BEHAVIORAL TOXICOLOGY OF THE EASTERN SUBTERRANEAN TERMITE, *RETICULITERMES FLAVIPES* (KOLLAR) (ISOPTERA: RHINOTERMITIDAE)

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DISSERTATION ABSTRACT

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The eastern subterranean termite *Reticulitermes flavipes* (Kollar) is one of the most economically important termite species in the United States. Behavioral toxicology has become very relevant in the control of termites because the non-repellent compounds used are specifically designed to exploit behavioral biology of termites for optimum performance. To describe insecticide-induced behaviors, termites were exposed continuously to filter paper that had been treated with 200 ppm indoxacarb, 50 ppm fipronil, 300 ppm chlorantraniliprole, and distilled water (i.e., control treatment). The neurotoxins, fipronil and indoxacarb elicited toxicity symptoms that progressed from incipient intoxication, through ataxia, and moribundity which culminated in death.

Chlorantraniliprole-treated termites did not exhibit ataxia but remained on their feet and became increasingly lethargic until they became moribund. Termites were exposed to 45, 90, 135, and 180 ppm (w/w) of indoxacarb-treated soil for 5, 10, 20, 40, 80, and 160 min to test for effects on onset of abnormal behaviors. Earlier onset of abnormal behaviors was observed at higher concentrations and longer durations of exposure to indoxacarb at both individual and group levels. Termites in the walking and tunneling studies were exposed to filter paper treated with fipronil (1, 10, and 50 ppm) and indoxacarb (50, 100, and 200 ppm) for 10 min. Total distance walked in 60 s was measured at 2-h intervals until cessation of walking. Distance walked remained fairly constant in the control treatments but declined significantly in the insecticide-treated termites. In termites treated with 100 ppm indoxacarb, the first significant decline occurred in 8 h (from 77.95 ± 3.81 cm to 41.92 ± 7.40 cm [$F = 24.66$, $df = 7$, $P < 0.0001$]) but it took only 2 h to decline from 73.70 ± 3.19 cm to 58.43 ± 3.80 cm ($F = 165.29$, $df = 7$, $P < 0.0001$) in termites treated with 200 ppm indoxacarb. A similar trend was observed among fipronil-treated termites. Tunneling ability was tested in transparent cylindrical plastic containers containing untreated soil (18% moisture w/w). Untreated termites constructed more and longer tunnels ($P < 0.05$) compared to those treated with insecticides. Distance tunneled by termites declined with time after exposure to indoxacarb ($F = 6.16$, $\mathrm{df} = 7$, $P < 0.0001$) and fipronil ($F = 6.04$, $\mathrm{df} = 7$, $P < 0.001$). Our results show that well-defined abnormal behaviors can be used in quantitative assessments of toxicity and thus complement mortality in the assessment of insecticide performance.

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CHAPTER I: GENERAL INTRODUCTION

Termites belong to the isopteran order of insects, so named because of the equal shape and venation of the fore and hind wings. Even though over 2,600 termite species have been described (Kambhampati and Eggleton 2000) only 183 species worldwide are known to attack buildings; 83 of these species cause significant damage (Su and Scheffrahn 2000). About 280 genera and seven families of termites have been recorded worldwide (Engel and Krishna 2004). Termites cause significant damage when they attack wood in the quest for cellulose, their staple food. In the United States, economic loss due to termites is conservatively estimated at $11 billion (Su 2002). Rhinotermidae, the most economically important family has an economic value of $ 3 billion per annum (Su 2002), $1 billion of which is attributable to the eastern subterranean termite (Oi et al. 2003) which has a wide geographical distribution (Su 1996). The Formosan subterranean termite is actually acknowledged as the most aggressive and potentially destructive termite species in the United States. They cause more and faster damage to structures than the *Reticulitermes* spp. mainly because their populations tend to be larger (Su and Scheffrahn 2000). They have also been known to displace areas originally occupied by *Reticulitermes* spp. when the two species cross paths (LaFage 1987).

According to Eggleton (2000) termites radiated from the tropical rainforest where the greatest species diversity is found. Studies in termite biodiversity and biogeographical distribution show nine regions namely: Australasia, Afrotropical, Malagasy, Nearctic, Oceania, Neotropical, Oriental, Palaearctic, and Papuan. Temperature and water conditions are major determinants of this distribution pattern. The United States falls within the Nearctic region which includes the section of the American continent located north of the Tropic of Cancer and South of the Arctic Circle. The list of five most important termite species in the United States used to include: *Cryptotermes brevis* Walker, *Incisitermes minor* Hagen, *Coptotermes formosanus* Shiraki, *Reticulitermes flavipes (Kollar)*, and *Reticulitermes hesperus* Banks (Kofoid 1934; Weesner 1965; Edwards and Mill 1986) but Forschler and Lewis (1997) revised the list by replacing *C. brevis* and *I. minor* with *Reticulitermes virginicus* (Banks) and *Reticulitermes tibialis* (Banks); The authors were not unanimous on the economic impact of *Incisitermes snyderi* Light, *Marginitermes hubbardi* Banks, *Heterotermes aureus* Snyder and *Reticulitermes tibialis* Banks. The limited distribution of these species however, renders them less economically important than the first group (Su and Scheffrahn 1990a). The importance of *R. flavipes*, *R. virginicus, R. hesperus, R. tibialis, and C. formosanus* in the United States is underscored by the fact that they are the subject of 90% of the treatment efforts (Forschler and Lewis 1997). The eastern subterranean termite, *R. flavipes*, and the Formosan subterranean termite, *C. formosanus* cause the most damage in the United States (Su and Scheffrahn 1990a). 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). The spread of termites is enhanced by human activities such as movement of wood products from termite-infested to termite-free locations.

Systematics

The insect orders, Mantodea (mantids) and Blattaria (cockroaches) constitute the superorder Blattodea which together with the Isoptera (termites) constitute the Subtercohort Blattarida (Boudreaux 1979). Termites, mantids, and cockroaches form a monophyletic group, the Dictyoptera. The isopterans which are members of the orthopteroid group of insect orders (Hennig 1981), are thought to share a common ancestor with the Blattaria. This is based on the fact that the proventriculus and female genitalia of Blattaria are morphologically similar to those in members of the termite family, Mastotermidae (McKittick 1965). *Mastotermes darwiniensis* Froggatt the only extant representative of Mastotermitidae (Emerson 1965) oviposits in masses that resemble oothecae of Blattaria (Hill 1942). *Mastotermes* and Blattaria both possess symbiotic protozoa (Thorne and Carpenter 1992); termites in the family Cryptocercidae (wood feeding cockroaches) actually share many protozoan species with *M. darwiniensis* (Emerson 1965) and are their closest relatives (Eickwort 1981). This degree of relatedness is however not supported by cladistic analysis of morphological and behavioral characters (Thorne 1991).

Higher termites have a terminally differentiated worker caste which distinguishes them from workers of lower termites which are immature forms that retain the ability to differentiate to soldier or reproductive caste phenotypes (Myles and Nutting 1988). In differentiating into soldiers these workers go through a presoldier stage (Noirot 1985; Henderson 1998). The higher termites, Termitidae (Lenz 1976) and Serritermitidae (originally serritermitinae, a subfamily of Termitidae) represent the most evolutionarily advanced termite families. Among the lower termites, Rhinotermitidae are the most evolutionarily advanced (Carpenter 1992; Inward et al. 2007), followed by the Kalotermitidae (drywood termites), Termposidae (dampwood termites), and finally the Mastotermitidae (Carpenter 1992). The view that the worker caste originated from a single basal source (Thompson et al. 2000) differs from the suggestion by Noirot and Pasteels (1998) that the worker caste evolved independently in Mastotermitidae, Hodotermitidae, and at least once in Rhinotermitidae and Termitidae (together). The cladogram of Inward et al. (2007) also suggests three independent origins of the true worker caste.

Subterranean termites have an important position in the evolution of termites. Pieces of evidence point to the two genera, *Reticulitermes* and *Coptotermes* as important transitional taxa between lower and higher termites. Evidence includes results from phylogenetic analyses (Austin et al. 2004; Inward et al. 2007; Lo et al. 2004) which show that the parent group of these genera is possibly a sister group of the Termitidae. Members of these two genera also exhibit features that are intermediate between higher and lower termites. These features include feeding habits characteristic of lower termites, nesting habits that are mid-way between the single-site nesting (in wood) of basal lower termites (Shellman-Reeve 1997) and the central nesting from which higher termites access multiple sources of food (Shellman-Reeve 1997).

Biology

As eusocial insects, termites exhibit reproductive division of labor, cooperative brood care, presence of non-reproductive helpers (Michener 1969), and overlapping generations. A number of theories have been put forward to describe evolution of eusociality, specifically the route towards it; suggestions include the subsocial and semisocial routes. The subsocial route involves the development of familial units initially made up of parents and offspring. The semi-social route involves an association of individuals from the same generation irrespective of whether they were related. According to Thorne (1997), the lack of evidence of exchange of reproductives between different established colonies makes the subsocial route more likely. The kin selection theory by Hamilton (1964) caused a significant advancement in knowledge on the evolution of eusociality in Hymenoptera societies. In this theory, relatedness and sex ratio were affected by the fact that female hymenoptera are diploid and males are haploid. The worker caste, all female, is more inclined to tend a brood of sisters who they are more closely related to than their mother. A combination of such genetic factors with various ecological features may promote eusocial evolution (Alexander et al. 1991; Bourke and Franks 1995). Hamilton's kin selection theory however falls short in explaining the eusociality of termites which are fully diploid. Full diploidy in termites eliminates the asymmetries in relatedness between sexes and generations indicating that some additional factors may be significant in the evolution of eusociality in termites (Thorne 1997). The need for generations to overlap arose from the reliance of termites, and cryptocercid cockroaches, on cellulolytic protozoan symbionts to digest the cellulose in wood (Cleveland et al. 1934). Newly eclosed individuals and those that have just molted lack these protozoa and must acquire them either by feeding on the hindgut fluids from nestmates or by eating freshly-cast exuviae which are rich in these protozoa (Thorne 1997).

Termites have three distinct castes: the soldier, worker, and reproductive caste phenotypes (Noirot 1985; Noirot 1990). These castes represent one of the major types of polyphenism in which individuals within a phenotype engage in a specific task and castes within a colony cooperate (Wilson 1971). Castes are morphologically distinct and equipped for their specific roles within the colony. Mechanism(s) underlying caste differentiation in termites is another aspect of termite biology that has been of interest to researchers. According to Wilson (1971), kin selection theory predicts that castes result from environmentally-induced differences in expression of genes from a totipotent genome. Thorne (1997), however, hypothesized that reproductive and worker castes are determined entirely by extrinsic factors, mainly pheromones. Hayachi et al. (2007) in their studies on *R. speratus* (Kolbe) proved that caste is controlled by both environment and a complex pattern of genetic inheritance. The authors proved this inheritance pattern when they obtained results that were consistent with an X-linked, one-locus-two allele model. Depending on their zygosity these two alleles had contrasting effects on the genotypic determination of sex in *R. hesperus*. One allele resulted in genotypic female nymphs in the homozygous condition and male workers when they occurred as single copies while the other resulted in male genotypic nymphs and female workers. The authors cited the existence of several termite species with ergatoids and/or workers of only one sex as indicators that the influence of genes on nymph and worker caste determination may not be limited to *R. speratus.*

Caste-specific body parts are exaggerated or reduced during postembryonic development by responding to extrinsic cues (Miura 2005). Examples include the enlarged and heavily sclerotized mandibles or frontal projections of soldiers for defense

(Deligne et al. 1981) and the wings and compound eyes of alates to aid in dispersal. Compounds eyes are needed for vision during flight and to scan for mates during nuptial flights by reproductives (Miura 2005), which are responsible for producing offspring and selecting a site for the new colony.

The worker caste in lower termites is an immature form that retains the ability to differentiate to other caste phenotypes (Myles and Nutting 1988) whereas in higher termites they are terminally differentiated and cannot develop into alates (Noirot 1990). The tasks of the worker caste include, 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, Sleigh 2002, Wilson 1975). 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 material or boli that result from allogrooming activities (Whitman and Forschler 2007).

Soldiers are formed by differentiation of workers through an intermediate presoldier stage (Noirot 1985, Henderson 1998). Relative to workers, soldiers have a reduced digestive tract and a head that is enlarged and sclerotized (Koshikawa et al. 2002). Colony defense falls under the purview of the soldier caste even though workers may be of assistance in some species. Methods of defense range from mechanical methods in the less advanced termites through the use of a combination of mechanical and chemical methods to the complete reliance on chemical methods in the most advanced groups. Mechanical defense include phragmosis, mandibular biting, mandibular snapping, autothysis, and abdominal dehiscence. Chemical defense involves the release of chemicals such as greases, irritants, contact poisons or glues from frontal, salivary or cibarial glands (Prestwich 1984). Termites that use phragmosis typically have cylindrically-shaped (phragmotic) heads used 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. According to Prestwich (1984), mandibular snapping involves striking the intruders with the mandibles whereas mandibular biting involves crushing, slashing, or piercing intruders with the mandibles. The similarity between mechanical defense by autothysis and that by abdominal dehiscence is limited to the explosive release of fluids through a ruptured body wall. Both mechanisms however differ in that autothysis involves explosive release of fluid from the labial gland reservoir through weaknesses in abdominal or thoracic walls, whereas abdominal dehiscence 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 either through an orifice in the front part of the head (e.g., fontanelle in *Coptotermes* spp.) or a frontal projection in the head called the nasus in *Nasusitermes* spp. In both genera, these sticky fluids are secreted from the large frontal gland of the soldier caste but whereas defensive fluid from *Coptotermes* is white or yellow and milky, that from *Nasusitermes* is clear (Stuart 1969).

Chemical stimuli are the most important stimuli for social behavior of termites, which are very sensitive to odors (Stuart 1969). Each termite colony acquires a distinctive odor that helps members to identify and deal with intruders. The alarm reaction is exhibited by termites introduced into a different colony because of these odor differences (Stuart 1969). Alarm is communicated by three main routes, sound (Smeathman 1781, Emerson and Simpson 1929), odor, and contact. Alarm behavior may either be general or specific depending on whether a large or small proportion, respectively of colony members is affected by the primary stimulus (Stuart 1963). Location of food is also communicated by way of odor trails made with trail substances (pheromones) secreted from the sternal gland (Stuart 1960) and stored in an external reservoir formed by the overlap of the 5th abdominal sternite by the 4th abdominal sternite. Termites press their abdomen against the substrate to release the trail substance onto it (Stuart 1964); the trail substance serves as a chemical road map to the location of food.

Ecology

A review of feeding habits show that termites feed on plant material: living, dead but fresh, dead decomposing, and soil rich in organic matter (humus). Specialized or incidental foods include fungi, algae, lichens, portions of termite nests rich in organic matter, members (including eggs) of their own colony, and skins or other parts of vertebrate corpses. Occasionally, other substances such as leather or plastics are also attacked (Lee and Wood 1971, Wood 1978). Lower termites depend on protozoans for cellulose digestion a function performed by bacteria in higher termites (LaFage and Nutting 1978).

Drywood termites (Kalotermitidae) as their name suggests are found within dry wood. These termites do not require contact with the ground or an above-ground source of moisture but obtain water directly from wood or as a metabolic product of its digestion (Potter 2004). All other castes except the alates restrict themselves to tunnels constructed within the wood. Damage inflicted by drywood termites lacks the mud tunnels and soil in the feeding galleries; galleries are large and constructed both along and across the wood grain. Piles of tiny fecal-pellets ejected from galleries through tiny holes, where termites are active, are the most obvious signs of infestation by drywood termites. These holes are usually plugged with fecal matter (Potter 2004).

Dampwood termites (Termopsidae) are also wood-dwelling species but unlike drywood species, they infest wood with very high moisture content. Suitable woods are usually damp or decaying and are usually in contact with the ground or in places where they are kept wet (Potter 2004). The absence of mud tunnels and plugging of openings with fecal material makes their feeding activity less conspicuous. The feeding pattern of dampwood termites depends on the condition of the wood. Tunnels or galleries are constructed across the grain in decayed wood but feed mainly on the springwood when the wood is sound (Potter 2004).

Subterranean termites are xylophagous and forage for cellulose-containing materials chiefly wood. They 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 but some species such as *C. formosanus*, are capable of building aerial nests with no connection to the ground. Nests are constructed of carton which is made from soil and masticated wood cemented together with saliva and excrement (Potter 2004). Moisture is obtained from leaking roofs, faulty plumbing, or condensation from air conditioning units (Chambers et al. 1988) and conserved by the carton material (Potter 2004) to provide an environment conducive for subterranean termites. Foraging is accomplished through the excavation of soil and the construction of underground tunnels that form a network of galleries around food sources. Galleries comprise of a number of satellites nests connected to a main nest by tunnels in a system that can extend to ≈100 m in *C. formosanus* (King and Spink 1969). Excavation involves the extension of the gallery through the formation, transportation and deposition of pills made of macerated material and shaped by the pressing action of maxillary and labial palps against the labrum (Whitman and Forschler 2007).

History of Termite Control

Subterranean termites (Rhinotermitidae) are by far the most economically important family of termites. Their cryptic (Su et al. 1998) and subterranean natures make them more difficult to control. The history of control strategies includes the use of wood preservatives, physical barriers, application of liquid termiticides for prophylaxis or remedial control, and the use of baits.

Richardson (1978) suggested that the treatment of wood has a history that is as long as the use of wood itself. Historical evidence of wood preservation can be found in the bible, history of ancient Greece, and among the Romans. Compounds used were pitch, olive oil and tar, respectively. Health concerns have resulted in reduced use of coal tar creosote and the cessation in the use of arsenic in parts of the world. Wood preservatives in current use include Copper naphthenate and borates. Copper naphthenate was first used as a wood preservative in Germany in 1889, but commercial use of the product began in 1911. Borates are inorganic minerals mined from naturally formed deposits in the earth; they 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. Metal termite shields which have remained popular in the tropics and parts of the United States, are probably the oldest physical devices that help to protect structures from attack by termites (Potter 2004). These shields are usually installed on foundations, pipes, piers, and other potential points of entry. Unlike the physical barriers, shields do not prevent entry of subterranean termites, but aid in their easy detection. This is achieved by forcing termites to build their tubes around the protruding edge and over the top of the shield all of which make their presence more visible. The use of particulate materials such as sand, granite, and basalt to protect structures is based on the findings of Ebeling and Pence (1957). The authors observed the inability of termites to tunnel through sand of a certain particle size. To be good physical barriers, 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.

Stainless-steel wire meshes are also becoming popular in the termite control industry. Termi-Mesh® developed and patented in Australia, 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).

Pesticide-impregnated sheeting with materials such as plastic films, fabrics, and rubberized roofing materials are also being evaluated and employed as termite barriers. Kordon Termite Barrier and Impasse Termite System (Syngenta Professional Products, Greensboro, N.C.) are in use in Australia and the United States, respectively; they are used in pre-construction treatments and are rolled out before the concrete slabs are poured. These products serve as both chemical and physical (moisture) barriers. According to Potter (2004) the toxicant is held in a dry state in the inner webbing of the plastic laminate. The active ingredients in Kordon Termite Barrier and Impasse Termite System are pyrethroids, deltamethrin and lamda-Cyhalothrin, respectively.

The use of chemical compounds to control subterranean termites was suggested at the latter part of the 19th century, but actual evaluation of candidate compounds began in the 1940s (Aventis 2003). The concept was to provide a chemical barrier to entry of subterranean termites into structures. Compounds used included calcium cyanide, sodium cyanide, and carbon disulfide. Chlordane, considered a toxic soil barrier termiticide (Forschler 1994), came into use in 1952 after years of efficacy tests. Chlordane and other cyclodienes: heptachlor, aldrin, and dieldrin became the preferred agents for control of subterranean termites into the 1980s. These repellent compounds function by repelling worker termites from tunneling toward the foundation of the structures (Su and Scheffrahn 1990b) and were very effective when applied correctly. Environmental persistence and public health concerns however, 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, even though less persistent than the cyclodienes were more toxic to vertebrates a fact which led to their ban by the EPA in 2000. Pyrethroids have a relatively long residual life, are effective at low use rates, and have low mammalian toxicity (Potter 1998). The repellent compounds that replaced the cyclodienes were generally less persistent but more expensive. Cost-driven sub-label rate applications of these chemicals led to frequent breaches of the chemical barriers by termites (Mampe 1994). Treatment of structures with repellent compounds was rigorous mainly because of the need to eliminate gaps or untreated regions that easily become highways by which termites enter and damage “protected” structures. Remedial control with repellent compounds was also complicated by the ability of termites to detect, seal off, or otherwise avoid the treated sections of the colony (Su et al. 1982). Environmental toxicity and harmful effects of repellent compounds on non-target organisms (Silver and Soderlund 2005, McCann et al. 2001) as well as the need for alternative compounds that were effective at low use rates

(McCann et al. 2001) also provided the impetus to develop and use non-repellent compounds and baits.

Non-repellent compounds are toxic but usually slow-acting compounds that can be applied as liquid treatments or formulated as baits. Compounds such as fipronil (Termidor, BASF corp., Research Triangle Park, NC), imidacloprid (Premise, Bayer Environmental Service, Montvale, NJ), and chlorfenapyr (Phantom, BASF Corp) became popular in the United States, (Anonymous 2002) at the expense of their repellent counterparts (Shelton and Grace 2003) and accounted for about 60% of the total amount of termiticides used in 2002 (Anonymous 2002). 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). Trenches are filled with finished solution at a rate of 15141.65 cm^3 per 304.8 cm (linear distance) per 30.48 cm of depth to the footer (Rambo 1985).

The bait technology consists of a toxicant formulated into cellulose matrix in a system that targets termites directly without releasing large quantities of insecticides into the environment as is the case with liquid termiticides. The first bait system, Sentricon® Termite Elimination System was developed by Dow AgroSciences in 1990 with hexaflumuron, a chitin synthase inhibitor, as active ingredient. Noviflumuron replaced hexaflumuron in later versions of the bait, including the latest, Recruit IV termite bait. Diflubenzuron, also a chitin synthase inhibitor, is the active ingredient in Exterra® Termite Interception and Bait System developed by Ensystex. Sulfluramid, a stomach poison, is the active ingredient in Firstline® Termite Bait System developed by FMC. Sulframid disrupts the metabolism of energy in termites by uncoupling oxidative phosphorylation (Valles and Koehler 1997). The active ingredient in Exterra is diflubenzuron, a chitin synthase inhibitor. Other bait systems were developed based on different active ingredients and station designs all of which are installed at regular intervals in the ground surrounding the structure. In their studies on the performance of baits against tree-infesting Formosan subterranean termite colonies, Henderson and Forschler (1997) reported the effectiveness of hexaflumuron, fipronil, sulframid, and mirex. Bait units require regular inspections to check the untreated cellulose component for termites which when present necessitate the replacement of the cellulose with bait compound. The performance of baits is, however, compromised by the presence of competing food sources such as the structure and natural food sources near it.

Termiticides currently used in the United States include: bifenthrin, cypermethrin, fenvalerate, permethrin, imidacloprid, indoxacarb, chlorfenapyr, fipronil, and hexaflumuron; chlorantraniliprole an anthranilimide is currently undergoing field trials as a termiticide. According to Su (2003, 2005) non-repellent compounds have been very effective against subterranean termites. Non-repellent compounds that are sufficiently slow-acting are suitable for remedial treatments because treated termites are able to transport the toxicant on or in their body to untreated parts of the gallery and even recruit other foragers to the treated zone (Su et al. 1982). According to the author, this helps to circumvent the difficult task of delineating and treating extensive and labyrinthine galleries associated with large colonies. Such unimpaired mobility of treated termites also precludes the accumulation of dead termites in the treated zone and thus prevent the avoidance of these zones by naïve nestmates.

Behavioral Response of Termites to Insecticides

Non-repellent termiticides are designed to exploit knowledge of the behavioral biology of termites to improve control outcomes. Compounds that are sufficiently slow-acting afford treated termites, ample time and opportunity to transport the toxicants on or inside their bodies to other parts of the colony where (chemically) naïve nestmates are contaminated through social interactions. Acquisition, transportation, and transfer of these toxicants to nestmates are affected by the behavior of termites under both normal and intoxicated conditions. Insecticide-induced behavioral response is a function of factors pertaining to the insect, the insecticide, and the environment all of which have been studied using various insecticides and insects. Available literature show a number of behavioral studies conducted using both non-social and social insects, each group with different information value. Studies on the insecticide-induced behavioral response of non-social insects provide vital information including the mode of action of the insecticide but do not reflect the group dynamics associated with social insects such as termites.

A review of insecticide-induced behavioral studies reveals very interesting dimensions that enrich the discussion and development of effective insect pest control schemes or strategies. A major problem with behavioral studies has been the lack of standard behavior definitions or their complete absence in study reports. Klotz and Reid (1993) defined moribundity to include ataxia (inability to coordinate movements), excitation, lethargy, and paralysis. Oi and Oi (2006) on the other hand equated moribundity with death and both conditions were differentiated from abnormal behavior such as ataxia, excitation (tremors/shaking), and lethargy. The authors defined lethargy as a condition in which insects curl their bodies and lie on their back or side but capable of getting back onto their feet when probed. Bostanian and Akalach (2004) listed: irreversible cessation of feeding, slight trembling, unstable movement, convulsions, paralysis, and death in *Orious insidiousus* Say (Hemiptera: Anthocoridae) and *Aphidius colemani* Viereck (Hymenoptera: Braconidae) treated with indoxacarb.

The range of insect behavioral response to insecticides depends on the biology and ecology of the insect, the concentration and mode of action of the insecticide and the substrate or medium of application of the insecticide. Jallow and Hoy (2005) in a two-way bioassay found that application of permethrin, a pyrethroid and sodium channel-blocker, to discs of cabbage leaf deterred the diamondback moth, *Plutella xylostella* L. from laying eggs on it. A multiple-choice bioassay revealed a positive correlation between concentration of permethrin and the degree of inhibition of oviposition. Imidacloprid appears to be one of the most studied insecticides when it comes to insecticide-induced behaviors. It is a neonicotinoid which acts as an agonist of acetycholine receptors but, unlike the natural receptors, it does not respond to acetylcholinesterase, a situation which results in persistent activation and overstimulation of cholinergic synapses which in turn cause hyperexcitation, convulsions, paralysis, and death of the insect. The reported behavioral effects on insects range from significant alterations in the probing and settling behaviors of thrips (Thysanoptera: Thripidae) on treated plants (Joost and Riley 2005) and tetanic muscle contraction in cat fleas within minutes of exposure to sublethal doses (Melhorne and Mencke 1999). Treatment of cat fleas with lethal doses of imidacloprid results in intense trembling of the legs and horizontal oscillations (symptoms of hyperactivity); this hyperactivity results in the destruction of nerves and muscles (Melhorn and Mencke 1999). Joost and Riley (2005) also used imidacloprid and two different species of flower thrips to demonstrate specificity of insect behavioral response. They observed that whereas certain feeding parameters were affected negatively by imidacloprid in *Frankliniella fusca* Hinds, they were enhanced (i.e., feeding increased) in *F. occidentalis* Pergande. Irrespective of rate of imidacloprid used, *F. occidentallis*, probed more frequently and for longer periods on treated plants relative to the control. *Frankliniella fusca* on the other hand probed more frequently on control plants relative to those treated with high concentrations of imidacloprid. The frequency of probing on non-treated plants was however similar for the two species. Compared to the control treatment, high rates of imidacloprid resulted in more frequent and longer durations of ingestion in *F. occidentalis* but these activities were depressed in *F. fusca* compared to the controls. The authors also observed that *F. fusca* preferred to settle on leaves of nontreated plants whereas *F. occidentalis* showed a preference for imidadcloprid-treated plants. *Frankliniella occidentalis* also failed to exhibit symptoms of intoxication even when exposed to high rates of imidacloprid but *F. fusca* exhibited intoxication symptoms that ranged from low (i.e., incipient intoxication symptoms) to high (i.e., advanced symptoms). The effects of imidacloprid on foraging behavior of honey bees became a subject of interest especially in the face of the colony collapse disorder that created a panic situation among those engaged in bee-keeping, bee-pollinated crop production, and the scientific community. Yang et al. (2008) found that imidacloprid actually impairs the foraging behavior of honey bees. The authors trained bees to forage on food (50% sucrose solution [wt: vol]) in artificial feeders placed 35 m from the nest. A clear definition of normal foraging behavior was based on the less than

300 s between subsequent visits to artificial feeders by untreated and undisturbed bees. Exposure to 12 solutions of imidacloprid (dissolved in dimethyl sulfoxide) and a control (50% sucrose solution [(wt: vol]) revealed a dose-dependent effect on the rewarding behavior of honey bees. This was evidenced in delays of at least 1.5 h in the return of some of the bees treated at low concentration whereas all the bees treated with higher concentrations of imidacloprid (i.e., 4,000 and 6,000 µg/liter) went missing. Lingering effects of imidacloprid-poisoning among returning bees caused irregular foraging behavior that was markedly different from what obtained prior to treatment. Yang et al. (2008) also reported a positive relationship between concentration of imidacloprid and onset of abnormal foraging behavior but an inverse relationship between concentration and percentage recovery of bees.

Beetles which constitute the largest group of insect pests have not been left out in behavioral response studies. Wise et al. (2007) compared the lethal and sublethal effects of imidacloprid to those of azinphosmethyl and esfenvalerate against adult Japanese beetles (*Popilia japonica* Newman). Azinphosmethyl (Guthion®) is an organophosphate insecticide which functions as a cholinesterase inhibitor; esfenvalerate, on the other hand, is a pyrethroid which exerts its insecticidal properties by modulating the sodium channel. The authors defined "knockdown" as the condition in which beetles twitched from an upside down position at the bottom of the arena. All three insecticides induced significant symptoms of knockdown within 96 h of exposure with no significant differences between insecticide treatments. There was a stark contrast between the high proportion of immobile beetles in the insecticide treatments and the low proportion in the untreated controls. The fact that the state of immobility observed in both the azinphosmethyl and esfenvalerate treatments was absent in the imidacloprid treatments attest to the importance of insecticide-inherent characteristics such as mode of action as a determinant of insect behavioral response.

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. Oi and Oi (2006) studied the toxicity effects of spinosad, indoxacarb, bifenthrin, and hydramethylnon baits against fire ants and reported some interesting results. Hydramethylnon belongs to the amidinohydrazone class of insecticides and functions as a metabolic inhibitor by blocking the biological process that forms Adenosine Triphosphate (ATP) in insects; this causes lethargy and cessation of feeding in target pests (Su et al. 1982). Indoxacarb is an oxadiazine that exerts neurotoxic effects on insects through a voltage-dependent blocking of the sodium channels in the insect neuron which results in nervous system disorders and death (Silver and Soderlund 2005). Like all pyrethroids, bifenthrin induces neurotoxic effects by blocking the insect sodium channel. Oi and Oi (2006) carried out their studies based on clearly defined behavioral symptoms and distinguished moribundity from abnormal behavior. Ants that were lethargic and unable to right themselves after being toppled over were considered moribund. Abnormal behaviors were defined to include tremors, shaky movements and ability of ants to stand or attempt to do so from a curled position or when put off their feet with a probe. Termites exhibiting these behavioral symptoms were assumed to be incapable of trophallaxis. Cumulative mortality profiles based on abnormal behaviors, moribundity, and death showed faster activity compared to profiles based on moribundity and death. Use of abnormal behavior in addition to mortality as a criterion of toxicity, resulted in visual toxicity symptoms that occurred 14 h earlier for the hydramethylon treatments; even though symptoms were observed 4 h earlier in the indoxacarb treatments, these differences were deemed statistically insignificant ($P > 0.05$). Toxicants that took a longer time to achieve 90% mortality when concentration was decreased were designated as slow-acting. Oi and Oi (2006) ascribed the sudden transition from normal behavior to high levels of behavioral impairment and moribundity/death in the indoxacarb treatments to the bioactivation of the compound into its toxic metabolite. The inhibition of the mitochondrial electron transport system and the resulting gradual decline in ATP production (Hollingshaus 1987) was accepted as an explanation for the longer delay in symptoms and death from the ingestion of hydramethylnon. Spinosad, on the other hand, derives its insecticidal properties from the direct stimulation of nicotinic acetylcholine receptors and the consequent prolonged hyperexcitation and neuromuscular fatigue (Oi and Oi 2006). According to the authors the low (i.e., <5%) disruption in normal fire ant behavior even up to 8 h after treatment with Spinosad® demonstrates that ample time is available for thorough distribution of the toxicant throughout the colony. Digging behavior of fire ants closely approximates the excavation behavior of subterranean termites during tunnel construction and is thus very relevant to the study of insecticide-induced behavioral responses in subterranean termites. Chen and Allen (2006) reported no significant differences between the amount of sand removed in controls and that in treatments with low concentration (i.e., 0.05 and 0.10 ppm) of fipronil; higher concentrations of fipronil-treated sand however resulted in statistically significant reductions in the amount of sand removed by fire ants. Fipronil is a phenylpyrazole that functions by blocking the γ-aminobutyric acid-gated chloride channel of the insect nervous system causing nervous system disorders. Chen and Allen (2006) also reported a positive correlation between worker mortality and amount of sand removed in the fipronil-treatments. Results also suggested that digging behavior had a concentration-dependent effect on the efficacy of fipronil. Chen (2006), in a separate study, reported that pyrethroids weakened or killed fire ants but did not appear to repel them from digging in treated sand. Treatment with pyrethroids (bifenthrin, cyfluthrin, deltamethrin, γ-cyhalothrin, and permethrin), pyrethrin, and a carbamate (carbaryl) resulted in less digging by fire ants. Exposure to acephate, a water-soluble organophosphate and cholinesterase inhibitor on the other hand resulted in digging efforts that were similar to control treatments. The authors reported that ants were not repelled by pyrethroids but ceased digging in response to dead or weakened nestmates.

Insecticide tests conducted on cockroaches are especially relevant to studies on termites because of similarities in morphology and ecology. Wang et al. (2004) demonstrated various behavioral and physiological mechanisms of resistance in three strains of the German cockroach, *Blattella germanica* (L.). The Jwax strain is a standard susceptible strain that had been reared in the laboratory for > 30 years; the Dorie strain was obtained from apartments with a 5 yr history of exposure to pyrethroid sprays, gel baits, bait stations, and boric acid. The Cincy strain was collected from apartments that had been treated with gel baits on a quarterly basis for 5 yr. When treated with hydramethylnon, fipronil, abamectin, and imidacloprid, these strains exhibited levels of resistance that corresponded with their history of exposure to insecticides (Wang et al.

2004). Abamectin is an avermectin that function by activating the chloride channel to release chloride ions which inhibit nerve cells and cause nervous system disorders and death. A topical assay revealed moderate (i.e., <10-fold at LD_{50}) levels of physiological resistance to abamectin and fipronil in the Cincy and Dorie strains with the Dorie strain exhibiting greater physiological resistance to abamectin. The Cincy strain however demonstrated high levels of resistance to abamectin and fipronil in a gel baits test, suggesting that the resistance may be more behavioral than physiological in nature. Blank gel tests performed on adult males and nongravid females helped to identify aversion behavior not attributable to active ingredients but inert components such as sugars. According to Wang et al. (2004) cockroaches with sugar-aversive traits are limited in the sources from which they obtain nutrients in human dwellings and suffer a reduced fitness as a consequence. The behavioral avoidance of repellent compounds described by Ebeling et al. (1966) and sugar-aversive traits are important mechanisms by which German cockroaches avoid compounds that have adverse effects on their fitness and survival.

One of the most profound behavioral studies on subterranean termites was carried out by Su et al. (1982). The authors grouped nine insecticides into three categories (i.e., types I, II, and III) based on the behavioral response induced in Formosan subterranean termites. The insecticides tested were pyrethroids (fenvalerate, resmethrin, and permethrin), organophosphates (Chlorpyrifos and Diazinon), an organochlorine (Chlordane), a carbamate (Cabaryl), a pyrethrin, and an amidinohydrazone (Amdro). These different classes of insecticides differ in their mode of action; organophosphates and carbamates are cholinesterase inhibitors, and organochlorines are GABA channel blockers. Amidinohydrazone is a metabolic inhibitor that inhibits the synthesis of Adenosine Triphosphate (ATP) causing lethargy and cessation of feeding. Su et al. (1982) reported that pyrethroids and pyrethrins constituted the type I group which caused termites to avoid zones treated at low concentrations, but completely seal off tunnels within a day after contact with zones treated at high concentrations. Type II compounds were clearly non-repellent because termites kept entering the treated zone up to 4 days after initial contact. Avoidance behavior including sealing off of tunnels that led to the treated zone was a natural response to the high number of dead termites that had accumulated in zones treated with these fast-killing compounds. This behavioral response resulted in a high percentage of survivors. The unique feature of the only type III compound, Amdro®, was that dead termites were scattered all over the arena instead of piling up in the treated zone. Termites exposed to this slow-acting stomach poison continued to feed in the treated zone several days after initial contact partly because of the non-repellent nature of these compounds and also because dead termites had not accumulated in the treated zone. Termites only exhibited avoidance behavior when a high number of dead and decaying nestmates were found scattered in the treated area.

It is abundantly clear that the identification of behavioral changes attributable to insecticides requires a good description and definition of normal behaviors. Henderson (2003), described some normal behaviors in subterranean termites: Walking was defined as an activity that involved lifting the legs, changing of location and orientation; termites were defined to have moved (instead of walked) if movement was limited to insect body and not the legs; termites that showed no movement of body and legs were defined to be resting; termites were said to be vibrating when rapid shaking/horizontal oscillation of the entire termite body was observed; antennae cleaning as the name suggest referred to the process by which a termite cleaned its own antennae. Antennae posture referred to the orientation (i.e., horizontal or otherwise) of the antennae. Henderson (2003) also defined digging as the excavation of tunnels in sand. Excavation is defined by Whitman and Forschler (2007) as gallery extension that is accomplished by picking, transporting, and depositing sand/soil particles; this is followed by the return of termites to the excavation site. Henderson (2003) defined grooming as the process by which termites cleaned other termites; Whitman and Forschler (2007) on the other hand defined this as allogrooming to differentiate it from autogrooming which refers to the process by which termites groomed themselves. According to Whitman and Forschler (2007), allogrooming is a rhythmic lateral movement of the mandibles while the head capsule remained in contact with various body parts of the termite being groomed. Allogrooming can either be non-vigorous in which case the groomed termite remains stationary or vigorous characterized by the physical displacement of the groomed termites often for a distance of several body lengths (Whitman and Forschler 2004). Trophallaxis was first described by Wheeler in 1918 as an exchange of food and information through stomodeal (mouth to mouth) or proctodeal (mouth to anus) contact (Sleigh 2002). Allofeeding refers to the consumption of food donated from the anus or mouth of a nestmate (Whitman and Forschler 2004). Termites were described to have consumed already ingested food if they ceased chewing without visible evidence that they had donated macerated material to another termite or deposited it on the substrate (Whitman and Forschler 2004). According to the authors, autofeeding allogrooming refers to the ingestion of a bolus formed during allogrooming while autofeeding regurgitation refers to the ingestion of the clear viscous fluid formed from regurgitated material. Using Formosan subterranean termites, Henderson (2003) described the behavioral changes that occurred with exposure to low concentrations of imidacloprid- or fipronil-treated sand. The author reported that imidacloprid-treated termites walked significantly less but spent more time resting compared to termites treated with fipronil and those that were untreated 4 h after treatment. Fipronil-treated termites were actually more active than the controls during the initial stages. The antennae of a significant number of the imidacloprid-treated termites were at right angles to the head and were unable to show normal searching patterns 9 h after exposure. According to Henderson (2003) these termites continued to act sickly and behave abnormally after 24 h. The ability of these termites to transfer toxicants to nestmates was speculated to be curtailed by the reduction in the distance they were capable of travelling. Fipronil-treated termites on the other hand failed to show any major behavioral changes until 24 h after exposure when nearly all of them were in an upside-down position either dead or dying with their antennae oriented horizontally. Also of immense importance are the effects of insecticides on soil excavation, tunneling, and other activities that form integral parts of the foraging process in subterranean termites. Thorne and Breisch (2001) reported that termites treated with high enough concentrations of imidacloprid lost their capacity to tunnel. This impaired tunneling ability was observed even among termites that had lost all visible signs of intoxication during interactions with untreated nestmates. The authors also reported reduced or impaired grooming, feeding, tunneling, and walking in dark southern subterranean termites (*Reticulitermes virginicus* Banks) exposed to sublethal doses of imidacloprid; higher concentrations or longer duration of exposure caused termites to lose their mobility, lay on their backs and occasionally twitch their legs and antennae until death. Thorne and Breisch (2001) reported impairment in 40% of untreated termites exposed to imidacloprid-treated nestmates. Impairment evidenced by a slow, staggering gait, the presence of termites on the surface of sand instead of inside tunnels and even death of untreated termites is indicative of transfer of imidacloprid residues between termites. The behavioral aversion of imidacloprid-treated zones by termites previously exposed to sublethal doses (10 or 100 ppm exposure for 4 h) of imidacloprid (Thorne and Breisch 2001) show the ability of termites to learn from past experiences with toxic substances. This would seem to be an important determinant of the performance of termiticides. If the concentration is too low, termites survive, learn to avoid the insecticide and the non-repellent becomes repellent.

The renewed interest in the development of environmentally-friendly organic insecticides has inspired research on their behavioral impact on target organisms. The slow-acting nature of these natural insecticidal compounds is in keeping with the current trends in chemical control of social insects. A typical example of such organic products is nootkatone, a eudalenoid sequiterpene ketone (Ibrahim et al. 2004). The compound is typically extracted from grapefruit but can also be produced using genetically modified organisms or the chemical/biochemical oxidation of valencene (Furusawa et al. 2005). The compound has repellent (Zhu et al. 2001, Maistrello et al. 2001a) and food deterrent properties (Maistrello et al. 2001b) and derives its insecticidal properties from the overstimulation of acetylcholinesterase activity and the consequent cholinergic neurotransmission dysfunction in the target insects (Ibrahim et al. 2004). The authors incubated Formosan termites that had been topically treated with nootkatone and its derivatives (1, 10-dihydronootkatone and tetrahydronootkatone) to study delayed effects on termite survivorship as well as feeding and tunneling activities. Topical treatment with all three compounds resulted in lethargy after first inducing shaking behavior in termites. Unlike its derivatives, nootkatone failed to induce any significant reductions in average food consumed by Formosan subterranean termites (Ibrahim et al. 2004). These toxic and repellent derivatives also caused significant reduction in tunneling and feeding behaviors when incorporated into sand barriers. Ibrahim et al. (2004) also reported "lingering behavior", characterized by a sharp reduction in activity profile during which termites formed close clusters on the surface instead of tunneling into treated sand. Lingering behavior was ascribed to reduced ability of termites to dig in insecticide-treated sand through possible adverse effects on the process of muscle contraction. Insecticide-treated termites traveled on the surface of the soil barrier to access food instead of tunneling through the soil as did control termites.

The studies reported in the preceding discussion reveal various aspects of insect-insecticide interactions and offer an insight into the ecological, physiological, and biochemical factors that may be relevant to the behavioral response of termites to specific insecticides given their mode of uptake and action. Available literature lacks a descriptive study on the behavioral response of termites to indoxacarb and chlorantraniliprole. The effects of insecticide concentration and duration of exposure on the onset and duration of well-defined abnormal behaviors as well as the tunneling and walking capacity of subterranean termites is yet to be reported. These pieces of information will result in better estimates of the distribution potential of non-repellent toxicants in the maze of tunnels in the subterranean habitat of Rhinotermitidae. The objectives of this study were to address the information gap indicated above by describing the range of abnormal behaviors in subterranean termites treated with different classes of non-repellent compounds. The study also sought to describe the effects of concentration and duration of exposure to indoxacarb on the onset of abnormal behaviors. A third objective was to study the effects of indoxacarb and fipronil intoxication on the tunneling and walking abilities of eastern subterranean termites.

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Dissertation Chapter Contents

The following chapters will describe and quantify the behavioral response of *R. flavipes* to non-repellent insecticides and how these impact the mobility of termites.

Chapter II deals with the description of abnormal behaviors in the eastern subterranean termite, *R. flavipes* exposed to three non-repellent insecticides: indoxacarb, fipronil and chlorantraniliprole which have different modes of action. Descriptions of all insecticide-induced abnormal behaviors and differences in behavioral response to the different insecticides are noted.

Chapter III concerns the onset of abnormal behaviors in eastern subterranean termite workers treated with indoxacarb. The specific effects of concentration and duration of exposure are reported.

Chapter IV focuses on the impact of indoxacarb and fipronil on the walking and tunneling abilities of *R. flavipes* workers. Tunneling behavior and time trends in tunneling ability of termites are described and discussed. The walking ability of insecticide-impaired worker termites is also reported.

CHAPTER II: DESCRIPTIVE STUDY OF NON-REPELLENT INSECTICIDE-INDUCED ABNORMAL BEHAVIORS IN *RETICULITERMES FLAVIPES* (Kollar)

Subterranean termites (family: Rhinotermitidae) are the most economically important family of termites in the United States where damage and efforts to control them are estimated at $3 billion annually (Su 2002). The most destructive species in the United States are the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), and the Formosan subterranean termite *Coptotermes formosanus* Shiraki (Su and Scheffrahn 1990); together with *R. virginicus* Banks, *R. hesperus* Banks, and *R. tibialis* Banks, these species are the subject of 90% of the treatment efforts (Forschler and Lewis 1997). Chemical methods of termite control have evolved significantly over the years but the transition from repellent to non-repellent termiticides has been one of the most revolutionary changes. Non-repellent compounds circumvent the difficult task of creating a continuous chemical layer between the soil and a structure and treating all parts of the maze of tunnels that constitute the underground gallery of rhinotermids. This is achieved by relying on foraging workers to transport the toxicant to parts of the gallery far removed from the point of application. The non-repellent and slow acting qualities of these compounds facilitate their transport and transfer to naïve nestmates through social interactions such as trophallaxis, grooming and care-giving activities such as brood care and care for intoxicated or otherwise incapacitated nestmates.

Avoidance behavior was the only element of behavior that was important in repellent-based control of subterranean termites. The introduction of non-repellent compounds however brought other aspects of termite behavioral biology into sharper focus. This was because broader aspects of termite behavior exerted significant effects on the performance of non-repellent compounds. Su et al. (1982) demonstrated these effects in a simple but profound study using insecticides from six different chemical classes: pyrethroids, organophosphates, organochlorines, carbamates, pyrethrins, and amidinohydrazones. Based on the behavioral response of termites, the authors re-categorized these insecticides either as repellent or non-repellent compounds. Slow-acting non-repellent compounds are defined as those in which increased concentration and/or duration of exposure causes earlier onset of adverse effects (Remmen and Su) such as mortality; decrease in these parameters results in later onset of adverse effects. According to the authors slow-acting, non-repellent compounds recorded the highest mortality partly because these properties allowed termites to continue tunneling into the treated soil and also because dead termites were distributed all over the arena instead of accumulating in the treated zone. Termites were therefore not alerted and did not learn of the presence of unfavorable conditions/compounds in the treated zone.

To be more comprehensive, behavior-based termite control needs to encompass the exploitation of the natural social behavior of termites as well as the use of well-defined and relevant behaviors for the assessment of insecticide performance. According to Kane et al. (2005), behavior describes the sequence of quantifiable actions involving cumulative effects of genetic, biochemical and physiological processes operating through the nervous system and aimed at maximal fitness and survival of the organism. Behavior is a unique manifestation of the connection between the physiology and ecology of an organism and its environment (Little and

Brewer 2001) which makes it a very important indicator of environmental changes such as presence of toxicants. Its usefulness as an indicator is further bolstered by what Kane et al. (2005) described as the nonrandom, highly structured and predictable sequence of activities that are associated with toxicity. To be relevant to toxicological assessments, behavioral responses must be: well-defined, measurable, ecologically relevant, and sensitive to a range of toxicants; the mechanism of response must also be understood (Rand 1985). Behavioral endpoints that are represented across difference species of organisms and are capable of distinguishing between classes of insecticides with different modes of action are particularly ideal as indicators (Kane et al. 2005).

The acceptance of behavioral endpoints as indicators of environmental toxicity in the United States began with the acceptance of avoidance behavior as legal evidence of injury to natural resources in 1986. This was under the proceedings of the Comprehensive Response, Compensation, and Liability Act of 1980 (NRDA 1986). The acceptance of other elements of behavior as indicators of toxicity marked an important milestone in the development of behavioral toxicology. Particularly noteworthy was the publication by the U.S. Environmental Protection Agency in 1991 listing behavioral response as a functional endpoint in neurotoxicity screening protocols (Duffard and Duffard 1996). These behavioral endpoints have been used as early indicators of environmental pollution, but can be adapted for assessment of insecticide toxicity and performance. This is particularly important given the long behavioral window between incipient intoxication and mortality of termites treated with non-repellent compounds.

There are very few descriptive studies that establish well-defined behavioral endpoints in subterranean termites treated with non-repellent compounds. The popular non-repellent compounds in the United States: fipronil (Termidor, BASF Corp., Research Triangle Park, NC), imidacloprid (Premise, Bayer Environmental Service, Montvale, NJ), and chlorfenapyr (Phantom, BASF Corp.) (Shelton and Grace 2003) have been well-researched; however, little attention has been focused on the behavioral responses of termites. One of the few studies that focused entirely on the insecticide-induced behavioral response of termites was carried out by Henderson (2003) using imidacloprid and fipronil. The quest for newer chemistries that were effective at low rates but with less adverse effects on the environment, led to the development of indoxacarb and chlorantraniliprole by DuPont (E.I. DuPont, de Nemours and Company, Wilmington, DE, USA). Indoxacarb is classified as a reduced risk pesticide (U.S. EPA 2000), and has undergone successful field trials as a termiticide in the United States. Provided it performs well in field trials, Chlorantraniliprole is another good option for the control of termites because of its environmentally friendly nature relative to other insecticides. The objective of this study is to describe and establish abnormal behavioral endpoints in termites treated with fipronil, indoxacarb and chlorantraniliprole to aid in the assessment of their toxicity against subterranean termites.

Materials and Methods

Termites

Groups of eastern subterranean termites were collected from field colonies in Auburn-Opelika (Lee County, Alabama) using open-bottom underground traps described by Hu and Appel (2004). Traps consisted of open-bottom plastic buckets (18 cm high, 13 cm internal diameter) provisioned with corrugated cardboard rolls (15 cm high and 11 cm in diam.) which were set in the ground. Termites were extracted by gentle tapping of rolls onto moist paper towel; workers that were at the 5th larval stage or older were selected for the study.

Chemicals

Formulated indoxacarb [Indoxacarb 150 SC (15% AI) (E.I. DuPont, de Nemours and Company, Wilmington, DE, USA)], fipronil (9.1% AI, BASF Corp., Research Triangle Park, NC), and chlorantraniliprole (18.4% AI, E.I. DuPont, de Nemours and Company, Wilmington, DE, USA) were used to prepare 24-h LC_{90} concentrations of insecticide-treated Whatman No.1 filter paper (Whatman International Ltd., Maidstone, England). Treatments were 200 ppm indoxacarb, 50 ppm fipronil, and 300 ppm chlorantraniliprole; distilled water-treated filter paper was the control treatment. These concentrations were selected based on previous unpublished studies by the authors. Filter papers (5.2 cm diam.) were treated in Petri dishes (5.2 cm in diam., 1.5 cm in height) after which they were air-dried for 24 h in a hood.

Bioassay

Groups of ten freshly-collected eastern subterranean termite workers of ≥5th instar (determined by size) were introduced into each experimental unit (Petri dishes containing treated filter papers); each treatment (i.e., insecticide) was replicated five times (N = 150). Experimental units were sealed with Parafilm® to maintain moisture and humidity maintained at 25 ± 1°C; units were kept in the light. Termite behavior was observed continuously for the first 8 h and then at hourly intervals until death of 90% of termites.

Data collected included description of abnormal behaviors, incidence and nature of behaviors such as alarm reactions (oscillatory movements), grooming/care-giving, and spatial distribution of termites (whether clustered or dispersed). Termites were noted to be clustered if they remained in a stable group in which individuals were separated by a distance of less than 0.4 cm. Similarities and differences in the behavioral response to the different insecticides were noted and described.

Results

In the first study conducted on eastern subterranean termites, it was observed that individual termites exhibited a number of different behavioral symptoms at each stage of abnormal behavior. Progression to more advanced behavioral stages was however uniform irrespective of the particular symptom exhibited in the previous stage. This observation led to the reclassification of the range of observed abnormal behaviors into three major stages i.e., incipient intoxication/state of disorientation, ataxia/lethargic behavior, and moribundity.

Termites exhibited one or more behavioral symptoms characteristic of each level/stage of intoxication. Transition from each of the variant symptoms to more advanced stages occurred without any intermediate symptoms/stages. This observation confirmed the reclassification of all insecticide-induced behaviors into behavioral stages, each of which is defined by a range of behavioral symptoms. The criteria for the stages were: nature of the behavior (e.g., sluggish movement, tremors), degree of mobility, and existence of alternative symptoms associated with same level of intoxication. There were only slight differences in the behavioral symptoms induced by fipronil and indoxacarb but chlorantraniliprole induced behaviors that were markedly different from these two compounds.

In all treatments, introduction of termites into the treated arena resulted in an initial burst of activity (mostly exploratory). Termites exposed to untreated filter paper did not exhibit any unusual symptoms or behaviors indicative of a reduced state of well-being or increased level of disorientation.

Upon introduction into the test arenas, termites in both untreated and insecticide-treated arenas explored the arena and kept moving around; termites intermittently bit on the filter paper until those treated with insecticides became uncoordinated in their movements at which point some of them held onto the filter paper with their mandibles for support. Chlorantraniliprole treatments were characterized by clustered termites, a behavior that was not observed among untreated controls. There were isolated occurrences of horizontal oscillatory movement (alarm behavior) in ≈10% of termites during the first 5 h after introduction to the arena.

The following is a description of the behavioral symptoms associated with fipronil and indoxacarb intoxication from the incipient stages until death:

Incipient Intoxication (Disoriented state). This was the first sign of intoxication in which termites were disoriented (changed directions frequently), moved sluggishly, or shook from side to side when walking. During the early parts of incipient intoxication, horizontal oscillatory movement also referred to as alarm reaction was exhibited by 10-20%, of termites similar to the controls; the later part of this stage was however characterized by a higher occurrence of alarm reaction among treated termites.

Ataxia/Uncoordinated Stage. Termites circled around the same spot, walked in reverse, hunched their dorsum, fell on their backs and kicked their feet. Whole-body movements accompanied kicking behavior. Termites at this stage usually righted themselves but lost their footing again in a cycle that continued until moribundity. The antennae were less active, drooped slightly and termites appeared to be antennating the substrate/arena floor.

Moribundity. Termites were unable to move a distance of at least their body length (≈5 mm). They either remained on their feet or more commonly on their back until death. Kicking was weaker, sparser, and not accompanied with the vigorous whole-body movements associated with the uncoordinated stage. Movement of the antennae (if any) was weak. The antennae assumed a distorted shape i.e., they were either bent at the tip, or were usually distorted and motionless. Some termites spotted the substrate with liquid excrement. Termites were defined to be dead when they failed to exhibit any response when probed.

Even though the above behaviors were exhibited by both fipronil- and indoxacarb-treated termites, there were a few differences. Fipronil-treated termites tended to cluster together, groom, and generally engaged in more social interactions than indoxacarb-treated ones. There were also differences in onset of abnormal behavior and release of stomodeal fluid (liquid excrement) visible on the white filter paper as yellow sticky spots. Spotting of the substrate occurred more frequently among fipronil-treated termites. Indoxacarb and fipronil, elicited more incidents of spotting compared to those treated with chlorantraniliprole, possibly due to the different modes of action of these compounds. Allogrooming was observed in ≈10% of termites treated with indoxacarb, or fipronil until 3 h after exposure. Alarm behavior was observed among ≈10% of termites from the first hour after exposure until 7 h after exposure. Termites treated with chlorantraniliprole displayed a range of behaviors distinct from that of fipronil and indoxacarb. The incipient stages of intoxication were characterized by sluggish movement; termites covered a distance of about 4 cm in about 10 s. This stage was followed by one characterized by very lethargic/zombie-like movements that persisted until moribundity. This behavior replaced the ataxia observed in fipronil and indoxacarb treatments. Termites walked at slow-motion pace and were able to cover a distance of 4 cm in about 30 s. At the advanced stage of lethargy, termites walked in slow-motion with raised fore-part (head and thorax) and assumed the take-off posture of a plane. There was a progressive reduction in walking speed until termites stopped moving (became moribund). Moribundity, the penultimate stage, was characterized by the inability of termites to cover a distance of at least their body length. Lethargic up and down and/or side to side movement of the head and thorax were observed. Termites retained an amazing ability to roll/turn sideways in order to resume their upright position and thus remained on their feet until death. Another distinct feature of chlorantraniliprole is the high level of uniformity in the behavioral symptoms exhibited by treated termites; the same set of symptoms was observed in termites at each observation period.

In the chlorantraniliprole treatments alarm behavior was exhibited by ≈14% of termites in the first 2 h after exposure. The frequency of alarm behavior reduced progressively until complete cessation in 2.5 h after treatment. Ataxia was not observed in the chlorantraniliprole treatment; termites in this treatment exhibited lethargic walking instead (Table 2.1).

Treatment with fipronil caused ≈65 % of termites to cluster together at any time during the study. Termites treated with fipronil spotted the substrate with proctodeal fluid and their antennae were distorted (i.e., folded backwards). During the latter stages of intoxication, the tip of the antennae became distorted or bent unlike the horizontal orientation in Formosan subterranean termites reported by Henderson (2003). During the early stages of ataxia, the antennae of indoxacarb-treated termites collapsed (i.e., became distorted). The antennae were sometimes folded beneath the mouthparts and termites appeared to be engaged in autogrooming. Unlike fipronil-treated termites, termites treated with indoxacarb held onto the substrate with their mouthparts during the advanced stages of ataxia. Chlorantraniliprole also elicited the release of proctodeal fluid in termites. The antennae of chlorantraniliprole-treated termites were kept in contact with the substrate either in a splayed or forward pointing orientation; bending or distortion was limited to the tip of the antennae.

Discussion

One of the most fundamental advantages of behavioral endpoints is that they are early indicators of intoxication when organisms are exposed to sublethal concentration of insecticides (Weis 1988). This has practical applications during inspection of structures after remedial treatment with insecticides. Recognition of behaviors induced by a specific insecticide helps to determine whether chemicals have been applied in the right places and also if termites have had contact with chemicals used to control them. Termiticide-induced behaviors such as ataxia, moribundity, release of fluid (proctodeal or stomodeal), reduced mobility, impaired tunneling (excavation), and avoidance of (repellent compounds) have significant impact on control efforts. Cessation of feeding as a direct consequence of neurotoxic or deterrent properties of insecticides aggravates the condition of treated termites through starvation. Reduced destruction of structures is an obvious advantage of this behavioral response. Chlorantraniliprole inhibits the normal contraction of the insect muscles (Cordova et al. 2006, Sattelle et al. 2008) whereas the two neurotoxic compounds cause nervous system disorders that seem to enhance the fluid release observed in the indoxacarb and fipronil treatments. Fluid release and trophallaxis is very significant in the successful horizontal transmission of insecticides. Trophallaxis is an important route of horizontal transfer that is affected by insecticides (Oi and Oi. 2006); this effect could either enhance or reduce the performance of the insecticide depending on its mode of action and other characteristics peculiar to the compound. Insect-growth regulators such as noviflumuron perform better than hexaflumuron partly because of the slower rate of clearance of noviflumuron from the termite body (Karr et al. 2004). According to the authors, their data suggest that the higher half-life of noviflumuron increases the likelihood that sufficient quantities of the compound are available in the termite to inhibit the molting process. Based on data obtained in their studies on the horizontal transfer of fipronil, Bagneres et al. (2009) suggested that metabolites of fipronil were capable of causing mortality in recipient termites when acquired by oral or dermal routes; the authors suggested that the degree of toxicity may be low relative to the parent compound. Indoxacarb, however, is metabolized into a more active form through the action of amidases and esterases (Silver and Soderlund 2005), a situation which makes the metabolized compound more potent against subterranean termites. The tendency of chlorantraniliprole-treated termites to cluster could either be a positive or negative characteristic under field conditions. A reduction in horizontal transfer would be expected if clustering is limited to toxified termites. Cluster formation will be a drawback if this is restricted to exposed termites and occurs at the expense of contact with naïve termites; this will have adverse effects on the areal coverage, transfer and general performance of chlorantraniliprole. In spite of the high concentration (300 ppm) of chlorantraniliprole used, onset of toxicity symptoms in termites exposed to this compound lagged behind those treated with indoxacarb and fipronil. The combined duration of induced behaviors, prior to death, were also considerably longer in the chlorantraniliprole treatments relative to fipronil and indoxacarb. According to Henderson (2003) delayed and/or long durations of toxicity symptoms increase the likelihood of transmission of insecticides to untreated nestmates by increasing the transmission periods.

The behavioral end points described in this study meet the biological relevance requirement suggested by Kane et al. (2005). Incipient intoxication is a stage during which termites are still capable of moving and interacting with their nestmates in ways that can enhance the horizontal transfer of toxicants. When termites become ataxic, they release fluids (stomodeal or proctodeal) and elicit some care-giving behavior from relatively healthy nestmates. Evidence suggests that intake of these fluids through contact or oral means facilitates the transmission of toxic compounds (Bagneres 2009). Moribundity is a stage during which termites are no longer capable of moving around; termites at this stage are less successful at eliciting care-giving behavior and can only contaminate their nestmates when touched or cannibalized. The significance of death as an endpoint cannot be overemphasized because it is the goal of all control efforts. Insecticide-induced behavioral symptoms serve as indicators of sublethal exposure and can complement traditional (mortality-based) indicators of toxicity that help to predict the performance of an insecticide more accurately. Information on insect-specific insecticide-induced behavioral symptoms in non-target (beneficial) insects can also serve as indicators of the environmental effects of toxicants (Kane et al. 2005).

Behavioral endpoints can also help to distinguish the relative performance of different classes of insecticides in situations in which retreatment is carried out with another compound or where different chemical compounds are applied to structures in close proximity. Analysis of behaviors will provide useful information on the relative performance of insecticides as well as resolve disputes concerning services provided by pest control firms.

The establishment of a link between biochemical and physiological processes and the provision of deeper insights into both individual and community level effects of insecticides makes behavioral endpoints very important (Brewer et al. 2001; Vogl et al. 1999). Given the fact that behavior-based chemical control of subterranean termites relies heavily on termite behavior under both normal and intoxicated conditions, indicators such as those described in this study deserve more attention in the quest for more environmentally friendly and sustainable control measures against termites. The behavioral endpoints described in this study can complement mortality-based methods in predicting the performance of non-repellent termiticides; these endpoints provide information on potential for horizontal transfer of toxicants. Practical applications of these insecticide-induced behaviors include determination of whether termites have had contact with the insecticide. Information on the prevalent insecticide-induced behavioral stage among treated-termites will be useful in estimating when termites will be eliminated from infested structure. Behavioral endpoints can also be used in identifying the active ingredients responsible for intoxication in termites; this will be particularly useful in cases where treatment failures necessitate retreatment by different pest control companies using different active ingredients.

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Table 2.1. Onset (h) and percentage of occurrence of abnormal behaviors in eastern subterranean termites exposed to three slow-acting insecticides

	Indoxacarb		Fipronil		Chlorantraniliprole	
Behavior	Time (h)	% occurrence	Time (h)	% occurrence	Time (h)	% occurrence
Incipient Intoxication	1.50	50.00	2.00	40.00	1.50	90.00
Ataxia	2.50	80.00	4.00	80.00	NA[a]	NA
Lethargic Movement	NA	NA	NA	NA	2.50	50.00
Moribundity	3.50	70.00	4.50	50.00	6.00	100.00

% occurrence= Percentage of termites exhibiting behavior

[a] Not applicable; did not occur.

CHAPTER III: EFFECTS OF INDOXACARB CONCENTRATION AND EXPOSURE TIME ON THE ONSET OF ABNORMAL BEHAVIORS IN THE EASTERN SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE)

Subterranean termites (Isoptera: Rhinotermitidae) are the most destructive pests of wooden structures which are attacked in the quest for cellulose, the staple food of termites. Termites cause more than $11 billion in economic loss and cost of control each year in the United States (Su 2002). The wide geographic distribution of the eastern subterranean termite, *Reticulitermes flavipes (Kollar),* is partly responsible for its status as the most economically important termite species in the United States (Su 1996).

Environmental toxicity, harmful effects on non-target organisms (Silver and Soderlund 2005) and the need for alternatives that were effective at low use rates (McCann et al. 2001) fueled the shift from conventional chemicals to baits and non-repellent liquid compounds. Non-repellent insecticides such as fipronil (Termidor, BASF Corp., Research Triangle Park, NC), imidacloprid (Premise, Bayer Environmental Service, Montvale, NJ), and chlorfenapyr (Phantom, BASF Corp.), became popular in the United States (Shelton and Grace 2003) and accounted for ≈60% of termiticides used in 2002 (Anonymous 2002).

Indoxacarb {(S)-methyl-7-chloro-2,5-dihydro-2-[[(methoxycarbonyl)[4-(trifluoromethoxy) phenyl] amino] carbonyl] indenol [1,2-e][1,3,4]oxadiazine-4a(3H)-carboxylate} has recently been tested for termite control (Hu 2005; Hu and Song 2005).

Desirable characteristics of this neurotoxic oxadiazine insecticide include little or no deleterious effects to beneficial insects (Silver and Soderlund 2005) and relatively low mammalian toxicity (U.S. Environmental Protection Agency 2000).

Most of the laboratory methods for evaluating the efficacy of insecticides focus almost entirely on mortality of insects. This criterion sometimes leads to toxicity indices that do not reflect the potential for horizontal transfer of insecticides. For social insects, efficacy is affected by the interaction of the insecticide, members of the colony, and the environment. Insecticide-based factors include inherent toxicity, type of substrate, concentration of insecticide, duration of exposure (Rust and Saran 2006) and mode of action; insect-based factors include behavioral biology and genetic factors. According to Mulrooney et al. (2007), environmental factors affect the behavior of termiticides in the soil which in turn affects the penetration of termiticide treatments. The effect of behavioral biology on efficacy is best addressed through behavior-based assessment of insecticides. Slow-acting and non-repellent insecticides such as indoxacarb allow enough time for interaction between intoxicated termites and their nestmates before death.

A number of studies have been carried out on attributes of indoxacarb that are relevant to their use as termiticides. One such study by Hu et al. (2005) reported higher percentage and earlier onset of death among recipient termites at higher donor-recipient ratios, dose or longer interaction time. Hu (2005) demonstrated the non-repellence of indoxacarb at concentrations ≤ 100 ppm and the significant effects of thickness and concentration of treated soil on mortality. There is no information on the description, onset, and duration of indoxacarb-induced behaviors in termites. We hypothesized that the relevant concentrations and durations of exposure affect onset of indoxacarb-induced behaviors.

The objective of this study was to determine the effects of indoxacarb concentration and exposure time on onset and duration of induced behaviors in eastern subterranean termites. We also compared the effects of indoxacarb exposure between individual and groups of termites.

Materials and Methods

Termites

Groups of eastern subterranean termites were collected from three colonies in Auburn (Lee County, Alabama) using traps described by Hu and Appel (2004). Open-bottom plastic bucket (18 cm high, 13 cm in diam.) traps were provisioned with corrugated cardboard rolls (15 cm high by 11 cm diam.) and set in the ground to trap termites.

Soil

Soil was obtained from the immediate surroundings of one of the field colonies. The soil was sieved to remove debris and sterilized in an autoclave (Fischer Scientific) at 80°C for 24 h, 2 d before the experiment.

Chemicals

Formulated indoxacarb [Indoxacarb 150 SC (15% AI) (E.I. DuPont, de Nemours and Company, DE, USA)] was used to prepare 45, 90, 135, and 180 ppm (w/w) concentration in soil (10% moisture content). Sterilized soil mixed with distilled water (10% w/w) was the control treatment.

Behavior Effects of Concentration and Duration of Exposure to Indoxacarb at the Individual Level

Five plastic Petri dishes (6.0 cm diam., 1.5 cm high) were filled with 0, 45, 90, 135 or 180 ppm indoxacarb-treated soil to a height of 0.9 cm. Forty, freshly collected termite workers (≥ 5th instar) from each of three colonies were introduced onto the treated soils with a fine brush. At 5, 10, 20, 40, 80, and 160 min after exposure, 5 termites from each treatment were transferred into five different experimental units (plastic Petri dishes, 3.5 cm diam., 1 cm high) provisioned with moist Whatman No. 1 filter paper. Experimental units were kept at 22 ± 2ºC in lidded plastic boxes (13.5 x 19 cm) with moist paper towel to maintain 85-90% RH. Experimental units were kept in the light and observations carried out at 2-h intervals for the first 3 d and then at 6- h intervals from days 4 to 31 to record onset (hours) of the various abnormal behaviors. Direct observation (i.e., with unaided eye) of behaviors was done through the transparent lid of the Petri dishes. Duration of each behavioral phase was obtained by subtracting its onset from that of the next phase.

Behavior Effects of Concentration and Duration of Exposure to Indoxacarb at the Group Level

The procedure used was similar to the individual test except that termites from each treatment (i.e., concentration and exposure time) were kept together in groups of 10 workers after treatment. One hundred worker termites from the same colony were introduced onto treated soil at 0, 45, and 90 ppm; this was done for all three colonies. At 5 min after treatment, 10 worker termites from each treatment were transferred into an experimental unit consisting of a 3.5 cm diam., 1 cm high plastic Petri dish. The procedure was repeated at 10, 20, and 40 min after treatment and was replicated twice per colony resulting in 72 experimental units (3 treatments, 4 exposure times, 3 colonies, and 2 replications per colony). The insecticide treatments were reduced to two in the group study to make observation easier. The two concentrations, 45 ppm and 90 ppm were selected to test the increased toxicity of insecticides among grouped termites as reported by (Valles and Woodson 2002). The onset (h) of abnormal behaviors and the percentage of termites showing each behavior were recorded at each observation period. Data collection in the group study was based on the assumption that termites progressed sequentially from incipient intoxication, through ataxia and finally moribundity before death. At each observation period, onset data on the most advanced behavioral symptom was assumed to have occurred in the termite that exhibited the most advanced symptom at the last observation period.

Statistical Analysis

Time data (i.e., onset of abnormal behaviors) were analyzed using the general linear models procedure (PROC GLM, SAS Institute 2001). Onset data were analyzed as a split plot with concentration as the main factor and duration of exposure as the sub-factor; significance was accepted at $P < 0.05$. Means were separated using the Duncan's Range Test of SAS (SAS Insitute 2001). The mean onset values were plotted using the three dimensional bar chart utility of SigmaPlot (SigmaPlot 8.0, SPSS Inc. 2002) to show effects of concentration and exposure time.

Results

Effects of Concentration and Duration of Exposure to Indoxacarb on Individuals

No abnormal behaviors (i.e., incipient intoxication, ataxia and, moribundity) were observed among termites in the control experiment. Higher concentrations and longer exposure to indoxacarb resulted in earlier onset of abnormal behaviors and death as well as higher percentages of termites showing these behaviors at any given time. Effects of concentration on onset of abnormal behaviors were most consistent and distinct when termites were treated for 20-40 min; 40 min was therefore used as a reference exposure time (unless otherwise stated) to compare concentration effects on onset of these behaviors in termites. Even though significant colony differences in onset of ataxia (F = 19.88, df = 2, P < 0.001), moribundity (F = 13.93, df = 2, P < 0.0001) and death (F = 11.51, df = 2, P < 0.0001) were observed, the general trend in concentration and exposure time effects were similar.

Incipient Intoxication

Onset of intoxication was generally earlier at higher concentrations and longer exposure times (Fig. 3.1A). Concentration effects on onset of intoxication were insignificant (P = 0.2896) when duration of treatment was 160 min (Fig. 3.1A); effects however resumed significance at the more advanced phases of abnormal behavior. Termites treated with 45 ppm indoxacarb for 5 to 40 min exhibited intoxication at comparable times (P > 0.05) but onset was significantly earlier at 80 and 160 min of exposure (F = 21.01; df = 5; P <0.0001) (Fig. 3.1A). In termites treated with indoxacarb for 40 min, the order of earliest to latest occurrences was 180 < 135 < 90 < 45 ppm at 19.22 ± 4.4, 92.67 ± 4.4, 123.11 ± 44.4, and 354.67 ± 20.8 h after treatment, respectively

(*F* > 21.38; df = 3; *P* < 0.0001). Effects of exposure time on onset of intoxication leveled off at 40 min in termites treated with 90 ppm indoxacarb; and at 20 and 10 min in the 135, and 180 ppm treatments, respectively. Among termites treated for 40 min, incipient intoxication lasted for 153.38 ± 27.87, 192.33 ± 73.70, 167.33 ± 59.37, and 57.67 ± 43.42 h in the 45, 90, 135, and 180 ppm treatments, respectively. This represented 65.48 ± 7.35, 51.85 ± 6.15, 39.58 ± 10.16, and 18.56 ± 8.65% of the total duration of abnormal behaviors prior to death, indicating a decrease in duration of incipient intoxication when termites were exposed to higher concentrations of indoxacarb.

Ataxia

Higher concentrations of indoxacarb generally elicited earlier onset of ataxia (Fig. 3.1B). Among termites treated for 40 min, ataxia was first observed in the 180 ppm treatment (76.89 ± 47.7 h), followed by 135 ppm (230.67 ± 59.6 h), 90 ppm (317.60 ± 69.1 h), and finally the 45 ppm treatment (501.38 ± 50.4) (*F* = 9.07; df = 3; *P* = 0.0002). Except for the 90 ppm treatment, longer exposure times resulted in earlier onset of ataxia (Fig. 3.1B). The most consistent exposure time effects were observed in the 180 ppm treatments in which increase in exposure time from 5 to 20 min resulted in earlier ataxia (*F* = 7.12; df = 5; *P* < 0.0001); longer exposure did not result in earlier onset. Among termites treated for 40 min, ataxia lasted for 30.43 ± 11.65, 57.89 ± 12.04, 172.56 ± 40.38, and 59.78 ± 10.40 h in the 45, 90, 135, and 180 ppm treatment, respectively; this represented 12.25 ± 4.01, 23.35 ± 3.50, 39.95 ± 9.01, and 48.20 ± 7.17% of the total duration of abnormal behaviors prior to death. Significant differences in onset of ataxia occurred between colonies (*F* = 19.88; df = 2; *P* < 0.001), but concentration and exposure time effects followed the same trend; both factors interacted significantly (*F* = 2.27; df

=1, 55; *P* = 0.0075) in their effects on onset of ataxia. The pre-moribund phases (i.e., incipient intoxication and ataxia) accounted for 60.46 ± 12.56, 66.84 ± 9.27, 70.70 ± 9.47, and 59.35 ± 8.30% of the total duration of abnormal behaviors in the 45, 90, 135, and 180 ppm treatments.

Moribundity

Concentration and duration of exposure decreased the time to onset of moribundity (Fig. 3.1C). When termites were exposed to indoxacarb for 40 min, onset of moribundity was similar for the 45, 90, and 135 ppm treatments (510.86 ± 59.7 h, 373.30 ± 76.5 h, and 403.22 ± 37.1 h); but moribundity occurred earlier (*F*= 7.68; df = 3; *P* = 0.0006) in the 180 ppm treatment (136.67 ± 43.8 h). Duration of exposure had no effects (*P* > 0.05) on onset of moribundity in termites treated with 45 ppm and 90 ppm indoxacarb (Fig. 3.1C). The 135 ppm (*F* =10.62; df = 3; *P* < 0.0001) and 180 ppm (*F* = 10.08; df = 3; *P* < 0.0001) treatments, however were responsive to duration of exposure. Time at which moribundity occurred was affected by termite colony (*F* > 13.93; df = 2; *P* < 0.0001) and the interaction of concentration and duration of treatment (*F* > 2.40; df = 15; *P* = 0.0048). Termites in the 45, 90, 135, and 180 ppm treatments were moribund for ≈43, 63, 82, and 49 h, respectively; these represented ≈22, 20, 21, and 30% of the total duration of abnormal behaviors prior to death. Termites treated with 45 and 90 ppm indoxacarb for 40 min exhibited onset of moribundity at comparable times (*P* > 0.05).

Death

Concentration had no significant (*P* > 0.05) influence on time of death among termites exposed to indoxacarb for ≤ 10 min (Fig. 3.1D). At exposure times ranging from 20-160 min, termites exposed to 45, 90, and 135 ppm died at similar times (*P* > 0.05) but those treated with 180 ppm indoxacarb died earlier. Time of death of termites exposed for 40 min to 45, 90, and 135 ppm was similar at: 553.86 ± 59.20 h, 436.44 ± 76.86 h, and 485.33 ± 43.72 h respectively; termites in the 180 ppm treatment, however, died earlier (185.11 ± 46.34 h) (F = 7.60; df = 3; P = 0.0006). Duration of exposure had no significant effect (P > 0.05) on time of death in the 90 ppm treatment (Fig. 3.1D). Duration of exposure was positively related to time of death in the 45 ppm (F = 2.48; df = 5; P = 0.0519), 135 ppm (F = 2.85; df = 5; P = 0.0277), and 180 ppm treatments (F = 6.08; df = 5; P = 0.0002) (Fig. 3.1D). In the 180 ppm treatment, no further acceleration of death was observed when duration of treatment was increased beyond 20 min. Even though there was a significant colony effect (F > 11.51; df = 2; P < 0.0001) on time of death, the general trend of concentration and exposure time was similar for all colonies. In the 45 ppm treatment, percentage of mortality was 55.6, 66.7, 88.9, 100, 100, and 100% when termite were treated for 5, 10, 20, 40, 80, and 160 min; corresponding mortality for termites in the 90 ppm treatment was 44.4, 77.8, 77.8, 100, 100, and 100% .

Effects of Concentration and Duration of exposure to Indoxacarb on Groups

Most (≥ 95%) termites in the control treatment remained normal throughout the study. A few (≤ 5.0%) untreated termites became sluggish, moribund, and finally died without exhibiting ataxia (Table 3.1).

Incipient Intoxication

Label rate indoxacarb (90 ppm) caused earlier onset of incipient intoxication than 45 ppm in termites exposed for 5 min (F = 10.35; df = 2; P = 0.0074), 10 min (F = 3.94; df = 2; P = 0.0362), 20 min (F = 272.84; df = 2; P < 0.0001), and 40 min (F = 716.84; df = 2; P < 0.0001) (Fig. 3.2 A). The most consistent and distinct concentration effects were observed among termites treated with indoxacarb for 20-40 min. Increased exposure generally resulted in earlier onset of incipient intoxication (Table 3.1; Fig. 3.2A). In the 45 ppm indoxacarb treatment, onset of incipient intoxication declined significantly from 242.86 ± 65.92 h among termites treated for 5 min to 42.22 ± 9.67 h among those treated for 40 min (*F* = 7.97; df = 2; *P* = 0.0009). The earliest onset of intoxication in the 90 ppm treatment occurred in termites treated for 40 min (16.14 ± 1.00 h) and the latest onset in those treated for 5 min (29.7 ± 6.5 h) (*F* = 7.50; df = 3; *P* < 0.0001). There were no colony differences in onset of incipient intoxication. Incipient intoxication lasted for 10.29 ± 1.17 and 11.13 ± 2.08 h which represent 10.07 ± 1.57 and 5.27 ± 0.68%, of the total duration of abnormal behaviors in the 45 and 90 ppm treatments, respectively. There was a significant concentration by exposure period interaction for the onset of incipient intoxication (*F* = 40.24; df = 5; *P* < 0.0001).

Ataxia

Termites treated at label rate (90 ppm) became ataxic earlier than those treated at half-label rate at all exposure times: 5 min (*F* = 8.66; df = 3; *P* = 0.0147), 10 min (*F* = 4.81; df = 3; *P* = 0.0488), 20 min (*F* = 232.71; df = 3; *P* < 0.0001), and 40 min (*F* = 10.81; df = 3; *P* = 0.0018) (Fig. 3.2B). Treatment for longer periods generally caused earlier onset of ataxia in 90 ppm (*F* = 9.38; df = 3; *P* < 0.0001) and 45 ppm (*F* = 9.48; df = 3; *P* = 0.0005). Termites from different colonies became ataxic at different times (*F* > 3.17; df = 2; *P* < 0.0468) but the trend of concentration and exposure time effects were similar. There was a significant interaction of concentration and period of exposure (*F* > 32.09; df = 5; *P* < 0.0001) for the onset of ataxia. Ataxia lasted for 104.67 ± 18.27 and 120.37 ± 15.80 h among termites treated for 40 min with 45 and 90 ppm indoxacarb, respectively; these represented 36.24 ± 2.61 and 37.01 ± 4.06%, of the total duration of abnormal behaviors prior to death. Incipient intoxication and ataxia accounted for a total of 46.32 ± 3.15 and 42.27 ± 4.11% of the duration of abnormal behaviors in the 45 and 90 ppm treatments, respectively.

Moribundity

Onset of moribundity was affected significantly by concentration in termites exposed for 10 min ($F = 4.11$; df = 2; $P = 0.0414$) and 20 min ($F = 5.55$; df = 2; $P = 0.0116$) (Table 3.1; Fig. 3.2C). Percentage of moribund termites was greatest in 90 ppm treatment and lowest in the control treatment. There were neither colony differences nor exposure time effects ($P > 0.05$) on onset of this behavior. Onset of moribundity occurred at comparable times ($P > 0.05$) in termites exposed to 45 and 90 ppm indoxacarb for 40 min. Duration of moribundity was 155.67 ± 20.08 and 177.00 ± 14.12 h which represented 53.68 ± 3.15 and 57.73 ± 4.11% of the total duration of abnormal behaviors in the 45 ppm and 90 ppm treatments, respectively.

Death

Concentration of indoxacarb and duration of treatment had direct effects on the total percentage of termites that died (Table 3.1) but insignificant effects on time of death within the 31-day duration of the study (Fig. 3.2D).

Termites at the individual level exhibited longer durations of incipient intoxication (i.e., 65.48 ± 7.35 and 51.85 ± 6.15% of the total time before death in the 45 and 90 ppm treatments which accounted for 10.07 ± 1.57 and 5.27 ± 0.68% of the total duration of abnormal behaviors at the respective concentrations. Duration of ataxia and moribundity were, however, longer among termites held in groups. At the individual level, termites treated with 45 and 90 ppm were ataxic 12.25 ± 4.00 and 23.35 ± 3.50% of the time, respectively but were ataxic 36.24 ± 2.61 and 37.01 ± 4.06% of the time when held in groups. Among termites exposed to 45 and 90 ppm, moribundity accounted for 22.27 ± 6.01 and 24.80 ± 4.31% of the time, respectively at the individual level, but accounted for 53.68 ± 3.15 and 57.73 ± 4.11% of the time among termites held in groups.

Discussion

Behavior Effects of Concentration and Exposure Time at Individual Level

Our findings are similar to those of Thorne and Breisch (2001), who reported impaired mobility of termites when exposed to sublethal doses of Imidacloprid. According to the authors, termites became immobile and lay on their backs with occasional twitching of legs and antennae as exposure times and/or concentrations were increased. Effects of concentration on onset of abnormal behaviors are also consistent with those reported for time of death by Hu et al. (2005), and the significant concentration effects of amidinohydrazone and avermectin B_1 (non-repellent compounds) on mortality and lethal time (LT) in termites reported by Su et al. (1987).

Onset of abnormal behaviors can complement simple mortality as indices of toxicity to provide more realistic estimates of field efficacy of insecticides. Various concentrations of indoxacarb elicited incipient intoxication at comparable times when termites were treated for ≤ 10 min; this was probably because the short duration of contact with the toxicant did not allow termites to acquire enough toxicant to exert significant (concentration) effects on the onset of this behavior. It appears that irrespective of concentration of indoxacarb, termites acquired enough toxicant to accelerate incipient intoxication to the fastest rate in ≤ 80 min; longer periods of exposure did not increase concentration effects. This was achieved through a reduction in the duration (but not the onset) of incipient intoxication. The fact that exposure time effects leveled off at 80, 40, 20, and 10 min for the 45, 90, 135, and 180 ppm treatments, respectively indicate that onset of incipient intoxication requires a certain dosage of indoxacarb; dosages higher than this do not result in earlier onset of the behavior. The longer duration of the incipient intoxication phase in the 45 and 90 ppm treatments increases the potential for horizontal transfer partly because termites are still capable of walking appreciable distances and participating in social interactions (e.g., care-giving and allogrooming) that still occur during this phase. We subscribe to the explanation offered by Osbrink and Lax (2001) in studies with fipronil, deltamethrin, chlorpyrifos, chlordane, and permethrin in which departures from the normal trend of concentration effects were ascribed to the possibility that portions of termite colonies might possess mechanisms that reduce uptake of termiticide; this include the release of insecticide through proctodeal or stomodeal fluid. The long duration of the incipient intoxication phase among termites exposed to 90 ppm indoxacarb (label rate) offers a greater potential for horizontal transfer of toxicants between nestmates. The fact that onset of ataxia, moribundity and death were not affected by duration of exposure further makes the label rate (90 ppm) relevant and useful in real field situations. Treatment of termites with 180 ppm indoxacarb for >20 min did not result in earlier onset of abnormal behaviors; termites probably acquired all the toxicant needed to accelerate these behaviors in the first 20 min after introduction. Approximately the same percentage of time was spent in the pre-moribund (incipient intoxication and ataxia) phases of abnormal behavior in the

45-135 ppm treatments. Termites exposed to lower concentrations of indoxacarb had longer duration of incipient intoxication whereas those exposed to higher concentrations had longer durations of ataxia and moribundity. Termites treated with 45-135 ppm died at comparable times which indicate that the insecticide can be effective over a large range of rates. Variable concentrations could result from unintended dilutions in field situations as a result of rainfall, other environmental conditions and natural degradation of the chemical over time. Colony differences in onset of ataxia, moribundity, and death were probably due to differences in susceptibility or ability to detoxify after treatment with indoxacarb, differences in size of termites, and age of colonies.

Behavior Effects of Concentration and Exposure Time at Group Level

Our results did not show the dramatic increase in insecticide toxicity, due to group effects reported by Valles and Woodson (2002). Even though the average period to onset of abnormal behaviors was shorter (at the group level), there was a marked reduction in the percentage of termites that showed these behaviors (Table 3.1); percentages ranged from 0-40 most of the time and only exceeded 80% in termites treated with indoxacarb for 40 min whereas at least 55% of termites exhibited insecticide-induced behaviors in the individual study. Social interactions (e.g., trophallaxis, allogrooming) and the concomitant redistribution of toxicants resulted in different onsets of abnormal behaviors. Termites that did not exhibit abnormal behaviors probably acquired less than the critical concentration of toxicant (not measured in the current study) than was required to induce these behaviors. This critical concentration is the behavior equivalent of the critical concentration required for the transfer of lethal doses in the mortality-based studies carried out by Shelton and Grace (2003). According to Whitman and Forschler (2007)

50% of allogrooming events result in allofeeding donations from groomed termites. Donation of indoxacarb-contaminated fluids decreased the concentration of indoxacarb which, in turn, led to delayed onset of abnormal behaviors in donor termites. It is possible that allogrooming extends beyond the infection-reducing role described by Rosengaus et al. (1998) to encompass roles that include the reduction of toxicants on the termite body. Grooming affects the relative percentage of acquired toxicants inside and on the termite body. This is particularly important for insecticides such as indoxacarb for which onset and duration of toxicity symptoms vary mainly by the amount of toxicant ingested (Oi and Oi 2006). According to Remmen and Su (2005), termites exposed to dual-mode insecticides (i.e., contact and ingestion) show earlier and more intense signs of toxicity than those exposed by either route alone. The lack of uniformity in the tendency of termites to move soil with their mouthparts or engage in allogrooming and trophallaxis, however, introduced variations that obscured the relative effects of concentration and exposure time on onset of toxicity symptoms. Effects of concentration and exposure time were also masked by the low percentage of termites that exhibited these behaviors at the group level (Table 3.1).

Effects of concentration and exposure time on onset of toxicity symptoms were more consistent for individual than groups of termites due to the absence of social interactions. At both individual and group levels, the most distinct concentration effects on onset of intoxication were observed among termites exposed for 40 min because it took ≈20-40 min for termites to burrow into the treated soil and in the process acquire sufficient quantities of indoxacarb to elicit the behavior.

Onset of abnormal behaviors is a reliable index of the toxicity of insecticide (especially those that are slow-acting). More reliable estimates of toxicity can be obtained by combining behavior and mortality-based indices. Termites might require about 20-40 min of exposure to 45, 90, and 135 ppm (field-relevant concentrations) to exhibit discernible differences in onset and intensity of toxicity symptoms.

The consistent performance of 90 ppm indoxacarb over a wide range of exposure times (at both individual and group levels) and its strategic position between two biologically relevant application rates (i.e., 45 and 135 ppm) probably account for the label rate. Given the fact that conditions in the field sometimes alter the concentration of applied insecticides, the choice of 90 ppm as the label rate is very strategic. Exposure time effects were generally weaker at the group level probably because of the redistribution of toxicants during social interactions. Latent and spill-over toxic effects characterize the onset, duration, and transition from one behavioral phase to the next. Information on time trends in onset and duration of abnormal behaviors, as well as mobility and interactive ability of intoxicated termites, contribute important information on the transfer and distribution potential of toxicants in a termite colony. These additional indices of toxicity are particularly important in the assessment of slow-acting insecticides which, according to Su et al. (1982), must be sufficiently slow-acting to be transported throughout the colony. Information on abnormal behaviors is important because the majority of social interactions such as allogrooming, casual contact, care-giving, and trophallaxis occur when termites are in the pre-moribund phases of intoxication

Studies to measure the efficacy of termiticides should include behavioral assays to determine effects on the incidence and duration of social interactions (e.g., allogrooming, care-giving, and trophallaxis) and the concomitant transfer of toxicants to nestmates. Highly toxic termiticides that have negative effects on social interactions will be less effective under field conditions. Incorporation of behavior assays in efficacy tests will help in selection of more relevant application rates that result in longer periods of incipient intoxication and other behavioral phases during which social interactions are appreciable enough to enhance transfer and therefore efficacy of the insecticides. The fact that laboratory assays are carried out in restricted arenas (unlike field situations) necessitates behavioral assessments to determine the contribution of behavior-based horizontal transfer to the total efficacy of the termiticide reflected in termite mortality.

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Table 3.1. Effect of indoxacarb concentration on onset (h) of abnormal behaviors in eastern subterranean termites (Mean ± SE) at the group level

Behavior	Exposure time (min)[a]	Control[b]	%	45 ppm	%	90 ppm	%
Disoriented	5	-	-	242.86 ± 65.92a	11.67 ± 4.77	29.71 ± 6.52b	13.33 ± 4.22
	10	137.33 ± 99.33a	5.00 ± 2.24	59.5 ± 16.38ab	6.67 ± 2.11	29.88 ± 7.37b	30.00 ± 14.61
	20	411.0 ± 3.00a	3.33 ± 3.33	256.33 ± 41.54b	10.00 ± 6.32	24.08 ± 0.85c	83.33 ± 6.67
	40	296.00 ± 8.00a	5.00 ± 3.42	42.22 ± 9.67b	15.00 ± 6.71	16.14 ± 1.00c	98.33 ± 1.67
Ataxic	5	-	-	280.29 ± 67.12a	11.67 ± 4.77	42.00 ± 10.16b	8.33 ± 4.01
	10	-	-	150.00 ± 82.49a	5.00 ± 2.24	47.82 ± 13.68b	21.67 ± 14.00
	20	-	-	337.00 ± 22.58a	10.00 ± 6.32	66.22 ± 8.67b	38.33 ± 13.02
	40	-	-	54.29 ±14.50a	11.67 ± 6.01	27.08 ± 2.42b	65.00 ± 14.32

Means within a row followed by the same letter are not significantly different (*P* > 0.05)

% = Percentage of termites showing abnormal behavior.

[a] Number of termites per exposure time = 60.

[b] Dash indicates that behavior did not occur.

Table 3.2. (Continued). Effect of indoxacarb concentration on onset (h) of abnormal behaviors in eastern subterranean termites (Mean ± SE) at the group level

Behavior	Exposure time (min)[a]	Control[b]	%	45 ppm	%	90 ppm	%
Moribund	5			414.00 ± 77.87a	10.00 ± 3.65	215.00 ± 121.00a	3.33 ± 2.11
	10	504.00 ± 0.00a	3.33 ± 2.11	315.30 ± 155.14ab	5.00 ± 2.24	156.7 ± 44.84b	21.67 ± 14.00
	20	450.00 ± 12.00a	3.33 ± 3.33	422.00 ± 29.75ab	10.00 ± 6.32	252.75 ± 34.70b	26.67 ± 12.02
	40	257.33 ± 8.67a	5.00 ± 3.42	161.00 ± 72.34a	10.00 ± 6.32	170.08 ± 26.54a	41.67 ± 14.00
Dead	5	-	-	480.70 ± 73.15a	10.00 ± 3.65	206.00 ± 101.37a	3.33 ± 2.11
	10	579.00 ± 51a	3.33 ± 2.11	360.00 ± 163.36a	5.00 ± 2.24	336.00 ± 37.52a	21.67 ± 14.00
	20	552.00 ± 24.00a	3.33 ± 3.33	483.00 ± 29.06a	10.00 ± 6.32	366.10 ± 43.00a	26.67 ± 12.02
	40	344.00 ± 8.00a	5.00 ± 3.42	316.67 ± 115.15a	10.00 ± 6.32	319.08 ± 22.85a	43.33 ± 14.53

Means within a row followed by the same letter are not significantly different (*P* > 0.05)

% = Percentage of termites showing abnormal behavior.

[a] Number of termites per exposure time = 60.

[b] Dash indicates that behavior did not occur.

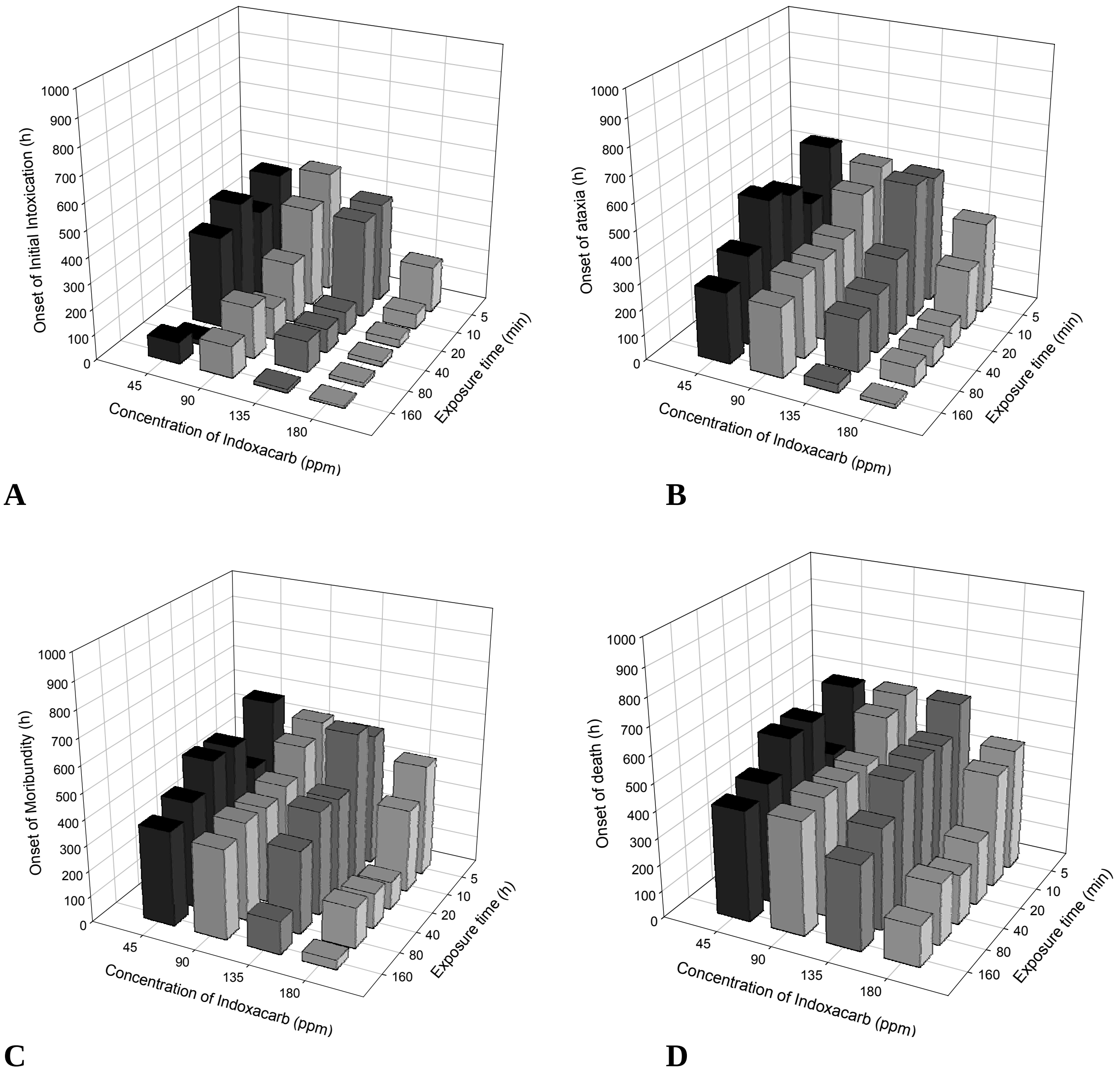
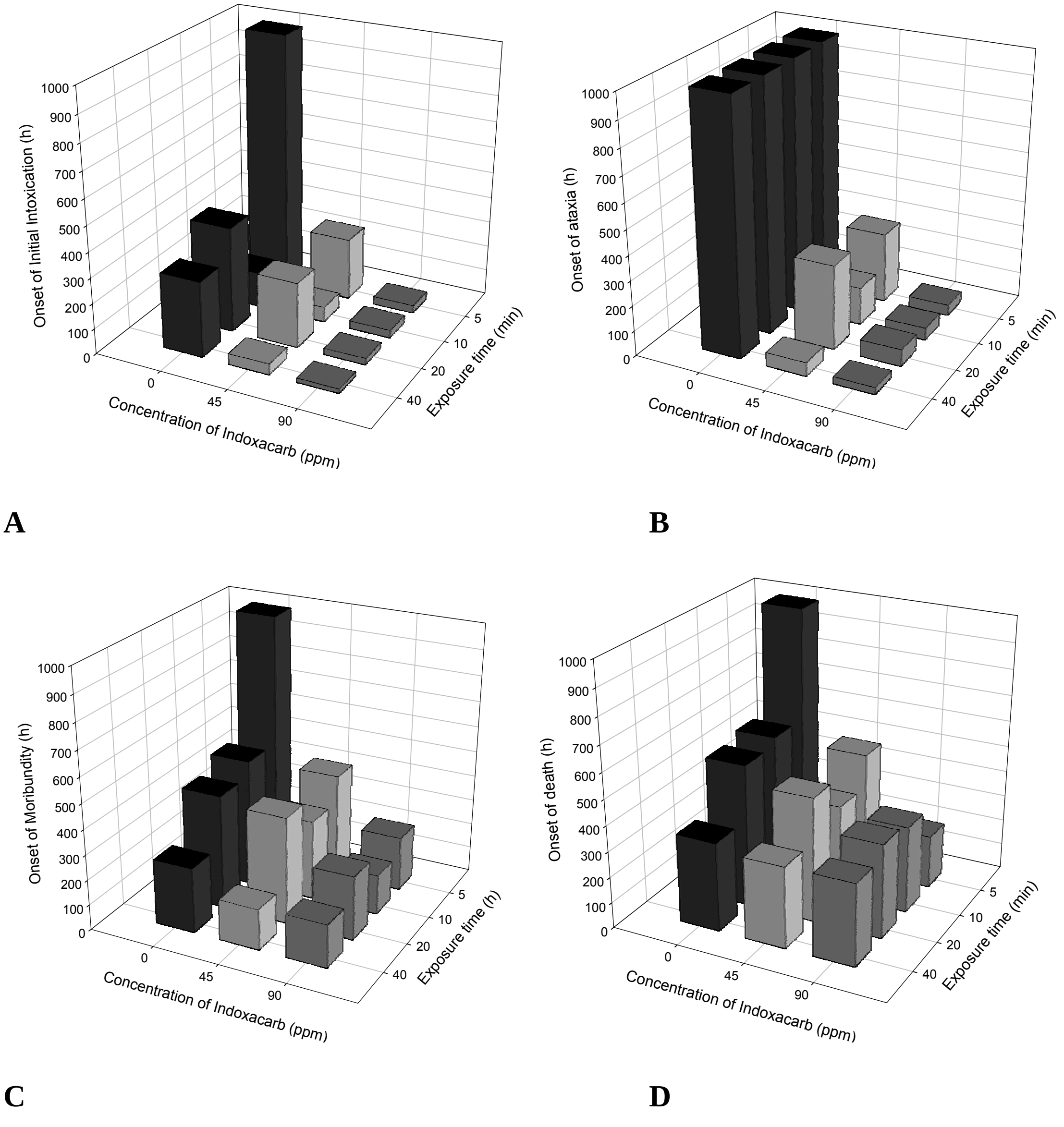


Figure 3.1. Relation between mean onset (h) of abnormal behaviors in eastern subterranean termites and indoxacarb concentration (ppm) and exposure time (min) at the individual level.

The response of different behaviors is indicated by symbols explained in the figure. Graphs A to D are for termites at the individual level; Graph A is incipient intoxication, B onset of ataxia, C onset of moribundity, and D onset of death.



A

B

C

D

Figure 3.2. Relation between mean onset (h) of abnormal behaviors in eastern subterranean termites and indoxacarb concentration (ppm) and exposure time (min) at the group level.

The response of different behaviors is indicated by symbols explained in the figure. Graphs A to D are for termites at the individual level; Graph A is incipient intoxication, B onset of ataxia, onset of moribundity, and D onset of death.

CHAPTER IV: EFFECTS OF NON-REPELLENT INSECTICIDES ON THE TUNNELING AND WALKING ABILITY OF THE EASTERN SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE) WORKER

Subterranean termites (Rhinotermitidae) forage for food and frequently attack structural wood for cellulose. The damage and cost of control of rhinotermids in the United States has been estimated at $3 billion per annum, an amount that attests to their economic importance (Su 2002). The eastern subterranean termite, *Reticulitermes flavipes* (Kollar), and the Formosan subterranean termite, *Coptotermes formosanus* Shiraki cause the most damage in the United States (Su and Scheffrahn 1990). The Eastern subterranean termite is however, undoubtedly the single most economically important termite species in the United States partly because of its wide geographical distribution relative to other species (Su 1996).

The history of termiticide use in the United States shows that structures were typically protected with repellent compounds until the development of non-repellent termiticides. Application requirements were rigorous to minimize the occurrence of untreated gaps in the chemical barrier. According to Perrott (2003) such under- or untreated sections became avenues by which foragers breached repellent-based chemical barriers**.** Uniform application is imperative with repellent compounds, but much less critical with non-repellent compounds. Environmental concerns associated with many of the conventional chemicals (Silver and Soderlund 2005) also led to the use of baits and non-repellent liquid alternatives. Desirable qualities of slow-acting, non-repellent compounds include their permissive nature that allows termites to continue tunneling in treated soils for considerable lengths of time before they become intoxicated, immobilized, and die. These compounds afford treated termites ample time and opportunity to interact and thereby contaminate untreated nestmates in locations that may be far removed from the treated zone. The relatively extended periods of mobility of termites exposed to slow-acting, non-repellent compounds enables them to continue foraging and interacting with naïve nestmates in a variety of locations. Foraging may be above-ground in shelter tubes (constructed out of soil, excrement, and salivary secretions); these tubes provide protection from predators, parasites, and environmental stresses (Chen and Henderson 1997). Underground foraging occurs through a network of tunnels constructed to optimize search patterns for food (Pitts-Singer and Forschler 2000).

Indoxacarb and fipronil have generated interest for a number of reasons. Fipronil is one of the most commonly used non-repellent compounds in the termite control industry. It is a phenylpyrazole that functions by blocking the γ-aminobutyric acid-gated chloride channel of the insect nervous system (Hainzl and Casida 1996). Indoxacarb is classified as reduced toxicity compound (US.EPA 2000) and successful field trials against termites have drawn attention to this oxadiazine; its mode of action is a voltage-dependent blocking of the sodium channel (Silver and Soderlund 2005). The efficacy and non-repellence of these two compounds (Hu 2005), the horizontal transfer of fipronil (Song and Hu 2006), and transfer of indoxacarb (Hu et al. 2005) have all been demonstrated in laboratory studies with termites. Effects of non-repellent compounds on the foraging range and activity of subterranean termites were studied under both laboratory (Su 2005a) and field (Hu et al. 2007) conditions. The influence of soil compaction (Tucker et al. 2004), moisture gradients (Su and Puche 2003), and presence of sound or decayed wood (Su 2005b) on tunneling behavior and ability of termites have been studied. There is still very little information on the effects of indoxacarb and fipronil on the tunneling behavior and tunneling ability of termites. These behaviors likely affect the size of the area protected by these compounds.

The purpose of this study was to describe the tunneling behavior of eastern subterranean termites treated with slow-acting, non-repellent insecticides. A second objective was to determine the effects of insecticide concentration and post-exposure time on termite mobility. The working definition of mobility encompassed both walking and tunneling activities of termites. We hypothesize that tunneling behavior will be different for low concentration of fipronil versus high concentration of indoxacarb and that insecticide concentration would have a strong negative correlation with termite mobility.

Materials and Methods

Termites

Groups of eastern subterranean termite workers were collected from a field location in Auburn-Opelika (Lee County, AL) in March and April 2008 using underground traps described by Hu and Appel (2004). Traps consisted of open-bottom plastic buckets (18 cm height, 13 cm in diam.) provisioned with corrugated cardboard rolls (15 cm high and 11 cm in diam.). Traps loaded with termites were brought to the laboratory and termites were extracted by gentle tapping of the traps to allow termites to drop onto a moist paper towel in a tray. Freshly collected termites were used for studies.

Chemicals

Formulate indoxacarb [Indoxacarb 150 SC (15% AI) E.I. Dupont, de Nemours and Company Wilmington, DE)] and fipronil (9.1% AI, BASF Corp., Research Triangle Park, NC) were used in this study. Plastic Petri dishes (5.2 cm in diam., 1.5 cm in height) provided with Whatman No. 1 filter paper (Whatman International Ltd, Maidstone, England) of the same diameter served as treatment chambers. Calculated amounts of fipronil and indoxacarb were used to prepare various concentrations of insecticide-treated filter paper. Treatments were 50, 100, and 200 ppm of indoxacarb-treated filter paper and 1, 10, and 50 ppm of fipronil treated-filter paper. The concentration of indoxacarb and fipronil were chosen based on previous studies by the author; the same concentrations of fipronil were also used by Yeoh and Lee (2007) but the insecticide was in soil medium as opposed to filter paper used in the current study. Filter paper treated with distilled water was the control treatment. Treated-filter papers were air-dried in a hood for 24 h and later moistened with 0.4 ml of distilled water prior to the test.

Test for Tunneling of Workers

Soil was obtained from the immediate surroundings of the field colony in Auburn (Lee County, AL). The soil was sieved to remove debris and sterilized in an autoclave (Fischer Scientific®) at 80°C for 24 h, 2 d prior to the experiment. Distilled water was added to the soil to achieve 18% moisture (w/w). Moist soil weighing 1075 g was used to fill a cylindrical plastic container (25 cm in diam., height 9 cm) to a height of 2 cm, resulting in a bulk density of approximately 1.09g/cm^3.

In a preliminary test, 100 workers of at least 5th larval instar were exposed to 200 ppm, indoxacarb-treated filter paper for 10, 20, 40, and 60 min; each treatment was replicated three times. Treated termites were transferred into transparent plastic cups (29.6 ml, Fill-rite Corp. Newark, NJ); these cups were inverted to deposit termites atop soil in the middle of cylindrical transparent plastic arenas (25.0 cm in diam., 9 cm high) each of which constituted an experimental unit. Cups were pressed gently into the soil to drive the rim to a depth of ≈1 cm. The resulting space at the external periphery of the rim was covered with soil after which the whole arena was covered with a lid and sealed with cellophane to prevent loss of moisture. Experimental units were placed atop a transparent glass shelf of an observation panel which consisted of a rectangular wooden framework on top of which was placed a transparent glass sheet. A mirror inclined at an appropriate angle beneath the glass sheet enabled the direct observation of activities at the base of each experimental unit. Tunneling activity and behavior were recorded at hourly intervals for the first 16 h after which observations were taken at 6 h intervals until complete cessation of tunneling. Progress in tunnel construction was tracked by tracing the path of the tunnels on the bottom of the transparent arena using colored markers. The labeling system used by Hedlund and Henderson (1999) and later by Pitts-Singer and Forschler (2000) was adopted to study the elaborate network of tunnels. Tunnels were defined based on their origin with respect to the site of introduction of termites. Primary tunnels originate from the introduction site and terminate at the edge of the arena opposite it; secondary tunnels branch off primary tunnels, and tertiary tunnels branch of secondary tunnels.

Based on our preliminary results, 10 min of exposure to insecticide deposits was selected to test effects of indoxacarb and fipronil on the tunneling ability of eastern subterranean termites. Observation and marking of tunnels were done at the same designated time intervals as described in the preliminary test. The test was concluded when tunneling had ceased in all treatments for 12 h. Termites that were alive but immobilized were placed on a sheet of Whatman No.1 filter paper [(55 mm diam. Whatman international Ltd, Maidstone, England)] to determine if they were still capable of walking.

Test of Walking Ability of Insecticide-intoxicated Subterranean Termites

Two hundred freshly-collected eastern subterranean termite workers were counted into groups of 10 termites and held in Petri dishes (5.2 cm in diam., 1.5 cm height) provisioned with Whatman No.1 filter paper (55 mm diam. Whatman international Ltd, Maidstone, England). The test arena consisted of a Petri dish (13.6 cm in diam.) provided with Whatman No.1 filter paper (125 mm in diam. [Whatman international Ltd, Maidstone, England]). With the aid of a compass and a black ink ballpoint pen (Paper mate Write Bros. Med. PT.), an 8.8 cm diameter circle was drawn on the filter paper in such a way as to achieve a uniform concentration of the trail substance (2-phenoxyethanol) contained in the ink.

Groups of termites were exposed to various concentrations insecticide-treated filter paper as above. Actual treatment was done by exposing groups of ten termites to each treatment for 10 min after which they were transferred into similar Petri dishes lined with untreated filter paper (moistened with 0.4 ml of water). Walking ability was measured by transferring individual termites into the test arena which was then covered. Termites were allowed sufficient time (≈15 s) to find the ink trail and the distance of trail following was measured. A pencil was used to mark the start and end points of termite movement in 60 s and a thread was placed along the path covered by the termites. The distance was marked on the thread and determined using a ruler. Tests were repeated at 2-h intervals until termites became immobilized. A separate test arena was used for each treatment (i.e., concentration and exposure time combination).

Statistical Analysis

Tunneling data were transformed by log 10 (x) to stabilize the variance and back transformed after analysis for presentation in tables. Regression analysis was used to determine the effects of insecticide concentration on the tunneling ability of termites post-treatment. Walking distance data for fipronil and indoxacarb were analyzed separately using the randomized split plot design of SAS (SAS Institute 2003). Concentration served as the main plot and post-exposure time as the sub-plot.

Results

Tunneling Behaviors

Irrespective of the concentration or type of insecticide used, termites tunneled to the base of the arena and then constructed tunnels that radiated out from that central location towards the periphery of the arena. Termites at the tunnel head picked up soil particles reversed or turned around to deposit soil particles on the tunnel wall away from the tunnel head. Other termites gained access to the tunnel head, either by squeezing past termites that were either reversing or walking back in a forward direction after turning around in distended portions of the tunnel. Reversing termites eventually turned around at portions of the tunnel that were enlarged. To create enlarged portions, tunneling was done at the tunnel head by two or three termites at the same time or tunneling was done at different parts of the tunnel head by different termites. Transport of soil particles from the tunnel head in enlarged portions was achieved by a simple turn-around and subsequent deposition of soil particles at earlier portions of the tunnel. Sometimes termites lingered at the tunnel head causing termites immediately behind them to hit their posterior; this was achieved through a series of forward and backward movements. This action usually elicited reversing or a turn-around and exiting of the tunnel head by the lead termite. Termites behind the lead termites apparently were cued by reversing or returning termites and reversed until lead termites arrived at enlarged areas where the next termite could squeeze past it to the tunnel head.

Tunneling declined significantly in the control treatments after a number of tunnels reached the periphery of the test arena; this occurred in spite of the presence of tunnel-free areas. Subsequent movement of termites consisted mainly of walking inside the constructed tunnels. Branching of tunnels occurred due to the occlusion of tunnel heads by moribund termites thereby preventing more active termites in older portions of the tunnel from reaching the tunnel head. The tandem posture of termites during excavation necessitates the withdrawal of lead termites and replacement by termites that are next in line. Failure to leave tunnel heads necessitated the branching of tunnels by relatively active foragers located behind moribund individuals. The majority of moribund termites were found at tunnel heads at the end of the study. Insecticide-induced reduction in physical ability generally caused foragers to seek paths of apparent “least resistance” for their excavation activities. Branching in this case was probably more of an attempt to optimize the expenditure of energy in tunnel construction to exploit the largest possible area with a dwindling energy reserve.

An interesting observation among the indoxacarb-treated termites was the “lone ranger phenomenon” in which tunneling was carried out at one or two tunnel heads by a single termite long after other termites had stopped tunneling due to ataxia or moribundity. This kind of tunneling typically involved: removal of soil particles from the tunnel head, walking in reverse, deposition of the soil particles in older portions of the tunnel, and walking forward to pick up another particle from the tunnel head. These “lone rangers” very seldom turned around to transport soil particles away from the tunnel head in a forward motion. Another interesting observation was that irrespective of treatment (i.e., insecticide-treated, or untreated), once termites made contact with the bottom of the arena, majority of them tunneled from an upside down position, with their dorsum in contact with the bottom of the arena. Termites walked upright inside the tunnels when they were not engaged in gallery extension or when tunneling had ceased.

Quantitative Indices of Tunneling Capacity

In the preliminary study, the total length of tunnels constructed by termites treated with 200 ppm indoxacarb was less than those in the controls ($P < 0.05$); tunnel lengths decreased by 54.17 ± 22.41, 55.86 ± 22.01, 70.98 ± 14.01, and 89.21 ± 5.26% in termites exposed to indoxacarb for 10, 20, 40, and 60 min respectively. Time to cessation of tunneling and the total number of branches were unaffected by duration of exposure to indoxacarb (Table. 4.1). In the indoxacarb treatments, the total length of tunnels constructed remained similar for exposure durations from 10-40 min but declined significantly when termites were treated with indoxacarb for 60 min. Based on the results in the preliminary test (Table 4.1) a 10 min exposure was selected to test the effects of insecticide concentration on tunneling; this enabled a conservative estimate of the duration of treatment required to incapacitate termites.

Untreated termites constructed more and longer tunnels ($P < 0.05$) compared to those in the indoxacarb (Fig. 4.1) and fipronil (Fig. 4.2) treatments. Concentration of indoxacarb did not affect the total number of branches constructed. The reduction in total tunnel length from 55.05 ± 4.87 cm (mean ± SE) in the 50 ppm indoxacarb treatment to 22.60 ± 8.16 cm in the 200 ppm indoxacarb treatment and the reduction from 45.60 ± 3.35 in the 1 ppm fipronil treatment to 1.68 ± 1.13 in the 50 ppm fipronil treatment were both deemed statistically insignificant ($P > 0.05$). The 15.25 ± 1.44 tunnels constructed in the 1 ppm fipronil treatment were statistically similar to the 4.00 ± 2.42 tunnels constructed in the 50 ppm fipronil treatment ($P > 0.05$).

Increase in number of branches was achieved either by branching of existing tunnels or construction of new primary tunnels. Time trends show that the number of branches constructed by untreated termites kept increasing until ≈96 h after introduction into the test arena (Figs. 4.3 and 4.4). Concentration effects in the indoxacarb treatments became more noticeable with time after exposure (Fig. 4.3), but such effects were statistically insignificant ($P > 0.05$). The number of branches leveled off as early as the first 2 h in the 10 ppm and 50 ppm fipronil treatments whereas a barely noticeable number of branches were still added until 25-30 h after exposure to 1 ppm fipronil (Fig. 4.4). The number of branches constructed in the 1 ppm fipronil treatments appeared to be more than those constructed in the 10 and 50 ppm fipronil treatments (Fig. 4.4) but these differences were statistically insignificant ($P > 0.05$).

Time trends in tunnel construction (Fig. 4.5) show untreated termites extended their tunnels more than the indoxacarb-treated termites at each time interval until ≈88 h when tunneling ceased or slowed down significantly because ≈40% of branches had reached the edge of the arena. Tunneling ceased in the 100 and 200 ppm indoxacarb treatment 64-72 h after treatment whereas termites in the 50 ppm indoxacarb treatment ceased tunneling 112-120 h after treatment. Distance tunneled by termites declined with time after exposure to indoxacarb ($F = 6.16$, df = 7, $P < 0.0001$); concentration had insignificant effects ($F = 0.95$, df = 2, $P = 0.3913$) even though reduction in tunneling distance at higher concentrations was a consistent trend. Tunneling was faster in untreated termites ($F = 99.99$, df = 1, $P < 0.0001$); tunneling declined with time after exposure to indoxacarb ($F = 6.17$; df = 7, $P < 0.0001$) but was unaffected by concentration of indoxacarb ($F = 0.95$, df = 2, $P = 0.3887$). Untreated termites constructed a greater number of branches compared to indoxacarb-treated individuals ($F = 80.92$, df = 1, $P < 0.0001$) but concentration ($F = 0.61$, df = 2, $P = 0.5474$) and time after treatment ($F = 1.90$, df = 7, $P = 0.077$) had insignificant effects. Untreated termites extended their galleries by a significantly greater distance ($P < 0.0001$, $F = 158.42$, df = 1), number of branches ($P < 0.0001$, $F = 102.41$, df = 1) and at a faster pace ($F = 158.68$, df = 1, $P < 0.0001$) compared to their fipronil-treated counterparts. Distance tunneled declined with hours after exposure ($F = 6.04$, df = 7, $P < 0.001$). Termites in the 10 and 50 ppm fipronil treatments tunneled similar distances ($P > 0.05$) but those in the 1 ppm fipronil treatments tunneled longer ($F = 8.54$, df = 2, $P = 0.0004$) distances. Number of branches constructed was similar at various times post-exposure ($F = 1.14$, df = 7, $P = 0.3440$) and for the 10 and 50 ppm treatment but the 1 ppm fipronil treatments recorded more tunnels (F = 13.96, df = 2, P < 0.0001). Termites treated with various concentrations of fipronil tunneled at rates that were significantly different (F = 8.55, df = 2, P = 0.0004). Among fipronil-treatments, tunneling was fastest in termites exposed to 1 ppm fipronil-treated paper. Tunneling slowed down with time after exposure (F = 6.05, df = 7, P < 0.0001). Tunneling in the 10 and 50 ppm fipronil treatments had ceased by 32 h and 16 h after treatment, respectively whereas the 1 ppm treatments tunneled until 56 h after treatment. Untreated controls however kept tunneling until 112 h when ≈30% of tunnels radiating from the central part of the arena had reached the edge. Approximately 65% of insecticide-treated termites that appeared to be immobilized at the end of the tunneling test were found to be capable of walking ≈20 cm/min.

Walking Ability

Distance walked by untreated termites remained fairly constant (F = 0.99, df = 7, P = 0.4439) throughout the study but declined significantly 8 h after treatment in both the 100 ppm indoxacarb and 1 ppm fipronil treated termites (Table 4.2). Untreated termites never ceased walking during the study but complete cessation of walking was observed 14 h after treatment in both insecticide treatments.

Discussion

The experimental set-up did not allow an unfettered view of the specific role of the various mouthparts in the excavation process but activities observed generally matched the descriptions of Ebeling and Pence (1957), Noirot (1970), Tucker et al. (2004), Whitman and Forschler (2007), and Li and Su (2009). Termites excavated soil particles instead of squeezing between pores because the soil particle sizes were < 1.7 mm which

Su and Scheffrahn (1992) reported was suitable for tunneling by eastern subterranean termites. Termites move along physical guidelines such as roots and pieces of wood probably because these guidelines might lead to other food sources (Swoboda and Miller 2004); positive thigmotaxis may explain the tendency to tunnel from an inverted position. This orientation places the dorsum against a more solid surface than the ventral surface. It is also possible that this orientation was induced by the nature of the arena; the hard plastic base of the arena differs from conditions in the field. On the other hand this orientation might reveal a tendency of termites to place their dorsum on a relatively harder surface than their ventral side. This behavior may have major effects on the orientation of tunnels and requires further study.

Tunneling behavior and capacity of intoxicated termites clearly reflected their decline in health; as reported by Strack and Myles (1997), intoxication effects were clearly observable without having to physically interfere with the test arena. Mobility of termites is an important determinant of the amount of horizontal transfer of toxicants which in turn affects the rate and magnitude of termite mortality. When tunneling capacity is compromised, as was the case among insecticide-treated termites in the current study, mobility of termites is restricted to walking in tunnels constructed prior to intoxication. Intoxicated termites are no longer able to forage for food in new territories. It is well-established that horizontal transfer of toxicants increases with increasing population density and contact frequency between individual termites (Valles and Woodson 2002). Probability of contact between treated and untreated termites is also known to decline with declining LT_{90} value of an insecticide. Higher LT_{90} values associated with higher insecticide concentrations, cause termites to become immobilized and lose their tunneling ability earlier. Tunnel construction involves the expenditure of energy which weakens the termite and accelerates the onset of moribundity; this may explain why most of the moribund termites were found at the tunnel heads. According to Shelton and Appel (2001), untreated eastern subterranean termites move continuously at a pace that is almost steady, with very short pauses from time to time; this is in stark contrast to the behavior under intoxication observed in the current study.

Branching of tunnels occurred probably to take advantage of more excavatable areas or due to an innate tendency of foraging termites to achieve a certain amount of coverage (i.e., tunnel density) of their surroundings. The importance of the latter factor was demonstrated by the ≈40% decline in tunneling among untreated controls after a number of tunnels reached the wall of the arena. This density which might be a function of both termite population and foraging area seems to reduce the tendency of foragers to continue tunneling. Foraging termites in field situation are, however, not restricted by arena walls and may not exhibit this behavior but may reduce their tunneling activities in response to other cues or events like the discovery of a rich source of food, or a huge rock. Eastern subterranean termite workers have however been reported to continue foraging without altering their speed significantly after discovering a food source (Delaplane and La Fage 1989). This is probably due to the attainment of a certain degree of coverage of the territory provided by the test arena. This is in consonance with the assertion of Pitts-Singer and Forschler (2000) that termites have an innate tendency to optimize the construction of tunnels to access new sources of food. The authors defined an optimal pattern as one that minimized search overlap but maximized the area searched using the number of branches constructed**.** As described by (Delaplane and LaFage 1989) tunnels constructed by eastern subterranean termites in this study were long and narrow; an adaption aimed at increasing the probability of finding other sources of food underground.

The significant reduction in cumulative tunnel length when termites were exposed to 10, and 50 ppm fipronil, compared to those treated with 1 ppm fipronil confirms the findings of Yeoh and Lee (2007) who conducted a similar experiment using continuous exposure to treated soil rather than limited exposure to filter paper. The number of branches leveled off as early as the first 2 h in the 10 ppm and 50 ppm fipronil treatments whereas very few branches were added until 25-30 h after exposure to 1 ppm fipronil

Termites that had ceased tunneling and walking in the arena were able to move appreciable distances when placed on filter paper. When treated with the same concentration of insecticide-treated filter paper, termites in the tunneling test retained their walking ability for longer periods compared with those in the walking test. This is probably because termites lost some of the toxicants through dermal contact with soil during the excavation process; the frequent transfer of termites from holding chambers to walking arenas may have weakened the test subjects; subjects in the control treatment, however appeared to be unaffected by these transfers.

There have been a number of suggestions on how to improve the performance of contact insecticides in soil. These include alteration in the duration and amount of active contact; active contact is defined as a method by which insects contact insecticides by tunneling through a band of insecticide-treated soil (Chen 2006). The author suggested the incorporation of chemicals that elicit or enhance digging into formulations of contact insecticides; this is expected to improved contact with insecticides and cause reductions in the amount of insecticides applied to achieve the same degree of control as conventional insecticide formulations. Termites in the current study acquired toxicants through treated filter paper instead of active transport but termites exhibited the expected trends in tunneling capacity mode of exposure notwithstanding.

The arena used in the current study is less restrictive dimensionally and spatially compared to artificial tubes which are desirable if the objective is to measure unidirectional tunneling. While branching of tunnels and other tunneling behavior are restricted in artificial tubes, the reduction in tunneling among untreated controls when tunnels reach the side arena side walls observed in the current study also constitutes environmental inhibition.

Tunnels are sometimes constructed through intact chemical barriers not because of low toxicity or poor performance of these compounds but because of high population of termites. Jones (1990) demonstrated that termites tunneled further into treated soil at higher population densities. According to Mulrooney et al. (2007) this penetration of barriers is not due solely to high population densities; environmental factors that affect the behavior of termiticides in the soil also play an important. Tunnel density (length and number of branches per unit area) seems to have an influence on the tendency of foragers to continue tunneling; higher tunnel densities seem to cause a decline in tunneling activity. The effect of olfactory gradients (Evans 2003) and stigmergic cues (Grasse 1959, O'toole et al. 1999) as determinants of tunnel orientation were not examined in this study and so their significance cannot be ruled out.

Tunneling and walking ability of intoxicated termites is an important determinant of their potential to transfer toxicants to naïve nestmates. It is very important to conduct further studies to test the interaction termites treated with indoxacarb and fipronil and naïve termites in soil treatments to determine effects on tunneling and walking distance.

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Table 4.1. Tunneling ability of eastern subterranean termite workers exposed to 200 ppm indoxacarb-treated filter paper (Mean ± SE)

Exposure time (min)	Total length of tunnels (cm)	Time to cessation of tunneling (h)	Number of branches
0	31.67 ± 2.17^{a}	∞	7.33 ± 0.46^{a}
10	14.33 ± 6.51^{ab}	8.70 ± 1.39^{a}	5.33 ± 0.46^{a}
20	13.53 ± 6.27^{ab}	9.03 ± 1.18^{a}	7.00 ± 0.48^{a}
40	9.73 ± 5.21^{ab}	8.73 ± 1.21^{a}	8.00 ± 1.02^{a}
60	3.63 ± 1.99^{b}	7.13 ± 1.85^{a}	3.33 ± 0.21^{a}
F	4.64	0.36	1.02
P	0.0223	0.7869	0.4429

Means within the same column with different letter superscripts differ ($P < 0.05$)

Total number of workers = 100

Number of replicates = 3

Table 4.2. Walking distance per min (cm) of indoxacarb-treated termites (Mean ± SE)

	Control	Indoxacarb		
Time after Exposure (h)	0 ppm	50 ppm	100 ppm	200 ppm
0	78.68 ± 3.42^{a}	88.41 ± 3.54^{a}	77.95 ± 3.81^{a}	73.7 ± 3.19^{a}
2	73.87 ± 2.22^{a}	87.6 ± 2.37^{a}	78.17 ± 5.66^{a}	58.43 ± 3.80^{b}
4	74.78 ± 2.32^{a}	77.94 ± 1.54^{b}	72.14 ± 6.74^{a}	31.75 ± 3.26^{c}
6	73.83 ± 1.67^{a}	69.13 ± 2.91^{b}	61.22 ± 9.30^{ab}	12.43 ± 3.06^{d}
8	71.90 ± 3.15^{a}	59.33 ± 3.78^{c}	41.92 ± 7.40^{bc}	0.00 ± 0.00^{e}
10	73.02 ± 2.71^{a}	50.13 ± 3.52^{d}	21.35 ± 6.62^{cd}	0.00 ± 0.00^{e}
12	69.95 ± 2.49^{a}	31.27 ± 5.91^{e}	15.40 ± 5.56^{cd}	0.00 ± 0.00^{e}
14	71.34 ± 2.81^{a}	10.3 ± 3.17^{f}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}
16	72.34 ± 2.49^{a}	0.5 ± 0.50^{f}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}
F	0.99	138.82	24.66	165.29
P	0.44	<0.0001	<0.0001	<0.0001

Means in the same column with different letter superscripts differ ($P < 0.05$)

10 replicates per treatment

Table 4.3. Walking distance per min (cm) of fipronil-treated termites (Mean ± SE)

	Control	Fipronil		
Time after Exposure (h)	0 ppm	1 ppm	10 ppm	50 ppm
0	78.68 ± 3.42^a	77.26 ± 3.60^a	79.08 ± 2.82^a	73.93 ± 4.05^a
2	73.87 ± 2.22^a	69.85 ± 3.37^a	40.7 ± 4.03^b	29.14 ± 1.74^b
4	74.78 ± 2.32^a	65.36 ± 5.56^a	15.4 ± 4.64^c	7.2 ± 2.72^c
6	73.83 ± 1.67^a	62.27 ± 5.41^a	0.00 ± 0.00^d	0.00 ± 0.00^d
8	71.90 ± 3.15^a	29.61 ± 10.27^b	0.00 ± 0.00^d	0.00 ± 0.00^d
10	73.02 ± 2.71^a	$16.64 \pm 9.08b^c$	0.00 ± 0.00^d	0.00 ± 0.00^d
12	69.95 ± 2.49^a	$7.40 \pm 3.82b^c$	0.00 ± 0.00^d	0.00 ± 0.00^d
14	71.34 ± 2.81^a	0.00 ± 0.00^c	0.00 ± 0.00^d	0.00 ± 0.00^d
F	0.99	26.80	150.58	210.20
P	0.44	<0.0001	<0.0001	<0.0001

Means in the same column with different letter superscripts differ ($P < 0.05$)

10 replicates per treatment

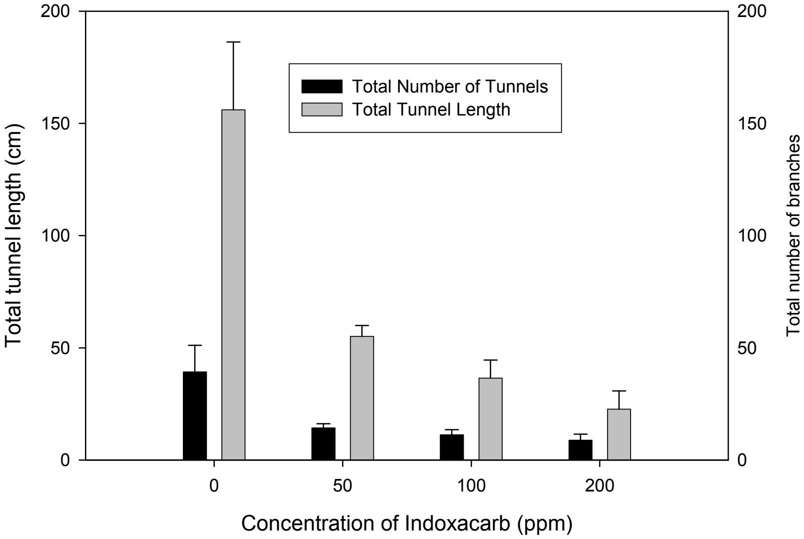

Figure 4.1. Effect of indoxacarb concentration on total length of tunnels and number of branches constructed by eastern subterranean termite workers.

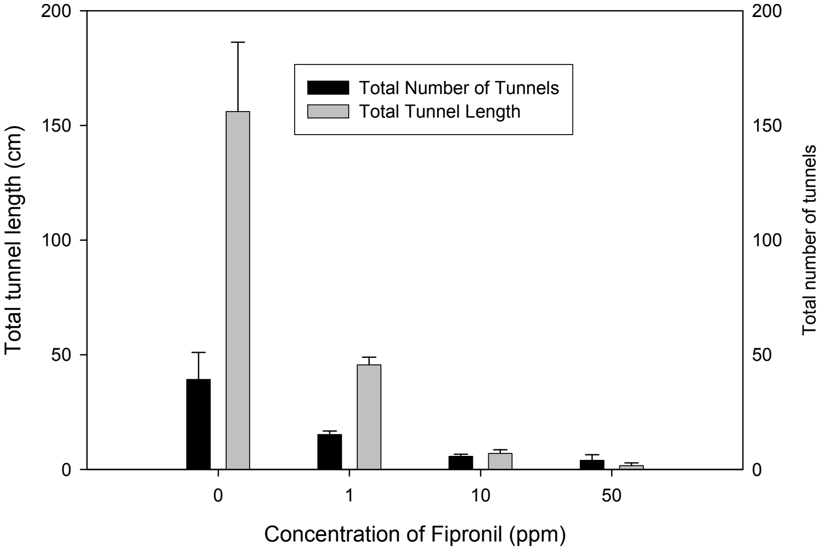

Figure 4.2. Effect of fipronil concentration on total length of tunnels and number of branches constructed by eastern subterranean termite workers

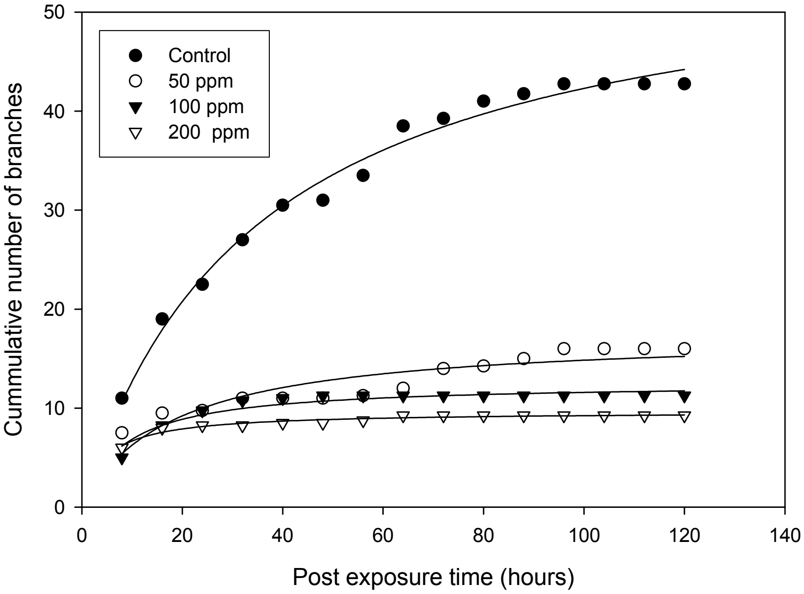

Figure 4.3. Effect of concentration of indoxacarb on total number of branches constructed by eastern subterranean termite workers at various times after exposure

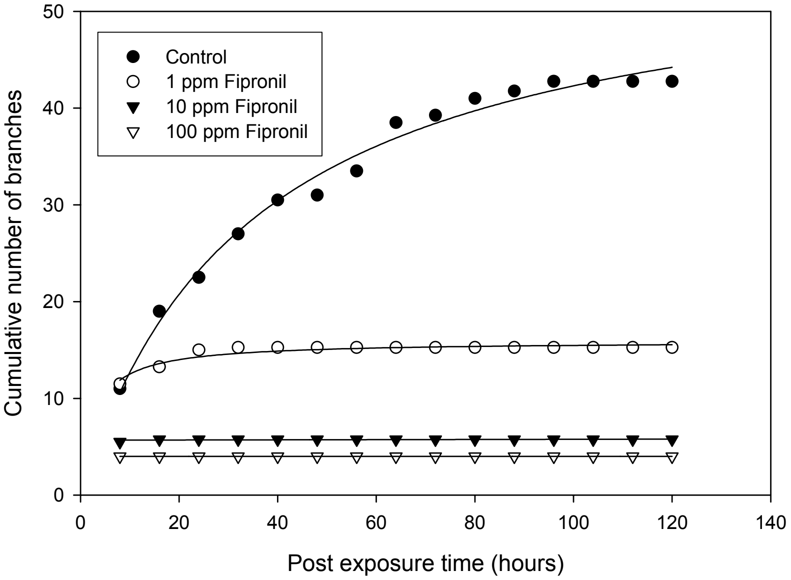

Figure 4.4. Effect of concentration of fipronil on total number of branches constructed by eastern subterranean termite workers at various times after exposure

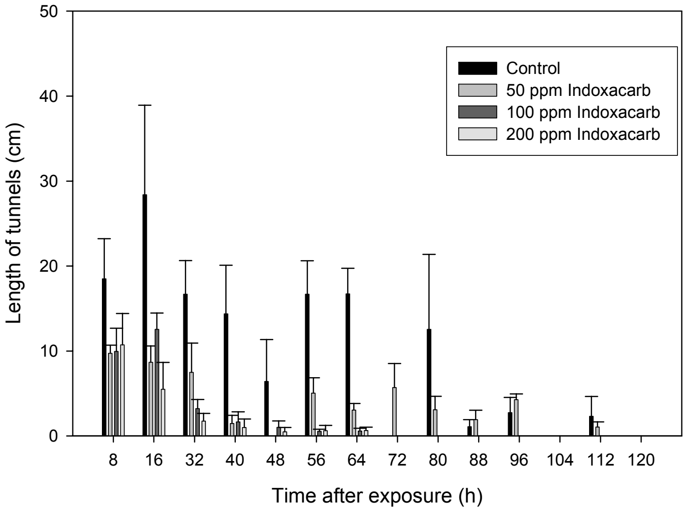

Figure 4.5. Effect of concentration of indoxacarb on length of tunnels constructed by eastern subterranean termite workers at various times after exposure

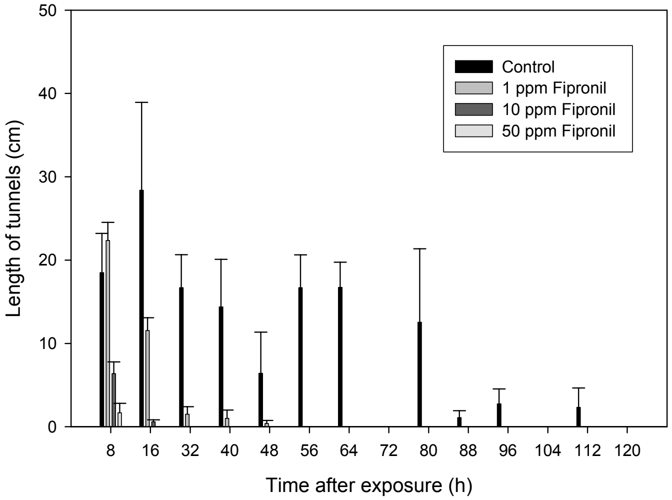

Figure 4.6. Effect of concentration of fipronil on length of tunnels constructed by eastern subterranean termite workers at various times after exposure