point where no population recovery could occur. Future studies will test this treatment concept on termite colonies in a laboratory setting, and subsequently in the field.

In conclusion, this study provided evidence of the effectiveness of treating aboveground tunnels using powdered formulations against *R. flavipes*. The application method of localized treatment is an important step in reducing termiticide exposure to humans and the environment. It is critical for termite control methods to be continuously researched and to reflect newer, more advanced technologies.

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