DIETARY MANIPULATION OF CUTICULAR HYDROCARBONS IN THE SUBTERRANEAN TERMITE, *RETICULITERMES FLAVIPES* (KOLLAR)

By

NGAN THANH THI NGUYEN RAWLINGS

Bachelor of Science in Biology
Midwestern State University
Wichita Falls, TX
2002

Master of Science in Biology
Midwestern State University
Wichita Falls, TX
2005

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DIETARY MANIPULATION OF CUTICULAR HYDROCARBONS IN THE SUBTERRANEAN TERMITE, *RETICULITERMES FLAVIPES* (KOLLAR)

Dissertation Approved:

Dr. Jack W. Dillwith

Dissertation Adviser

Dr. Mark E. Payton

Dr. Bradford M. Kard

Dr. Carmen M. Greenwood

Dr. Mark E. Payton

Dean of the Graduate College

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CHAPTER I

INTRODUCTION

Termites are eusocial, paurometabolous insects found on every continent except Antarctica. They are a major economic pest and can cost 3 billion dollars or more in the United States in prevention and treatment each year (Su and Scheffrahn 1990, Lewis 1997). Subterranean termites in the genus *Reticulitermes* are the most economically destructive structural pest in North America, damaging millions of homes and other wooden structures annually. Termite damage to wooden structures can remain non-detected for an extended period so that when damage is discovered it is already severe or too late for repairs. Wooden structures may need extensive repairs or replacement, thus expending the country's lumber supply.

The economic impact of termite damage has prompted entomologists to study and apply control strategies. A component of these studies is the development of biochemical and molecular tools for termite identification. One of the approaches that have been extensively studied is the use of cuticular hydrocarbon profiles.

The cuticle of insects, including termites, is coated with a thin, waxy layer of lipids that is used for chemical communications to elicit alarm, mating and aggregation behavior, and for identification of nestmates and recognition of intruders. This cuticular lipid layer also acts to conserve water and prevent desiccation by working as a protective

barrier to the environment. A major component of the cuticular lipid layer in termites is long chain hydrocarbons. Cuticular hydrocarbons are non-volatile and specific to each species. A large number of papers have been published using termite hydrocarbon profiles for chemotaxonomy. However, studies by Brown and co-workers (2005) showed that established methods for analysis of termite cuticular hydrocarbons allowed significant contamination with hydrocarbons from the internal hydrocarbon pool. This observation suggested that the internal hydrocarbon pool might be altered by dietary hydrocarbons and thus makes current methods of termite hydrocarbon analysis unusable for chemotaxonomy. Since it is widely believed that insect hydrocarbon profiles are determined by the needs of the insect and are maintained by *de novo* biosynthesis, it was not expected that dietary hydrocarbons would have an impact on cuticular hydrocarbon composition. Surprisingly in preliminary experiments it was found that not only were dietary hydrocarbons incorporated into the internal hydrocarbon pool but they were also incorporated into the cuticular hydrocarbon pool.

The overall goal of the study described here was to determine how the termite cuticular hydrocarbons can be manipulated and altered with an artificial hydrocarbon diet. If the cuticular hydrocarbon profile can be altered based on diet manipulations, the alterations may have deleterious effects on individual termite's ability to conserve water by changing the termite's cuticular permeability. The altered cuticular hydrocarbon profile may disrupt chemical cues for nestmate recognition, thus disrupting colony organization. Such modifications of termite biology have obvious potential to be exploited for termite control. Currently used baiting stations that are in commercial use could easily be adapted to deliver diets containing hydrocarbons. Such a system would

have the advantage of being environmentally friendly and would be safe to non-target organisms.

CHAPTER II

RESEARCH OBJECTIVES

The effects of adding pure hydrocarbons to the eastern subterranean termite diet were investigated by completing the following three objectives.

I. Determine Optimal Diets for Altering Termite Cuticular Hydrocarbon Composition - This objective was designed to determine whether cuticular hydrocarbon profiles can be altered by adding a dietary hydrocarbon, a combination of hydrocarbons, or different concentrations of hydrocarbons. We hypothesized that cuticular hydrocarbon profiles of *Reticulitermes flavipes* can be altered by adding hydrocarbons to their diet.

II. Characterize the Up-take, Turnover, and Transfer of Dietary Hydrocarbons - Based on our preliminary results, we hypothesize that dietary hydrocarbons remain in the termite system for weeks after they have been incorporated onto the cuticle. We also hypothesized that termites fed dietary hydrocarbons can transfer these hydrocarbons to their non-treated nestmates through their social interactions.

III. Evaluate the Effect of Altered Termite Worker Hydrocarbon Profiles on Water Balance and Cuticular Permeability in Termites - We hypothesize that altered cuticular hydrocarbon profiles will affect the water balance and cuticular permeability of the termite that will result in an increase in mortality.

CHAPTER III

LITERATURE REVIEW

I. Termites - Termites are a widely distributed and diverse group of eusocial insects found almost all over the world. Currently, there are over 2600 identified species within seven different families (Kambhampati and Eggleton 2000). In the Unites States, there are approximately 45 termites species (Su and Scheffrahn 1990, Lewis 1997). Termites reside in the Order Isoptera which means "equal wings" (Pearce 1997). Unlike ants (which termites are sometimes mistakenly called), termite forewings and hindwings are of nearly equal size and their proximal abdomen is not as narrowed or constricted as in ants (Pearce 1997, Kambhampati and Eggleton 2000).

I. I. Life Stages - Unlike social insects in another order, e.g., Hymenoptera, termites are paurometabolous with a primary king and queen in their colony (Pearce 1997). The specific life stages of termites are complex but can consist of eggs, larvae, workers, nymphs, pre–soldiers, soldiers, and reproductives (Krishna 1969, Pearce 1997, Rosin 2000). The term "larvae" when applied to termites can be misleading because it does not have the same meaning as in holometabolous insects (Krishna 1969). "Larvae" is used to describe immatures without external wing buds from workers and nymphs (immatures with external wing buds) (Krishna 1969, Pearce 1997, Rosin 2000). Larvae

gradually develop into workers. "Workers" can molt either into pre-soldiers with a terminal stage as soldier, nymphs, ergatoids (tertiary or supplementary reproductives with no external wing buds), or remain as workers for their entire life span (Su et al. 2001). Nymphs can develop into nymphoids (secondary or supplementary reproductives with small external wing buds), alates (winged primary reproductives), or undergo a regressive molt (pseudergates) (Su et al. 2001). When climatic conditions are ideal and the colony has reached a certain population density, alates will swarm to form new colonies (Krishna 1969). After their dispersal swarm, alates detach their wings by rubbing them against a solid object or twisting their abdomen to break their wings off at the basal sutures (Krishna 1969, Pearce 1997). When primary reproductives die or become incapacitated, they are replaced with supplementary reproductives (Pearce 1997, Rosin 2000).

I.II. Diet – All termites need cellulose, a compound commonly found in wood, in their diet. In their quest for cellulose, they become a major pest for humans because they consume and destroy wooden components found in homes, buildings and other wooden structures. Termites are xylophagous (wood-feeders), geophagous (soil- or organic eater), or harvesters (harvesters or grass-feeders) (Krishna 1969, Pearce 1997).

Xylophagous termites are usually considered "primitive" or "lower" termites but some "higher" termites are also xylophagous (Krishna 1969). Xylophagous termites generally consume dead wood, although a few species of termites do feed on living wood (e.g. some *Coptotermes sp.*) (Krishna 1969). One family of termite, Macrotermitinae, consumes wood that is defecated as pellets. The pellets are deposited and arranged in a comb-like structure where a fungus (*Termitomyces sp.*) breaks down the pellets that are re-ingested by the termites for absorption of more nutrients (Pearce 1997). Harvester

termites feed mainly on grass, but a few species (e.g., *Acanthotermes sp.*) feed on wood or leaves when their preferred food is unavailable (Krishna 1969). Geophagous termites are not as selective as harvester termites and are found only in the family, Termitidae (Krishna 1969).

Nitrogen is needed by all termites to synthesize amino acids and protein (Pearce 1997). Termites can obtain nitrogen through fungi found on wood, fungi grown in fungus-combs, or through nitrogen fixation from bacteria in the hindgut (Pearce 1997). Another source of nitrogen is uric acid that is stored in the fat bodies of the termites and is broken down into ammonia when nitrogen is needed (Pearce 1997).

I.III. Nutrients Absorption – Termites have a crop where ingested food is ground and broken down (Pearce 1997). Then food is passed into the midgut and hindgut of the termite where the food is absorbed (Krishna 1969, Pearce 1997). "Lower" termites have flagellate protozoa in their hindguts that break down cellulose into acetate, carbon dioxide, methane, and hydrogen (Pearce 1997). Acetate is then utilized by termites as an energy source (Krishna 1969). "Higher" termites generally have bacteria instead of protozoa in their hindgut to breaks down the cellulose into acetate, but there are some higher termites that have protozoa in their hindgut (Krishna 1969). There are currently no reports in the literature about how hydrocarbons that might occur in the diet are processed or absorbed. Because hydrocarbons are stable it is reasonable to assume that they would go through the digestive tract in an unaltered state.

I.IV. Cuticle Structures– Termites rely on chemical and mechanical cues for communication. Sensilla are hair-like structures that are found on the cuticle of termites. Sensilla on the antennae and mouthparts can be used as chemoreceptors to detect food

(Pearce 1997). Sensilla can also be used as mechanoreceptors to detect movement and contact with another object (Krishna 1969, Pearce 1997). Mechanoreceptors are usually located on appendages and all over the body (Pearce 1997). There are two types of chordontonal sensilla on the second antennal segment whose function is to respond to movement and to monitor gravity (Krishna 1969, Pearce 1997). Subgenual organs located on the legs of termites have cilia that detect vibrations and are used by termites to sense an alarm (Krishna 1969, Pearce 1997). Termites also have pores on their cuticle that secrete lipids that include hydrocarbons that are used for nestmate recognition and as well protection against desiccation (Pearce 1997). Without cuticular hydrocarbons, termites would be susceptible to desiccation because they are soft-bodied insects with poor water retention capacities (Krishna 1969).

I.V. Behaviors – Because termites are eusocial insects, they exhibit social behaviors that include grooming and trophallaxis. Termites groom each other with their mandibles and palps to remove debris and parasites and to relay chemical and sensory information (Pearce 1997). Grooming can lead to trophallaxis where food is transferred from one termite to another (Pearce 1997). There are two types of trophallaxis, stomodeal and proctodeal. In stomodeal trophallaxis feeding, semi-liquid food is transferred from the mouth of one termite to another whereas in proctodeal trophallaxis, a termite receives secretions from another termite's anus (Pearce 1997)

II. Insect Hydrocarbons - The insect cuticle has a thin, epicuticular layer of species-specific lipids composed of wax esters, sterols, sterol esters, alcohols, fatty acids, alkyl esters, glycerides, aldehydes and hydrocarbons (saturated, unsaturated, and methyl branched) (Blomquist et al. 1987, Bagneres et al. 1991, Singer 1998, Howard and

Blomquist 2005). Hydrocarbons are usually the predominant lipids in the insect epicuticle and can measure 11-43 carbons length, with either an even or odd number of carbon atoms (Blomquist et al. 1987, Singer 1998).

II.I. Cuticular Hydrocarbons - Cuticular hydrocarbons serve as a protective barrier against microorganisms and agricultural chemicals, and maintain water balance and fluidity, thus preventing desiccation (Blomquist et al. 1987, Howard and Blomquist 2005, Klochkov et al. 2005). Due to their relatively higher melting point or transitional temperature, longer chain hydrocarbons are more effective in preventing water loss (Gibbs 1998). Short chain methyl-branched, or unsaturated hydrocarbons exhibit reduced cuticular lipids melting points thus increasing water loss (Gibbs 1998). Several studies have been conducted on the cuticular permeability of termites that include members of *Reticulitermes* and *Coptotermes* (Shelton and Appel 2001, Shelton and Grace 2003). Cuticular hydrocarbons are synthesized *de novo* by insects (Blomquist and Dillwith 1985, Howard and Blomquist 2005). They are non-volatile, and each species of insects has its own unique cuticular hydrocarbon composition (Blomquist and Dillwith 1985).

II.II. Internal hydrocarbons - In addition to cuticular hydrocarbons, insects have an internal pool of hydrocarbons (Schal et al. 1998, Schal et al. 2001). Most insects have higher concentration of internal hydrocarbons than cuticular hydrocarbons. Some of the hydrocarbons found in the internal pool are the same as those present on the cuticle, but several others differ. Internal hydrocarbons can be found in the oenocytes, hemolymph, fat body, digestive tract, and gonads (Schal et al. 1998, Fan et al. 2002). It is believed that lipophorin, a reusable lipoprotein, transports hydrocarbons through the hemolymph (Schal et al. 1998, Young et al. 1999, Schal et al. 2001, Fan et al. 2002, Fan

et al. 2004). Little is known about the physiological role that internal hydrocarbons fulfill in insects. One hypothesis is that the internal hydrocarbon is transported by lipophorin through the hemolymph to areas of the integument that do not synthesize hydrocarbons (Young et al. 1999). Fan et al. (2004) isolated and characterized lipophorin from *R. flavipes,* and demonstrated that lipophorin was used to transport newly synthesized hydrocarbons to the cuticle. One study found that internal hydrocarbons were incorporated into the cuticle after each molt during the larval development of the cabbage looper, *Trichoplusia ni* (Dwyer et al. 1986, Blomquist et al. 1987).

II.III. Hydrocarbon Biosynthesis - Insects are believed to synthesize hydrocarbons from cells in their epidermal layer through an elongation-decarboxylation metabolic pathway, in which a long chain fatty acid is decarboxylated to form a one carbon unit shorter hydrocarbon (Major and Blomquist 1978, Howard and Blomquist 1982, Blomquist and Dillwith 1985, Lockey 1991). Chu and Blomquist (1980) discovered that tetracosanoic acid was incorporated into n-tricosane in the Pacific dampwood termite, *Z. angusticollis*. In an earlier study, Major and Blomquist (1978) provided evidence of the biosynthesis of n-alkanes in insects through the elongation-decarboxylation pathway but not the condensation-reduction pathway. They demonstrated that hexacosanoic acid was directly decarboxylated into n-pentacosane in *P. americana*, and tetracosanoic acid into n-tricosane in *P. fuliginosa* (Major and Blomquist 1978).

II.IV. Dietary hydrocarbons - Currently, diet is believed to have minimum-to-no effect on cuticular hydrocarbon composition, but there is increasing evidence to suggest otherwise. In an early study, it was suggested that dietary hydrocarbons are

incorporated into the cuticular lipid layer of the tobacco hornworm, *Manduca sexta* (Nelson et al. 1971). Another study with the tobacco hornworm found that there was some correlation between cuticular lipids composition and the plants that the tobacco hornworms were fed (Espelie and Bernays 1989). Using labeled dietary n-alkanes, Blomquist and Jackson (1973) discovered that dietary hydrocarbons can be incorporated in cuticular lipids of the grasshopper, *Melanoplus sanguinipes*. In another grasshopper, *Schistocera america,* those that were reared on different plants showed differences in their cuticular hydrocarbon profiles (Espelie et al. 1994). In the Argentine ant, *Linepithema humile*, the type of prey consumed by the ant determined the cuticular lipid composition and demonstrated that hydrocarbons came directly from their prey (Liang and Silverman 2000). Buczkowski et al. (2005) found that ants consuming different prey displayed agonistic behaviors toward ants of the same species, but when they were fed the same prey, agonistic behavior was reduced. Honeybees that were fed a mixture of sucrose and pollen had a larger quantity of cuticular hydrocarbons than those that were fed only sucrose or pollen (Francis et al. 1989). Rojas et al. (2005) altered the free fatty acid composition of the subterranean termite, *Coptotermes formosanus,* with diet. A different study of *C. formosanus* found that cuticular hydrocarbon profiles changed with the type of wood consumed, and the resulting hydrocarbon patterns correlated with inter-colony aggression (Florane et al. 2004). There are no reports of the effects of dietary hydrocarbons of *R. flavipes* cuticular hydrocarbon composition.

II.V. Hydrocarbons in Chemotaxonomy - The unique hydrocarbon composition for each insect species is potentially a useful tool for chemotaxonomy (Blomquist and Dillwith 1985, Lockey 1991). *Nasutitermes exitiosus* (Hill), *R. flavipes* (Kollar),

R. virginicus (Banks) and *Zootermopsis angusticollis* (Hagen) have each had their cuticular hydrocarbon composition completely characterized (Moore 1969, Howard et al. 1978, Blomquist et al. 1979, Howard et al. 1982b). Their cuticular hydrocarbons profiles were markedly different from one another and could be used to distinguish and identify these species. Brown et al. (1990) were able to determine quantitative and qualitative differences in cuticular hydrocarbon profiles of some species of Australian *Coptotermes* that appear morphologically similar. Haverty et al. (1992) used cuticular hydrocarbons to distinguish five species of termites in the genera *Coptotermes sp.* and *Nasutitermes sp.* that were found around the Pacific Rim and Caribbean islands. Jenkins et al. (2000) used cuticular hydrocarbons phenotype and mitochondrial haplotypes to separate three sympatric species of *Reticulitermes* in Georgia, USA. They may have identified a new taxon of *Reticulitermes* using these techniques coupled with differences in morphological characters.

II.VI. Termite Cuticular Hydrocarbons - Cuticular hydrocarbons in termites include alkanes, alkenes, alkadienes, monomethylalkanes, and dimethylalkanes (Blomquist et al. 1979). The first termite cuticular hydrocarbon composition described was that of *Nasutitermes exitiosus* (Hill) by Moore in 1969 (Haverty et al. 1992). Moore (1969) found that most cuticular hydrocarbons are unsaturated, with the predominant hydrocarbon identified as nonatriacontatetraene. *Zootermopsis angusticollis* (Hagen) has cuticular hydrocarbons that consist mainly of n-alkanes, 3-methylalkanes, 5-methylalkanes, and dimethylalkanes with chain lengths of the major components ranging from C_{21} to C_{25} (Blomquist et al. 1979). Howard et al. (1982b) identified the cuticular hydrocarbon components of *Reticulitermes virginicus* (Banks) as n-alkanes, 2-, 3-, 11-,

13-, and 15- methylalkanes, 11,15-dimethyalkanes, (Z)-9-alkenes, (Z, Z)-7-9-dienes, and (E/Z)-6-9-dienes with carbon chain lengths varying from C_{21} to C_{40}. *Reticulitermes flavipes* was reported to contain n-alkanes, 2-methylalkanes, 3-methylalkanes, 5-methylalkanes, Z-9-pentacosane, and Z, Z,-7, 9-pentacosadiene (Howard et al. 1978).

There are several *Coptotermes sp.* whose cuticular hydrocarbons have been identified. Brown et al. (1989) described the cuticular hydrocarbon profiles of *Coptotermes acinaciformis*, *C. frenchi*, and *C. lacteus* plus one undescribed species that resembled *C. lacteus*. Hydrocarbons in *Coptotermes formosanus* are n-alkanes (n-pentacosane, n-hexacosane, and n-heptacosane), 2- and 3-methylalkanes (chain length ranging from 23-27 carbons), 9-, 11-, 12-, 13-, and 15- internally branched methylalkanes (carbon chain lengths of 25-29, 33, 35, 37, 39, 41, and 43), and 13, 17- or 15, 19-dimethylalkanes of 33, 37, 39, 41, and 43 carbon chain lengths (Haverty et al. 1990, McDaniel 1990, Haverty 1996). McDaniel (1990) noted that a trimethylalkane, 13, 15, 17-trimethylnonacosane, can possibly be a chemotaxonomic marker for this termite because it has not been found in other *Coptotermes* species.

III. Functions of Insect Hydrocarbons - One of the many functions of cuticular lipids in insects is their critical role in preventing desiccation by controlling cuticular permeability and water balance. Cuticular hydrocarbons are believed to be the major component in cuticular lipids involved with in water conservation in insects (Blomquist et al. 1987, Gibbs 1998). These hydrocarbons vary in chain lengths and are saturated, unsaturated, or methyl-branched (Blomquist et al. 1987, Gibbs 1998). Cuticular hydrocarbons are also used for chemical communication to recognize nestmates and to prevent intruders from infiltrating the colony.

III.I. Role in Water Balance - Several studies found a correlation between cuticular hydrocarbons composition and climate (Blomquist et al. 1987, Gibbs 1998). Cuticular hydrocarbon composition changed in developmental response changes and seasons (Blomquist et al. 1987). Long-chain hydrocarbons have higher melting points thus maintaining their solid, impermeable barricade for water loss in insects and are found in greater quantities in insects in arid, warm climates (Blomquist et al. 1987, Gibbs 1998). In a study of Namib tenebrionid beetles, wax blooms that were predominately made up of hydrocarbons prevented water loss and aided in thermoregulation. Beetles found in warmer climates had more wax blooms covering their bodies than their counterparts in cooler climates (McClain et al. 1985, Blomquist et al. 1987). Another study found differences in composition of cuticular hydrocarbons in the stonefly, *Pteronarcys californica*, in both the aquatic, immature life stage and the terrestrial, adult life stage (Armold et al. 1969, Blomquist et al. 1987). Hadley (1977) discovered that the desert beetle, *Eleodes armata*, has different cuticular hydrocarbon compositions during the summer and winter season (Blomquist et al. 1987). In summer, beetles had larger amounts of cuticular hydrocarbons and more long-chain hydrocarbons compared with the winter (Hadley 1977, Blomquist et al. 1987). Several species of termites from Rhinotermitidae, Kalotermitidae, and Termitidae collected from the British Virgin Islands exhibited similar characteristics. Termites collected from drier climates had greater amounts of long-chain cuticular hydrocarbons than termites collected from relatively wetter areas (Collins et al. 1997, Haverty et al. 1997). Another study by Woodrow et al. (2000) found that nymphs of the drywood termite *Crytoptermes brevis* had an increase in nC29 and a decrease in nC25 at higher temperatures. Clearly there are

correlations between cuticular hydrocarbon composition and the ability of termites to regulate water balance. To our knowledge there are no reported studies on the manipulation of termite cuticular hydrocarbon composition with diet.

III.II. Role in Chemical Communications - Solitary insects use cuticular hydrocarbon to recognize conspecifics for mating and reproduction (Singer 1998). *Drosophila* includes several species of flies that use cuticular hydrocarbon recognition to stimulate mating behaviors in males and as an anti-aphrodisiac in females (Bartelt et al. 1986, Scott 1986, Scott et al. 1988, Schaner et al. 1989, Oguma et al. 1992, Singer 1998). Another chemical communication example is found in the male rove beetle, *Aleochara curtula*. Malnourished male rove beetles used cuticular hydrocarbon that contained female pheromones to deceive and distract other male rove beetles to perform mating behaviors (Peschke 1987). Meanwhile, they take advantage of the reduced competition for food to feed on blow fly maggots found on dead carcasses (Peschke 1987, Singer 1998).

Social insects such as ants, bees, wasps and termites use cuticular lipids for nestmate recognition and caste distinction (Singer 1998, Liang and Silverman 2000, Howard and Blomquist 2005, Klochkov et al. 2005). There have been several studies on cuticular hydrocarbons as chemical cues for nestmate recognition in the invasive Argentine ant, *Linepithema* humile. In one study, ants that were treated with cuticular hydrocarbons from a different species were rejected or attacked by former nestmates, and a higher degree of aggression was displayed when a higher concentration of the hydrocarbon was applied (Torres et al. 2007). Another study found that diet altered the cuticular hydrocarbon profiles of these ants and resulted in agonistic behaviors between

altered ants and their former nestmates (Liang and Silverman 2000). Termites are another group of insects where cuticular hydrocarbons are believed to serve as nestmate recognition cues (Howard et al. 1982b, Blomquist and Dillwith 1985, Bagneres et al. 1991, Delphia et al. 2003, Kaib et al. 2004).

Several social insects are associated with inquilines that live within their colony by mimicking their host's cuticular hydrocarbons profiles. Termitophilous beetles such as *Trichopsenius frosti* possess cuticular hydrocarbons that are identical to their termite host, *R. flavipes* (Howard et al. 1980, Blomquist and Dillwith 1985). Howard et al. (1982a) found that another subterranean termite, *R. virginicus*, has three termitophiles associated staphylinid beetles, with cuticular hydrocarbons matching their host. An identical hydrocarbon profile provides an effective way for these beetles to integrate into a termite colony.

III.III. Agonistic Behavior in Termites - Several laboratory bioassays have demonstrated agonism between inter- and intra-specific species of termites. Bagneres et al. (1991) demonstrated that cuticular hydrocarbons were used for recognition cues for *Reticulitermes grassei* and *Reticulitermes banyulensis*. Both species are naturally aggressive toward each other. After the cuticular lipids were removed from both termites, all agonistic behaviors ceased. When cuticular hydrocarbons of one species were placed in lures and presented to different species, agonistic behavior resumed. Another example is a study that used soldiers from two Rhinotermitidae species, *Reticulitermes speratus* and *C. formosanus* (Takahashi and Gassa 1995). They reported that agonistic behavior between inter-specific workers, and also between conspecific

workers that were treated with heterospecific cuticular hydrocarbons (Takahashi and Gassa 1995).

Not all species of termites displayed agonistic behaviors toward different termite species. Fuller et al. (2004) did not find any agonistic behavior between termites from different colonies of the "higher" Caribbean termite, *Nasutitermes acajutlae*. Behavioral tests on the fungus-growing termite, *Macrotermes falciger*, reported that when workers from different colonies were paired together, a full range of behaviors from no discernible alarm to overt aggression and death from fighting were observed. There was a positive correlation in the numbers of deaths and greater differences in the cuticular hydrocarbon composition between colonies, but no mortality between workers from neighboring colonies (Kaib et al. 2002). Dronnet et al. (2006) suggest that the lack of intra-specific aggression displayed from an introduced termite *R. santonensis* was a result of similar cuticular hydrocarbon profiles. This supports the importance of cuticular hydrocarbons as recognition cues.

IV. Termite Damage and Management - Termites are infamously known for their destruction of wooden components in residential and commercial structures. Subterranean termites are a major economic pest and can cost 3 billion dollars or more per year in the United States for prevention and control (Su and Scheffrahn 1990, Lewis 1997, Su 2002). Costs of structural replacements and repairs from termite damage are estimated to be as high as $11 billion per year, and this may increase (Su 2002, Smith 2008). The eastern subterranean termite, *Reticulitermes flavipes,* is considered the most economically destructive structural pest in North America (Potter 2004).

Currently, there are several methods used to manage termites. These include liquid termiticide applications to soil, baiting systems, wood preservatives, and physical barriers (Lewis 1997, Smith 2008). The most commonly used management method is soil termiticides. Soil termiticides are applied either as a granular or liquid treatment to the soil around and underneath a wooden structure to repel and kill termites. Chlordane, an organocholorine, was once widely and successfully used to control termites until it was removed from the market for environmental concerns in the late 1980's. Other classes of soil termiticides include organophosphates, pyrethroids, and neo-nicotinoids (Lewis 1997).

Non-chemical, particulate barriers such as limestone, silica sand, or crushed granite or basalt, and physical barriers using stainless steel mesh are sometimes used to exclude termites from reaching a wooden structure (Lewis 1997, Smith 2008). Another strategy for termite management is the use of baiting systems. Baiting uses a cellulose matrix that is treated with a low dose of toxicant that termites ingest and transfer to other termites within their colony through grooming and trophallaxis (Lewis 1997, Smith 2008).

In some baiting systems, monitoring stations without an active ingredient (a.i.) are first installed to detect termite activity. When activity is detected, baits with a.i. are then placed inside the stations (Brown 2005). Some baiting systems bypass the initial monitoring phase and immediately install a.i.-containing baits in the stations (Brown 2005). Slow-acting toxicants, juvenile hormone analogs, and chitin synthesis inhibitors are the three a.i. categories of chemicals generally used for bait systems (Su 1994, Brown

2005). Su et al. (2000) used a chitin synthesis inhibitor, hexaflumuron, to eliminate four colonies of *Coptotermes havilandi* within 3-5 months after bait station installation.

Currently employed strategies for termite control have shown varying degrees of success. However, more testing on new chemical formulations that can be used for baiting and soil-applied termiticides, and new or improved physical barriers as well as novel approaches to successfully control termites is still needed. Potentially effective new measures that may lead to effective prevention and control, and that will have a reduced impact on the environment is an area where more research needs to be continued and explored.

CHAPTER IV

MATERIALS AND METHODS

Termite Collection and Laboratory Rearing - During this study two different collections of termites were used. For preliminary studies termites were collected from the Tallgrass Prairie Preserve and for detailed studies termites were collected from a site inside the Noble Research Center. Field collections of *R. flavipes* were carried out using the methods of Brown et al. (2004). Large cylindrical, below-ground monitoring stations (traps) were built using 20.3 cm diameter PVC pipes. The pipes were cut to 20. cm in length with a chop saw. Using a router, six vertical slits (0.6 cm wide × 15.2 cm long) were cut into the sides of the PVC pipes to allow for proper drainage and served as entrance portals for termites. Corrugated cardboard was cut and tightly rolled to fit inside each monitoring station to serve as bait (17.8 cm dia. × 19.7 cm tall). Bait carriers were constructed using PVC pipe but without vertical cut into the sides. The carriers were slightly longer in length (25.4 cm) and fitted on both ends with PVC caps. They were used to transport the termites from the field to the laboratory.

Termites were collected from several sites in the Tallgrass Prairie Preserve, 10 km north of Pawhuska, Osage County, Oklahoma, and in Stillwater, Oklahoma. Monitoring stations were placed at least 30 m apart to ensure that the termites collected were from

different colonies. Termite monitoring stations were vertically inserted into pre-drilled holes in the ground with their tops at ground level. Holes for each trap were drilled using a power auger, and plant materials such as roots or leaves were removed from the hole to reduce fungus and mold growth. Stone pavers (27.9 cm × 27.9 cm) were used to cap the traps to maintain humidity levels and provide protection against weather. A steel T-post was vertically driven into the ground next to each trap and marked for easy site identification.

Traps were checked for termites every two weeks. If termites were present, the entire roll of cardboard was removed and placed into a carrier and brought back to the laboratory. To determine the number of termites per trap, termites were removed from the cardboard and counted using a hand-held vacuum aspirator system (Kambhampati and Eggleton 2000). Collected termites were placed into individual 37.9 L capacity galvanized steel trash cans that contained a mixture of white sand and vermiculite (1:1, wt:wt) substrate. The substrate was maintained at a depth of 7.6 cm with at least 25% moisture level (Kard et al. 2003). When needed, a new roll of cardboard with dampened Monterey pine (*Pinus radiata)* boards (24.8 cm × 18.4 cm) stacked on top was placed inside the galvanized steel trash can as food for the termites (Kard et al. 2003).

A second termite collection site was located in infested shelving in the Oklahoma State University insect museum. A termite trap made out of a 5.4 L Rubbermaid container (34.3 cm × 21.6 cm × 10.3 cm) with a lid was placed on top of a wooden shelf where termite activity was known. Termites entered through pre-cut holes in the lid to feed on the pre-moistened stacks of paper towels inside the container. The Rubbermaid

container was switched out every two weeks and the termites maintained in a galvanized trash can as described above.

Hydrocarbons – The hydrocarbons used in this study were chosen because they are readily available in pure form from commercial sources. Even chain length n-alkanes and Z-9-tricosene were obtained from Sigma-Aldrich, St. Louis, MO. Odd chain length n-alkanes were obtained from Fluka Chemical Co., Milwaukee, WI. Z, Z-6, 9-heptacosadiene, 3-methyl pentacosane, and n-pentacosane (n-alkane) were isolated from the American cockroach, *Periplaneta americana*, using the cuticular hydrocarbon extraction method described below. The American cockroach was chosen because it is a relatively large insect and contains a simple mixture of hydrocarbons that added two additional hydrocarbon types to the study, a 3-methyl alkane and an alkadiene. These three hydrocarbons make up 97% of the cuticular hydrocarbons with Z, Z-6, 9-heptacosadiene predominant (65%) and lesser amounts of 3-methyl pentacosane (20%) and nC25 (12%) (Baker et al. 1963). Extraction of sixteen adult cockroaches produced the equivalent of 15.4 μmoles of total cuticular hydrocarbon.

A. Dietary Hydrocarbon Setup - Three layers of filter paper (42.5 mm Whatman No. 1) was treated with a solution of known hydrocarbons in mixed 2.0 ml of hexane. The amount of hydrocarbons used was based on initial experiments with 5.0 mg of nC23 (15.4 μmoles). Weights of other hydrocarbons investigated were adjusted to an equal number of μmoles. Treated filter paper was allowed to air-dry under a hood to remove hexane and then moistened with 0.1 ml of water. Filter paper was placed in Petri dishes (60 ×15 mm, Fisher Scientific, Pittsburgh, PA) and fifty termites added, then placed between two plastic serving trays to minimize light and to maintain a high humidity.

Three feeding replicates were conducted for each hydrocarbon. Filter paper treated with hexane only served as a control. Termites were fed treated filter paper for two weeks. After two weeks, the treated filter paper was removed and replaced with non-treated filter paper for a chase period of seven days. After the chase period, termites were removed and their internal and external hydrocarbons isolated and analyzed. Mortality was monitored and recorded during all experiments.

B. Cuticular Hydrocarbon Extraction - Using a soft, moist paintbrush to prevent cuticle damage, live termites were transferred to a hexane-rinsed 7.0 ml scintillation vial and stored at -20˚C until hydrocarbon extraction processing. Total cuticular lipids were extracted for 10.0 minutes in 5.0 ml of hexane containing 50 µl of n-triacontane (0.2µg/µl) as the internal standard. Hexane containing dissolved cuticular lipids was then transferred to a new 7.0 ml scintillation vial. One ml of methanol was added back to the extracted termites. Termites were then stored at -20˚C until needed for internal hydrocarbon extraction.

C. Cuticular Hydrocarbon Isolation - The total cuticular lipid extract was dried at 60˚C under nitrogen and re-dissolved in 200 µl of hexane. Cuticular hydrocarbons were isolated using a silica gel (100-200 mesh, Sigma-Aldrich, St Louis, MO) mini-column (14.5 mm × 6 mm ID) (Dillwith et al. 1981). The extract was added to the mini-column followed by 200µl hexane rinse. Hydrocarbons were eluted using 6.0 ml of hexane, and this fraction was dried under nitrogen and stored at -20˚C until analyzed.

D. Internal Hydrocarbon Isolation - The internal hydrocarbon extraction protocol was modified from Bligh and Dyer (1959) for this experiment. Termites from the cuticular hydrocarbon extraction were transferred to 7.0 ml glass homogenizer chilled on ice and

homogenized in 1.0 ml of methanol containing 50 µl of n-triacontane (0.2 µg/µl) as the internal standard. The homogenate was chilled for 10 minutes then transferred to a 5.0 ml glass centrifuge tube. Each 7.0 ml glass homogenizer was rinsed with 1.0 ml of chloroform, and the rinse added to the centrifuge tube. One ml of distilled water was added to aid in phase separation and the centrifuge tube was vortexed for 30 seconds followed by centrifugation at 5,000 rpm for five minutes. The chloroform layer was removed and placed in a new 7.0 ml vial. The sample was extracted two more times using 1.0 ml of chloroform and the extracts combined. Total lipid extract was dried under nitrogen and re-dissolved in 200 µl of hexane. Hydrocarbons were isolated as described above and stored at -20˚C until analyzed.

E. Hydrocarbon Analysis - Cuticular and internal hydrocarbons were analyzed using a HP 5890 gas chromatograph. One micro-liter of sample was injected onto a J&W DB-1 (30 m x 0.25 mm, 0.25 µm film thickness) column with a splitless injector. Injector temperature was 250˚C, and helium at a flow rate of 1ml/min was used as the carrier gas. The temperature program was set initially at 180˚C for 1.75 minutes then increased at 3˚C/min to a final temperature of 320˚C and held constant for 18 minutes. Detection was with a flame ionization detector run at 350ºC and peaks were identified by comparison with hydrocarbon standards. Components were quantified using the internal standard (nC30) and Agilent ChemStation software.

Objective 1. - Determine Optimal Diets for Altering Termite Cuticular Hydrocarbon Composition.

I.I. Effects of N-alkanes - N-alkanes tested were commercially available. Odd-chained n-alkanes included nC17 to nC35 in two carbon increments. The series of even-

chained n-alkanes that were tested ranged from nC18 to nC38. *R. flavipes* naturally have nC23, nC24, nC25, and nC27 in their cuticular and internal hydrocarbon profiles. Data from cuticular and internal hydrocarbons that are normally found in *R. flavipes* (nC23, nC24, nC25, and nC27) were analyzed with SAS software. Two population t-tests were used to compare the means of the treatments with the controls. Cuticular and internal hydrocarbons that are not normally found in *R. flavipes* fail to show a positive response so one-population t-tests were used to test treated means values for significant differences from zero (control means). All treatment means were compared with each other using analysis of variances (ANOVA) and protected pair-wise t-tests. A significance level of 0.05 was used for all tests and comparisons.

I.II. Effects of Alkenes and Methyl-branched Hydrocarbon - Z-9-tricosene, Z, Z-6, 9-heptacosadiene (alkenes), and methyl-branched 3-methyl pentacosane were used. Z-9-tricosene was commercially obtained, whereas Z, Z-6, 9-heptacosadiene, 3-methyl pentacosane, and n-pentacosane (nC25) were isolated from the American cockroach, *Periplaneta americana*, using the cuticular hydrocarbon extraction method described above. Cuticular and internal hydrocarbon profiles were examined for Z, Z-6, 9-heptacosadiene, 3-methyl pentacosane, and nC25 and if any appear, concentrations were determined. Treatment values were compared with control values using a two population sample t-test with a significance level of 0.05.

I.III. Effect of Dietary Hydrocarbon Combinations - Different combinations of hydrocarbons were fed to the termite to evaluate if combinations of hydrocarbons were incorporated into both cuticular and internal hydrocarbon pools. The amount of each hydrocarbon that was added to each combination equaled 15.4 μmoles, so the number of

molecules was the same for each hydrocarbon in the mixture. The combinations used were nC23 and nC27 and nC23, nC29, and nC31. Another experiment was performed using a total of 15.4 µmoles of nC23, nC29, and nC31. Specific hydrocarbons were selected to give a range of chain lengths and some were selected because they were present normally on the cuticle and others were selected because they are not normally present. By including different hydrocarbons in the diet possible selective absorption was tested. Determining the possible interaction of hydrocarbons during absorption was also a goal of this experiment. Data was analyzed using SAS software and two population sample t-test.

I.IV. Optimize Hydrocarbon Concentration Levels - A series of dilution experiments was conducted using nC26 to determine the minimum amount of dietary hydrocarbon needed to elicit a change in cuticular hydrocarbon profiles in *R. flavipes*. Concentrations used were 0.05, 0.1, 0.5, 1.0, 5.0, and 10.0 µmoles. In our earlier experiments, 15.4 µmoles was shown to cause a change in cuticular hydrocarbon profiles. This experiment was designed to determine if adding smaller concentrations of nC26 produced an effect on the cuticle. To determine LD_{50} using nC26, concentrations tested included 25.0, 50.0, 75.0, and 100.0 µmoles. Three replicates for each concentration were tested.

Objective II. - Characterize the Up-take and Time Dependent Loss of Dietary Hydrocarbons.

Neutral Red Dyed Filter Paper - A 0.5% (wt/wt) Neutral Red dye solution was made by adding 0.1797g/100.0 ml of Neutral Red dye to 100.0 ml of acetone in a 250.0 ml glass beaker. The beaker was covered with aluminum foil and the dye solution was

mixed on a stirring plate until dye was dissolved. A piece of Whatman No. 1 filter paper, 42.5-mm diameter was placed in a Petri dish (60 × 15 mm) and 0.5 ml of dye solution was applied. The pipette containing dye solution was moved over the filter paper in a manner as to evenly distribute the dye solution. Three applications of 0.5 ml of solution were applied and the filter paper was allowed to dry completely under a fume hood between each application. After the final application, the filter paper was dried under a fume hood until all moisture had evaporated before dyed filter paper could be used.

II.I. Determining Hydrocarbon Up-take - Three hundred termites were fed nC21 or nC26 impregnated into filter paper as described in the dietary hydrocarbon setup section. Twenty termites were removed every 24 hours for two weeks. Cuticular and internal hydrocarbons were extracted as described in the cuticular hydrocarbon extraction, cuticular hydrocarbon isolation, and internal hydrocarbon isolation sections. Daily up-take or incorporation of nC21 and nC26 during the two weeks was determined using gas chromatography. There were three replicates for each time interval. Means for each day were compared with each other using ANOVA and protected pair-wise t-tests. A significance level of 0.05 was used for all comparisons.

II.II. Determining Hydrocarbon Time Dependent Loss - Four hundred termites were fed dietary nC21 or nC26 as described in the dietary hydrocarbon setup section. After two weeks of feeding on treated filter paper, groups of twenty termites were removed at intervals during the chase period. These termite groups were removed after one day, three days, seven days, and at one-week intervals thereafter for sixty-three days with three replicates for each time interval. Means for each time interval were compared

with each other using protected pair-wise t-tests and ANOVA with a significance level of 0.05.

II.III. Hydrocarbon Transfer in Termites - One hundred fifty termites were fed dyed filter paper (Neutral Red) treated with nC21 or nC26 (Brown et al. 2008; Su et al. 1991; Esenther 1980). The dyed, treated termites were mixed in with an equal number of non-dyed, non-treated termites from the same colony. Twenty termites from each group (dyed, treated termites, and non-dyed, non-treated termites) were removed after three days, seven days, and two weeks. The cuticular and internal hydrocarbon content of each group was analyzed. One hundred fifty termites that were fed on non-treated, dyed filter paper were also tested to ensure that the dye had no effect on hydrocarbon transfer in termites. The experiment was repeated three times.

Objective III - Evaluate the Effect of Altered Termite Worker Hydrocarbon Profiles on Water Balance and Cuticular Permeability.

III.I. Cuticular Water Loss and Cuticular Permeability in Termites – Cuticular permeability (CP) of termites was determined by determining water loss rates (Shelton and Appel 2001, Shelton and Grace 2003). Twelve groups of twenty termites each were fed for two weeks on filter paper that had been treated with either 15.4 µmoles of nC17, nC22, nC26, or a combined total of 15.4 µmoles of nC25, Z, Z-6, 9-heptacosadiene, and 3-methyl pentacosane extracted from the American cockroach, *Periplaneta americana.* Termites fed only hexane-treated filter paper were used as the control. Each treatment was replicated three times. Ten termites were weighed individually to the nearest 0.01 mg using a Mettler AT20 electronic balance and placed into individual 7.0 ml scintillation vials within a desiccator containing anhydrous calcium

sulfate (Drierite) (relative humidity 0-2%) and placed in an oven at 30˚C (Shelton and Appel 2001, Shelton and Grace 2003). Termites were removed (at two hour intervals for the first12 hours, and then after 24 hours), weighed, and returned to the desiccator. Time of death was noted when termites did not respond to tapping on the side of the vials. After 24 hr, the oven temperature was increased to 50ºC for 48 more hours to obtain dried mass (Shelton and Appel 2001, Shelton and Grace 2003). Percent total body water lost and percent mortality were calculated at each time interval. Percent total body water lost data was analyzed with SAS using Dunnett's procedure that compared each treated values with control value at each time interval. Percent mortality was analyzed with PROC FREQ in SAS by creating contingency tables to run Fisher's exact test that compared each treatment value to the control values at each time interval.

Cuticular permeability (CP) was calculated by determining the difference of initial and 2 hour masses (µg) divided by saturation deficit (31.824 mmHg at 30˚C and 0% humidity), and estimated body surface area expressed as μgH_20 lost$\cdot cm^{-2\cdot h-1}\cdot mmHg^{-1}$(Shelton and Appel 2001, Shelton and Grace 2003). Surface area was calculated using Haagsma's combined surface area model for termites expressed as $(cm^2) = 0.0886 + 26.85$ mass (g) – 214.21 mass $(g)^2$ (Shelton and Appel 2001). CP data were analyzed by using ANOVA and protected pair-wise t-tests using SAS software.

III.II: Effects of Altered Hydrocarbon Profiles at Different Humidities – The effects of altered hydrocarbon profiles on worker termite water balance were determined at 0, 22, 55, 75, and 100% relative humidity. Filter paper previously treated with a total of 15.4 µmoles of either nC17, nC22, nC26, or a combined total of 15.4 µmoles of nC25, Z, Z-6, 9-heptacosadiene, and 3-methyl pentacosane extracted from the American

cockroach, *Periplaneta americana*, was fed to 25 termites for two weeks. Control termites were fed hexane-only treated filter paper. Twenty fed termites were removed and placed in an open Petri dish (60 ×15 mm) containing one non-treated filter paper and placed in a 5.4 L. Rubbermaid container (34.3 cm × 21.6 cm × 10.9 cm) containing a salt solution to adjust relative humidity (Young 1967). Petri dishes were placed on plastic racks inside the containers to avoid contact with the salt solutions. These saturated salt solutions have an equilibrium temperature range adequate for termite survival. The salt solutions covered the entire bottom of each container for maximum surface area and included $CH_3COOK.1{\cdot}5H_2O$, NaBr, NaCl, and water. This resulted in 22, 55, 75, or 100% relative humidity in different containers (Young 1967). Containers were placed inside an oven at 25 ± 1˚C. Mortality was checked and recorded at 24 hr intervals for five days and treatment was repeated three times. Values for each treatment mean were compared with each other for a given time interval at specific humidity by using a protected pair-wise t-test and ANOVA in SAS software. For all comparisons, a significance level of 0.05 was used.

CHAPTER V

RESULTS AND DISCUSSION

I.I. N-Alkanes – Cuticular hydrocarbons are one of many components that make up cuticular lipids in insects and serve many purposes that include protection from the environment, chemical communication, and maintaining water balance (Howard and Blomquist 1982, Blomquist and Dillwith 1985, Blomquist et al. 1987). Hydrocarbons are also found internally in insects (Schal et al. 1998, Schal et al. 2001). Internal hydrocarbons are usually qualitatively similar to those found on the cuticle but are found in more abundant amounts (Schal et al. 1998). Physiological functions of internal hydrocarbons are unknown but it is speculated that internal hydrocarbons are backup reserves for hydrocarbons found on the cuticle (Schal et al. 1998). In the present study, the cuticular hydrocarbons were extracted with hexane using a method known to extract only cuticular hydrocarbons (Brown 2005). The internal hydrocarbons were then extracted with chloroform and methanol. This procedure yielded two hydrocarbon fractions with minimal contamination between fractions. Figure 1A shows the cuticular hydrocarbons isolated from our *R. flavipes* laboratory colony. The components present are very similar to those reported by Howard et al. (1978). However, it should be pointed out that in the present study the identity of all individual hydrocarbons was not determined because the focus was on changes in specific compounds included in the diet.

Figure 2A shows the normal internal hydrocarbons from *R. flavipes.* It should be noted that the cuticular and internal hydrocarbon pools have different compositions even though they contain some common components. For example, nC23 and nC25 are present in both pools. As indicated by the detector response and the internal standard (nC30), there were relatively more hydrocarbons in the internal hydrocarbon pool. There were three times the amount of nC23 and nC25 that were found in the internal hydrocarbon pool when compared with the cuticular hydrocarbon pool in non-treated termites (Figs. 1A, 2A).

Preliminary data indicated feeding nC23 to *R. flavipes* resulted in it being incorporated into the cuticular hydrocarbon pool with an increase of almost nine times the amount of nC23 compared with the control (Figs. 1A, 1B). Dietary nC23 was also incorporated into the internal hydrocarbon pool (Fig. 2B). In the internal hydrocarbon pool, there was approximately five times the amount of nC23 in the treated group compared with the control (Figs. 2A, 2B).

Another study was conducted by feeding either nC23, nC27, or nC31 impregnated filter paper. Both nC23 and nC27 are normally found in *R. flavipes* whereas nC31 is not. In preliminary data, dietary nC23 elicited an increase in nC25 in the cuticular and internal hydrocarbon pools. In this study, the concentrations of cuticular nC25 and nC29 were measured along with nC23, nC27, and nC31 to determine if there was an increase in nC25 or nC29 even though they were not added to the diet. When nC23 was added to the diet, it increased from 13 µg/gm termite to 199 µg/gm termite in the cuticle (Fig. 3). Also, nC25 doubled from 27 µg/gm termite to 59 µg/gm termite (Fig. 3). Dietary nC23 also caused minimal increases in nC27, nC29, and nC31 (Fig. 3). When dietary nC27

was added, it increased compared with the control but did not have any effects on nC23, nC25, nC29, or nC31 (Fig. 3). Dietary nC31 was also incorporated into the cuticle when compared with the control, but like dietary nC27, it did not elicit any effects on the other hydrocarbons (Fig. 3).

Dietary nC23, nC27, and nC31 were also incorporated into the internal hydrocarbon pool. Dietary nC23 increased nC23 from 36 µg/gm termite to 828 µg/gm termite (Fig. 4). As was observed for the cuticular hydrocarbon pool, dietary nC23 also caused an increase in nC25 in the internal pool. There was an increase in nC25 from 140 µg/gm termite to 385 µg/gm termite (Fig. 4). Dietary nC23 also elicited an increase in nC27 but not nC29 or nC31 as was observed in the cuticular hydrocarbon pool (Fig. 3, 4). Both dietary nC27 and nC31 caused an increase in nC27 and nC31 when compared with the control, but did not cause an increase in nC23, nC25, or nC29 (Fig. 4). Quantitatively, there is more nC23, nC25, nC27, nC29, and nC31 found in the internal hydrocarbon pool than in the cuticular hydrocarbon pool when comparing the cuticular and internal controls or the cuticular and internal treatments (Fig. 3, 4).

Currently, the most accepted theory for hydrocarbon biosynthesis in insects is the elongation-decarboxylation pathway where fatty acids are elongated and then reductively decarboxylated to form a hydrocarbon one carbon shorter than the parent fatty acid (Howard and Blomquist 1982, Blomquist and Dillwith 1985, Blomquist et al. 1987). In our preliminary study, dietary nC23 caused an increase in nC25 and nC27 in the internal hydrocarbon pool as well as increasing nC29 and nC31 in the cuticular hydrocarbon pool. Dietary nC23, an n-alkane, could not be elongated to form longer n-alkanes according to the current accepted theory of hydrocarbon biosynthesis, which leads to the conclusion

that dietary nC23 may have an unknown mechanism that triggers hydrocarbon biosynthesis in the termite. Further experiments are needed to clarify this interesting observation but were beyond the scope of the present study.

Another preliminary experiment was designed to determine the optimal chase period (time where termites were feeding on non-treated filter paper) following 2 weeks of termites feeding on treated filter paper. The chase period was included in the feeding experiments to remove any unabsorbed hydrocarbon from the termite gut. The chase period resulted in a more accurate assessment of hydrocarbons that were actually incorporated into the two pools. Figure 5 shows the amount of nC23, nC25, nC27, nC29, and nC31 in μg hydrocarbon/gm termite in the cuticular hydrocarbon pool after feeding nC23 for two weeks followed by a chase period of 4 days, 1 week or 2 weeks. The 1-week chase period was determined optimal for the feeding experiments because there were minimal differences between 4 days, 1 week, and 2 weeks when dietary nC23 was used, with a trend showing a slight decline of hydrocarbon over time (Fig. 5). The 1 week chase period would also decrease an experiment's running time. Figure 6 shows how the different chase periods affect the amount of nC23, nC25, nC27, nC29, and nC31 found in the internal hydrocarbon pool. The internal hydrocarbon amounts for the 4 days chase period was less than the 1 week and 2 week chase period but that may be a result of variability and having only one sample replicate (Fig. 6). The 1 week and 2 week chase periods reflected the same trend that was observed in the cuticular hydrocarbon pool with a slight decrease in the amount of hydrocarbons from the 1 week to the 2 week chase period (Fig. 6).

To determine the influence of chain length on the incorporation of dietary

hydrocarbon into the two hydrocarbon pools, chain lengths between 17 and 38 were fed to termites. In preliminary experiments, 5.0 mg (15.4 μmoles) of nC23 were used and this resulted in good incorporation. In these experiments, the amount of each hydrocarbon was normalized to 15.4 μmoles so the same numbers of molecules were fed. Both odd- and even-chain length n-alkanes were incorporated into the cuticular hydrocarbon pool (Fig. 7) with means ranging from 29.63 μg hydrocarbon/gm termite to 398.33μg hydrocarbon/gm termite (Table 1, 3). Figure 7 shows that the shortest chain length n-alkanes tested, nC17 and nC18, were statistically similar to the longest chain length n-alkane tested, nC38. There was a general increase in incorporation from nC17 up to nC22 followed by a decrease in longer chain length hydrocarbons. The exception to this trend was nC36 which showed a much higher incorporation than hydrocarbons with similar chain lengths. The reason for this is not clear, but this result was obtained several times. Results from ANOVA using comparisons from protected pair-wise t-tests determined that the more moderate chain length n-alkanes nC20-21, nC24-nC29, nC31, and nC34 were statistically similar (Fig. 7). N-alkanes that were not normally found in *R. flavipes* were analyzed using one population t-test determine that nC20-nC31 and nC34-nC36 were statistically significant (Table 1). All four n-alkanes (nC23, nC24, nC25, and nC27) that are normally present in *R. flavipes* were significant using two population t-test that compared treated values with control values (Table 3). The incorporation of hydrocarbons in general follows the chain length distribution found in the cuticular hydrocarbon pool.

Figure 8 and Table 2 show the incorporation of dietary n-alkanes into the internal hydrocarbon pool. The pattern of incorporation was different than for the cuticular

hydrocarbon pool. There was a greater incorporation of the shorter and longer chain lengths compared with the medium ones. There was a particularly high incorporation of nC17 (7059.72 µg/g termite). This was higher than incorporation of nC17 into the cuticular hydrocarbon pool (37.95 µg/g termite). N-alkane incorporations that were statistically significant in the internal hydrocarbon pool included nC17 to nC21, nC23 to nC25, nC27, and nC31to nC36 (Table 2, 4). The study also found greater concentrations of the dietary alkanes in the internal hydrocarbon pool than in the cuticle, with means ranging from 180.61µg hydrocarbon/gm termite to 7059.72 µg hydrocarbon/gm termite (Table 2, 4). The greater incorporation of longer chain hydrocarbons into the internal pool reflects the occurrence of longer chain hydrocarbons in the internal pool of control insects (Fig 2A).

The n-alkanes studies suggest that there is a selective mechanism in the termite system in the transfer and deposition of hydrocarbons from diet into the cuticular and internal hydrocarbon pools. In the cuticular n-alkane studies, the shorter and longer chain length n-alkanes were incorporated less in the cuticle than the more moderate chain length n-alkanes. But in the internal n-alkane studies, the shorter chain length n-alkanes (nC17-nC20) were found in the greatest quantities. It is known that cuticular hydrocarbons play a role in water balance by preventing desiccation in soft-bodied termites (Howard et al. 1982a, Blomquist and Dillwith 1985, Blomquist et al. 1987). Perhaps the differences found in the incorporation of the different chain length n-alkanes in the cuticular and internal hydrocarbon pools are results of the termite trying to maintain its cuticular water balance by selectively incorporating n-alkanes from the internal hydrocarbon pool into the cuticle that will disrupt water balance the least. This

could explain why there were more shorter chain-length n-alkanes found internally because longer chain-length hydrocarbons are believed to decrease water loss due to higher melting points (Gibbs 1998).

The differential incorporation of hydrocarbons into the two hydrocarbon pools provides convincing evidence that the appearance of hydrocarbons on the cuticle of termites previously fed specific hydrocarbons is the result of absorption from the diet rather than mechanical transfer from the hydrocarbon impregnated filter paper. If mechanical transfer was taking place, it would be expected that there would be a distribution reflecting the melting points of the hydrocarbons. It would also be expected that incorporation into the internal and external pools would be the same.

I.II. Alkenes and Methyl-branched Hydrocarbons – The cuticular hydrocarbons in *R. flavipes* are composed of n-alkanes, one alkene, one alkadiene and 2-, 3-, and 5-methyl-branched alkanes (Howard et al. 1978). To determine how termites would process dietary alkenes, Z-9-tricosene, a readily available alkene and sex attractant in the female house fly, was tested because it is not found naturally in *R. flavipes*. Figure 9 shows that 197 µg of Z-9-tricosene/gm termite was found on the cuticle. Using the one-population t-test, results showed incorporation of that Z-9-tricosene was statistically significant in the cuticular hydrocarbon pool compared with the control. Figure 10 shows that 4,545 µg of Z-9-tricosene/gm termite was found in the internal hydrocarbon pool. The incorporation of Z-9-tricosene was also statistically significant in the internal hydrocarbon pool when compared with the control. Again, there is a striking difference between the distributions of dietary Z-9-tricosene between the two pools. Howard et al. (1978) reported that *R. flavipes* cuticular hydrocarbons contain twenty-five percent Z-9-

pentacosene. Therefore, it is not surprising that Z-9-tricosene, which is 2 carbons shorter, would be incorporated. It would be interesting in future experiments to isolate the Z-9-pentacosene from termites and compare its uptake in diets to Z-9-tricosene.

Another study used a combination of dietary nC25, Z, Z-6, 9-heptacosadiene, and 3-methyl pentacosane extracted from the American cockroach, *Periplaneta americana*. These three hydrocarbons make up 97% of the cuticular hydrocarbons with Z, Z-6, 9-heptacosadiene predominant (65%) and lesser amounts of 3-methyl pentacosane (20%) and nC25 (12%) (Baker et al. 1963). NC25 and 3-methyl pentacosane are naturally found in *R. flavipes* but Z, Z-6, 9-heptacosadiene is not. Howard et al. (1978) reported that nC25 makes up twenty percent of cuticular hydrocarbons in *R. flavipes* worker while 3-methyl pentacosane makes up five percent. *R. flavipes* does contain Z, Z-7, 9-pentacosadiene (22%) in the cuticular hydrocarbon pool. Figure 11 shows 133 µg nC25/gm termite was found on the cuticle of treated termites compared to 38 µg nC25/gm termite in the control. There was 70 µg methyl pentacosane/gm termite and 34 µg Z, Z-6, 9-heptacosadiene/gm termite in the treated termites compared with 12 µg methyl pentacosane/gm termite and 0 µg Z, Z-6, 9-heptacosadiene/gm termite in the controls (Fig. 11). Using the two population sample t-test, dietary nC25, Z, Z-6, 9-heptacosadiene, and 3-methyl pentacosane were statistically significant compared with the control and showed all three hydrocarbons were incorporated into the cuticular hydrocarbon pool. The level of incorporation corresponds to the composition of the cuticular hydrocarbon pool and not the composition of the dietary hydrocarbon. For example, although Z, Z-6, 9-heptacosadiene was the predominant hydrocarbon in the diet, it was poorly incorporated into the hydrocarbon pool. The occurrence of relatively

large amounts of Z, Z-7, 9-pentacosadiene in the termite did not result in a corresponding larger incorporation of Z, Z-6, 9-heptacosadiene. These results clearly show a very selective processing of dietary hydrocarbons that is guided by naturally occurring hydrocarbon composition.

Figure 12 shows that 442 µg nC25/gm termite, 523 µg 3-methyl pentacosane/gm termite, and 43 µg Z, Z-6, 9- heptacosadiene/gm termite were found in the internal hydrocarbon pool in the treated samples. In the control samples, 102 µg nC25/gm termite, 61 µg 3-methyl pentacosane/gm termites, and 0 µg Z, Z-6, 9-heptacosadiene/gm termite were found. Only dietary nC25 and 3-methyl pentacosane/gm termite were significant and proven to be incorporated into the internal hydrocarbon pool (Fig. 12). Only one of the three treated samples showed Z, Z-6, 9-heptacosadiene in the internal hydrocarbon pool. It may be that Z, Z-6, 9-heptacosadiene was incorporated but in trace amounts. These results again show the specificity of incorporation of dietary hydrocarbon, e.g., 3-methyl pentacosane is preferentially incorporated into the internal hydrocarbon pool while nC25 is preferentially incorporated into the cuticular hydrocarbon pool. There is no information about the connection between the two hydrocarbon pools but the results obtained in this experiment indicate that if the two pools are connected, the interaction is complex. Movement of hydrocarbons between the pools would be specific. There was greater incorporation of the alkanes into the internal hydrocarbon pool compared with the cuticular pool, suggesting that the internal pool may serve as a reservoir for dietary hydrocarbons before they are required on the cuticle. Additional studies with labeled hydrocarbons will be required to sort out the relationship between hydrocarbon pools.

I.III. Hydrocarbon Combinations – Dietary n-alkanes were found to be incorporated in both cuticular and internal hydrocarbon pools (Fig. 7, 8), but our previous experiments were set-up to test the effects of individual dietary n-alkanes. NC23 and nC27 are both naturally occurring in *R. flavipes* with greater quantities of nC23 found in both cuticular and internal hydrocarbon pools. In the treatment containing 15.4 μmoles each of nC23 and nC27, results show that both n-alkanes were incorporated in the cuticle and internal hydrocarbon pool when compared with the control (Fig. 13, 14). There were 83 μg of nC23 in the cuticle of the treated termites compared with only 3 μg in the control termites (Fig. 13). NC27 had similar results with 66 μg in the treated termites and only 1 μg in the controls (Fig. 13). Incorporation of both nC23 and nC27 was statistically significant using the two population sample t-test in the cuticle, thus determining that incorporation of both n-alkanes did occur in the cuticle. Even though there was absorption of the hydrocarbons from the mixture, the amount of hydrocarbon absorbed was less than when the hydrocarbons were fed individually (Table 1). In both experiments the same amount of each hydrocarbon (15.4 μmoles) was fed. Clearly as the total amount of hydrocarbon in the diet increased, there was less absorption of each hydrocarbon. The total hydrocarbon absorbed in the mixture experiment was less than the amount of each hydrocarbon absorbed in the single compound experiments, 149 μg total vs. 302.78 μg for nC23 and 217.94 μg nC27.

In the internal hydrocarbon pools, 265 μg of nC23 and 218 μg of nC27 were found in the treated samples with only 32 μg of nC23 and 17 μg of nC27 in the controls. Two population sample t-tests show significant incorporation for the mixture of nC23 and nC27 when compared with the controls (Fig. 14). As was observed in the individual

dietary n-alkane experiments, quantitatively more hydrocarbons were found in the internal hydrocarbon pools than in the cuticular hydrocarbons pools. These experiments show that feeding a combination of hydrocarbons elicits changes in both hydrocarbon pools. Interestingly, feeding the mixture resulted in a level of incorporation into the internal pool that was nearly identical to the incorporation observed in the single hydrocarbon feeding study (Table 2). These results again show the selective nature of incorporation into the two pools.

Our previous experiments examined the effects of dietary hydrocarbons when 15.4 μmoles of each hydrocarbon were tested. A second hydrocarbon combination experiment tested nC23, nC27, and nC31 at two different concentrations. This experiment included nC31, a long-chain n-alkane not naturally found in *R. flavipes*. A mixture of 15.4 μmoles each of nC23, nC27, and nC31 showed significant incorporation of each compound when compared with the control in the cuticular hydrocarbon pool (Fig. 15). There was 405 μg of nC23, 280 μg of nC27, and 236 μg of nC31 in the treated samples with only 14 μg of nC23 and 0 μg of nC27 and nC31 in the control (Fig. 15). NC27, normally present in *R. flavipes* in the cuticle, was not observed in the controls, but it may have been present in trace amounts. The results of this experiment contrast the results of the previous experiment where two compounds were fed. In this experiment the incorporation of each compound from the mixture was similar to the levels incorporated from feeding the single compound (Table 1). Whether this difference is real or is the result of variation in the system will need to be determined.

All three hydrocarbons were significant had significant incorporation in the internal hydrocarbon pool with 278 μg of nC23, 209 μg of nC27, and 98 μg nC31 when

compared with 52 µg of nC23, 19 µg of nC27, and 0 µg of nC31 in the control (Fig. 16). Similar results were found in the experiment that tested mixtures with a combined total of 15.4 µmoles of nC23, nC27, and nC31 (Fig. 15, 16). Results show 204 µg of nC23, 222 µg of nC27, and 99 µg of nC31 in the cuticle and 309 µg of nC23, 179 µg of nC27, and 81 µg of nC31 in the internal hydrocarbon pool (Fig. 15, 16). At a superficial glance, it appears that adding greater concentrations of dietary hydrocarbons resulted in more incorporated on the cuticle. However in the internal hydrocarbon pool, adding greater concentrations of dietary hydrocarbons did not necessary result in increased incorporation internally. It may be that there is a specific balance needed in the internal hydrocarbon pool that causes any excessive dietary hydrocarbons to be deposited in the cuticle. This explains why additional amounts of dietary hydrocarbons were reflected in the cuticle and not in the internal hydrocarbon pool. Further studies will need to be conducted to examine the relationship of how mixtures and varying concentrations affect incorporation in both hydrocarbon pools.

I.IV. Hydrocarbon Concentrations – In earlier dietary hydrocarbon experiments, 15.4 µmoles of hydrocarbon was used because results showed significant incorporation at that concentration. To determine if there is a dose dependent incorporation of hydrocarbon, different levels of nC26 were fed. There was clearly a dose dependent incorporation for this one hydrocarbon into both the cuticular and internal pool (Fig 17, 18). To more clearly define the incorporation of each hydrocarbon, similar dose-response experiments will need to carry out.

Side Observation on Mites Attacking Termites – This dose experiment was one of the last experiments to be conducted. During that period, there were difficulties with

the termite colony that was used in this experiment. The termites were collected solely from infested wooden shelves in an OSU room. In prior experiments, thousands of termites were collected from the shelves at one time. In these latter experiments, the termites only numbered in the low hundreds. Occasionally, there were no termites in collected from the termite trap. These changes may be a result of frequent collection from this site over several years or other unknown factors such as variable environmental conditions. This concentration experiment was repeated several times because of high termite mortality from mite infestations. It was observed that termites were turning pink on the head and front legs after 3 days. The infected termites appeared desiccated when compared with healthy termites because of their noticeably flatter abdomen. A few days later, infected, pink termites were dead and their heads were decapitated. Under closer inspection, thousands of mites were found feeding in the hollow cavities of the dead termites' heads and on their bodies. The mites were congregating only around the pink, dying, and dead termites. There were also numerous mite eggs inside the hollow head and around dead termites. This phenomenon that causes the pink coloration in the head and front legs has not been reported before. The mites also turn pink but the color was only observed in their abdominal cavity. *R. flavipes* is sometimes associated with phoretic mites (Wang et al. 2002). The most common mite species in *R. flavipes* is *Australhypopus sp.* This species has a phoretic stage where they attach to the termite head and mouthparts, but they do not cause harm to the termites because they lack feeding mouthparts at this life stage (Wang et al. 2002). Wang et al. (2002) observed that in a weakened termite colony, mites morph into a different life stage where they can feed on dead and dying termites and lay eggs near the termites. The mites in the nC26

concentration experiments were not conclusively identified, but it is possible that the mites are from this genus because they were found on the head and mouthparts of dying termites. Wang et al. (2002) did not report any pink coloration in their infested termites, so the mites from this experiment may be a different species or the pink coloration may have been a fungal or bacterial infection. Further studies are needed to accurately identify the mites and the cause of their peculiar pink coloration.

To determine the toxicity of fed hydrocarbons to termites, 25.0, 50.0, 75.0, and 100.0 μmoles of nC26 were fed to termites. There was not a dose dependent increase in mortality over the range of concentrations tested and none of the concentrations resulted in 50% mortality so it was not possible to determine an LD_{50} (Fig. 19). Another experiment tested nC26 at 1000.0 μmoles, but fifty percent mortality was not observed at this concentration. It was not repeated because the concentration was so high that the hydrocarbons were visibly flaking off the filter paper. Clearly this data shows that dietary hydrocarbons are not toxic to termites when they are maintained under high humidity conditions.

It should be noted that the mortality experiment was repeated numerous times due to mite infestations. One interesting observation was that experiments that had to be repeated several times were those using higher hydrocarbon concentrations (50.0 μmoles to 100.0 μmoles of nC26), although mite infestation did occur at lower concentrations. There was one set of experiments that tested mortality using the same concentrations but with nC17 instead of nC26. At lower concentrations (0.05 μmoles – 25.0 μmoles), there were few mortalities, but at higher concentrations (50.0 μmoles – 100.0 μmoles), there were relatively high mortalities from mite infestation. Further studies are needed using a

different colony to determine if there is a connection between high dosage of dietary hydrocarbons and mite infestation. It may be possible that incorporation of dietary hydrocarbons disrupts cuticular water balance and weakens termites, thereby making them more susceptible to mite infestation. If so, mites can potentially be used as a biological control agent in conjunction with dietary hydrocarbons.

II.I. nC21 and nC26 Up-take – Earlier studies determined that dietary hydrocarbons were incorporated in the cuticular and internal hydrocarbon pools after 2 weeks of feeding followed by a 1 week chase period (Fig. 7, 8). In order to determine the rate of uptake of hydrocarbons from the diet, nC21 and nC26 (both not naturally occurring in *R. flavipes)* were used to determine daily up-take. In the nC21 cuticular up-take experiments, results show that incorporation of nC21 into the cuticle begins to occur within the first 24 hours of feeding (Fig. 20). Using results of comparisons of protected t-tests obtained from ANOVA, nC21 concentrations on the cuticle remained relatively stable after 4 days (Fig. 20). Dietary nC21 was also incorporated in the internal hydrocarbon pool within the first 24 hours. NC21 levels were relatively stable after 3 days of feeding in the internal hydrocarbon pool (Fig. 21).

As was observed with nC21, nC26 was incorporated into the cuticle within the first day of feeding with no significant differences between the time intervals of the experiment (Fig. 22). Dietary nC26 was present in the internal hydrocarbon pool within the first 24 hours and levels remained relatively consistent during the 14 days (Fig. 23). Once they were ingested, dietary hydrocarbons were quickly incorporated into the cuticular and internal hydrocarbon pool. Comparing nC21 with nC26, hydrocarbon incorporation into the cuticular and internal hydrocarbon pools was variable. This

indicates that there may be a selective mechanism for the incorporation and transfer of dietary hydrocarbons in the internal and cuticular hydrocarbon pools so that the cuticular water balance can be maintained.

II.II. nC21 and nC26 Time Dependent Loss – Dietary hydrocarbons are incorporated quickly in the cuticular and internal hydrocarbon pools (Fig. 20–23). In the preliminary chase studies (Fig. 5, 6), nC23 was still present in both the cuticular and internal hydrocarbon pool after 2 weeks of feeding on hydrocarbon impregnated filter paper that was followed by a chase period of 2 weeks.

In this study, the turnover rate or the rate at which the dietary hydrocarbon was lost during the chase period was determined using nC21 and nC26 for a chase period of 63 days. In the nC21 turnover experiments, results show that nC21 remained in the cuticle for at least 63 days after two weeks of feeding (Fig. 24). The amount of nC21 after a chase period of 1 day was 522 µg/gm termite and nC21 steadily decreased to 27 µg/gm termite at 63 days (Fig. 24). Using comparisons from protected pair-wise t-tests, nC21 levels were relatively stable from day 3 to day 63 (Fig. 24). Dietary nC21 remain in the internal hydrocarbon pool throughout the 63 days (Fig. 25). The amount of nC21 after 1 day was 1675 µg/gm termite. This decreased to 498 µg nC21/gm termite at 63 days. Results from protected pair-wise t- tests show that nC21 decrease steadily after 21 days (Fig. 25).

In the nC26 turnover experiment, dietary nC26 remained in the cuticular hydrocarbon pool for at least 63 days (Fig. 26). After 1 day, 627 µg nC26/gm termite was in the cuticle. It decreased to 91 µg/gm termite after 63 days. Figure 26 shows that after 3 days, nC26 decreased steadily over time in the cuticle. As was observed in the

cuticular hydrocarbon pool, dietary nC26 was present in the internal hydrocarbon pool for at least 63 days (Fig. 27). NC26 decreased from 1488 µg/gm termite after 1 day to 552 µg/gm termite at 63 days. Comparisons of protected pair-wise t- tests indicated nC26 decreasing steadily throughout the 63 days (Fig. 27). Once incorporated into the termite system, dietary hydrocarbons stay in the cuticular and internal hydrocarbon pools for at least 63 days.

II.III. nC21 and nC26 Transfer – Termites are eusocial insects and exhibit social behaviors that include grooming and trophallaxis (stomodeal and proctodeal). It was determined that dietary hydrocarbons are quickly incorporated in the internal and cuticular hydrocarbon pools and they remained in the system for at least 63 days (Fig. 20-27). To determine if the incorporated dietary hydrocarbons can be transferred from treated termites to their non-treated nestmates through their social behaviors, experiments were conducted with nC21 and nC26 because they are not normally found in the cuticular and internal hydrocarbon pools of *R. flavipes*. In the nC21 and nC26 transfer studies, dietary nC21 and nC26 were incorporated into treated termite's cuticular and internal hydrocarbon pools by feeding them dyed, nC21 or nC26 impregnated filter paper for 2 weeks followed by a 1 week chase period. The dye was used to distinguish treated termites from their non-dyed, non-treated nestmates. The controls showed that nC21 and nC26 did not appear in either the treated or non-treated cuticular and internal hydrocarbon pools. NC21 and nC26 were found on the cuticle of non-treated termites at 3, 7, and 14 days (Fig. 28). The only source of nC21 and nC26 can only come from their treated nestmates. NC21 and nC26 were also found in the internal hydrocarbon pool of non-treated termites that were mixed with their treated nestmates at 3, 7, and 14 days

(Fig. 29).

It was observed that grooming occurred between the dyed, treated termites and their non-dyed, non-treated nestmates when they were mixed together in a Petri dish. Trophallaxis was not observed, but it was not the focus of the study and therefore, strict adherence for observing signs of either behavior was not conducted. Dietary nC21 and nC26 were transferred from treated termites to their non-treated nestmates in this study. Social behaviors that include grooming and trophallaxis may explain how the dietary hydrocarbons were transferred. Since the hydrocarbons appear in the internal pool of non-treated termites, ingestion of the hydrocarbons must have occurred.

III.I. Cuticular Water Loss and Cuticular Permeability – Cuticular hydrocarbons are one of the main components of cuticular lipids needed to maintain cuticular water balance and prevent desiccation while serving many other vital functions in insects (Blomquist et al. 1987, Gibbs 1998). Earlier experiments showed incorporation of different dietary hydrocarbons into the cuticular and internal hydrocarbon pools of *R. flavipes* (Fig. 7, 8), so experiments were designed to determine how dietary hydrocarbons affect cuticular water balance in *R. flavipes*.

There was a clear effect of dietary hydrocarbons on water balance in *R. flavipes* (Fig. 30, Table 5). Termites that were feeding on Z, Z-6, 9-heptacosadiene, 3-methyl pentacosane, and nC25 impregnated filter paper initially had a higher percentage of total water loss after two hours in a desiccator than the other treatments (Table 5). However, the most total body water loss for the control termites occurred within the first two hours of the desiccation experiment (Table 5). These data confirm previous published literature of water loss in insects that utilized a two compartment water loss model where water

was initially loss from the cuticle and then internally, and that water lost occurred within the first two hours (Wigglesworth 1945, Shelton and Grace 2003). In the two compartment water loss model, the higher rate of water loss occurred in the cuticle (Wigglesworth 1945, Shelton and Grace 2003). After twelve hours, all four treatments had significantly higher percentages of total body water loss than the control (Table 5). After 24 hours, the four treatments were still significantly higher than the control (Table 5). The addition of the dietary hydrocarbons and its incorporation into *R. flavipes's* cuticle disrupted its cuticular water balance and caused significant water loss in a warm, arid environment.

Dietary hydrocarbons also had an effect on *R. flavipes* mortality in the desiccation experiments (Fig. 31, Table 6). Termites feeding on filter paper treated with nC17 and nC22 had significant mortality after 10 hours when compared with the control (Table 6). After 24 hours, all four treatments had 100% mortality and were significantly different from the control (Table 6). Only half of the control termites suffered mortality after 24 hours (Fig. 31, Table 6). Dietary hydrocarbons affected the cuticular water balance in *R. flavipes*, leading to rapid body water loss and 100% mortality within 24 hours exposure in a dry and warm environment.

Cuticular permeability (CP) values indicate that termites fed Z, Z-6, 9-heptacosadiene, 3-methyl pentacosane, and nC25 impregnated filter paper had the greatest water loss from the cuticle during the first two hours of the desiccation experiment than nC22, nC26 and the control (Table 7). The next largest CP values were nC17, nC22, and then nC26 (Table 7). Control samples had the smallest CP values that indicate the least amount of water lost from the cuticle occurred with control samples

(Table 7). The addition of a methyl-branched hydrocarbon and an alkene seemed to alter the cuticular hydrocarbons of *R. flavipes* to an extent where it disrupts its cuticular water balance and thus allowing more water lost from the cuticle. The chain length of the dietary hydrocarbon also exhibited an effect on CP in this experiment. The shorter chain length nC17 had a larger CP value than nC22 or nC26. In this experiment, the addition of a short chain hydrocarbon, an alkene, and a methyl-branched hydrocarbon affect the CP of *R. flavipes* that disrupts its cuticular water balance and thereby, causing an increase in water lost from the cuticle. The values obtained for cuticular permeability in non-treated termites were similar to those reported by other authors for *R. flavipes* (Sponsler and Appel 1990, Shelton and Appel 2001). The results obtained in these experiments are also consistent with the results of studies conducted with model mixtures of compounds that predict that incorporation of methyl branched alkanes and alkenes will have the greatest effect on lowering the melting point of a hydrocarbon mixture and thus increase cuticular permeability to water (Gibbs 1995, Gibbs and Pomonis 1995, Gibbs 2002). The increasing permeability of the cuticle with the incorporation of shorter chain hydrocarbons is also consistent with the models. The termite system that has been developed in the present study is the first one described where it has been possible to intentionally change hydrocarbon composition of an insect and observe the results on cuticular permeability. There is potential in this system to provide novel insights into the functional role of cuticular hydrocarbons in water balance and to finally test some of the models that have been proposed.

III.I. Mortality at Different Humidities – Dietary hydrocarbons can alter the cuticular water balance of *R. flavipes* at 0% relative humidity and 30˚C, but *R. flavipes*

thrive in moist and cooler conditions. The effects of dietary hydrocarbons in *R. flavipes* in their natural habitat are unknown, but in this experiment the temperature was adjusted to match field conditions that *R. flavipes* normally thrives in at five different relative humidities (0%, 25%, 55%, 75%, or 100%). The effects of different relative humidity and altered cuticular hydrocarbons profiles on mortality were examined in this experiment.

Dietary hydrocarbons also have an effect on mortality at 0% relative humidity and 25±1˚C after 24 hours when treated values were compared with control values (Fig. 32, Table 8). This confirms results from the desiccation experiment after 24 hours where nC17. nC22, nC26, and Z, Z-6, 9-heptacosadiene, 3-methyl pentacosane, and nC25 termite's percent mortalities were statistically significance from the control mortalities (Table 6). At 75% humidity, Z, Z-6, 9-heptacosadiene, 3-methyl pentacosane, and n25 had less percent mortality than the other treatments (Table 11). No statistical significance was found between the treated percent mortality and the control at 55% relative humidity (Table 10). It appeared that at a relatively moderate relative humidity, altered cuticular hydrocarbon profiles did not have an effect on mortality. At 25 and 100% relative humidity, no significant differences were found between all five treatments (Table 9, 12). Future studies should focus on total body water loss and mortality in the first 24 hours since water loss occurs the most during the first few hours (Wigglesworth 1945, Shelton and Grace 2003). Additional studies need to be conducted under parameters that *R. flavipes* normally thrives in to fully understand how water balance is affected by altered cuticular hydrocarbon profiles.

CHAPTER VI

CONCLUSIONS

Cuticular hydrocarbons are a major component in cuticular lipids that help maintain water balance in insects. It is also used in insects as chemical cues for mating, and nestmate recognition. Previously, it was believed that cuticular hydrocarbon profiles cannot be altered and are species-specific. In this study, pure hydrocarbons were added to *Reticulitermes flavipes*'s diet to examine their effects on several factors that include changes in cuticular and internal hydrocarbon profiles, water balance, and cuticular permeability.

Cuticular and internal hydrocarbon profiles in *R. flavipes* were altered with dietary hydrocarbons. This is the first reported instance of dietary hydrocarbons and its effects on cuticular and internal hydrocarbon profile. N-alkanes with chain lengths ranging from nC17 to nC38, alkenes, and methyl-branched hydrocarbons were all incorporated in both hydrocarbon pools after 2 weeks of feeding on dietary hydrocarbon impregnated filter paper. Adding a dietary hydrocarbon or a combination of hydrocarbons successfully altered cuticular and internal hydrocarbon profiles. Alkenes and methyl-branched hydrocarbons were tested and they were all incorporated in both cuticular and internal hydrocarbon pools. Dietary hydrocarbons that were tested in

minute concentrations (0.05, 0.1, 1.0, 5.0, and 10.0 μmoles) were incorporated in the cuticular and internal hydrocarbon pools. Relatively large concentrations of dietary hydrocarbon did not cause significant mortality and a LD_{50} could not be determined.

Dietary hydrocarbons appear in the cuticular and internal hydrocarbon pools after 24 hours. Once they are incorporated, dietary hydrocarbons remain in the termite system for at least 63 days. Through social behaviors that include grooming and trophallaxis, dietary nC21 and nC26 were transferred by treated termites transferring it to their non-treated nestmates after 3, 7, and 14 days.

Altered cuticular hydrocarbon profiles reduce *R. flavipes*'s ability to conserve water at 0% relative humidity and 30˚C. Significant total body water loss occurs within the first 24 hours that leads to 100% mortality after 24 hours at both 25 and 30˚C and 0% relative humidity in treated samples when compared with the control. CP values indicate that adding a short chain hydrocarbon (nC17) or an alkene and methyl branched hydrocarbon (Z, Z-6, 9-heptacosadiene, 3-methyl pentacosane) cause a more rapid cuticular water loss in the first two hours of a desiccation experiment compared with the control. At a moderate relative humidity (55%), altered cuticular hydrocarbon profiles did not have an effect on percent mortality at 25˚C. Altered cuticular hydrocarbon profiles seemed to have the most effect at 0% relative humidity in the first 24 hours.

This present study shows that cuticular hydrocarbon profiles are not necessarily stable as was once believed and that cuticular and internal hydrocarbon profiles can be altered with dietary hydrocarbons. These dietary hydrocarbon studies are a starting place for other studies that include: behavior studies between termites fed dietary hydrocarbons and their non-treated nestmates, identifying the mites that were observed in the

concentration experiments; identifying the cause behind the pink coloration in mite infested termites, studies on how dietary hydrocarbons could be utilized in termite baits for termite management, and identifying the mechanisms of how dietary hydrocarbons are incorporated into the cuticle. The ability to alter cuticular hydrocarbon profiles in termites through dietary hydrocarbons has never been demonstrated before and can lead to exciting possibilities in termite management strategies that are environmentally friendly.

CHAPTER VII

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Table 1. Incorporation of different dietary n-alkanes into the cuticular hydrocarbons of *Reticulitermes flavipes* fed for 2 weeks followed by a 1 week chase period.

Treatment [b]	Treated Mean [a]	t-value	Pr > |t|
nC17	37.95 ± 16.15	0.92	0.3663
nC18	34.69 ± 09.31	0.84	0.4083
nC19	60.31 ± 02.87	1.46	0.1556
*nC20	203.40 ± 23.38	4.92	<0.0001
*nC21	199.48 ± 44.95	4.83	<0.0001
*nC22	398.32 ± 23.32	9.64	<0.0001
*nC26	266.43 ± 23.10	6.45	<0.0001
*nC28	252.68 ± 68.11	6.11	<0.0001
*nC29	187.30 ± 34.06	4.53	<0.0001
*nC31	163.03 ± 22.20	3.95	0.0005
nC32	81.42 ± 25.54	1.97	0.0588
*nC33	90.88 ± 22.25	2.20	0.0363
*nC34	113.48 ± 49.10	2.75	0.0104
*nC36	344.88 ± 107.87	8.35	<0.0001
nC38	29.63 ± 11.45	0.72	0.4793

[a] Mean ± standard error of the mean; unit: µg hydrocarbon/gm termite (n=3)
[b] Asterisks (*) indicate significant difference ($p < 0.05$), based on one population sample t-test.

Table 2. Incorporation of different dietary n-alkanes into the internal hydrocarbon of *Reticulitermes flavipes* fed for 2 weeks followed by a 1 week chase period.

Treatment [b]	Treated Mean [a]	t-value	Pr > |t|
*nC17	7059.72 ± 383.15	32.77	<0.0001
*nC18	1878.60 ± 298.43	8.72	<0.0001
*nC19	1692.26 ± 166.56	7.86	<0.0001
*nC20	1702.85 ± 209.85	7.90	<0.0001
nC21	714.64 ± 305.12	3.32	0.0025
*nC22	398.32 ± 23.32	1.85	0.0750
*nC26	413.45 ± 18.13	1.92	0.0652
*nC28	263.84 ± 57.11	1.22	0.2309
*nC29	220.93 ± 24.96	1.03	0.3139
nC31	1021.84 ± 363.14	4.74	<0.0001
nC32	620.22 ± 262.45	2.88	0.0076
*nC33	529.80 ± 59.05	2.46	0.0204
*nC34	728.95 ± 61.49	3.38	0.0021
*nC36	886.42 ± 257.14	4.11	0.0003
nC38	295.56 ± 128.32	1.37	0.1810

[a] Mean ± standard error of the mean; unit: µg hydrocarbon/gm termite (n=3)
[b] Asterisks (*) indicate significant difference ($p < 0.05$), based on one population sample t-test.

Table 3. Incorporation of dietary nC23, nC23, nC25, and nC27 into the cuticular hydrocarbon pool of *Reticulitermes flavipes* fed for 2 weeks followed by a 1 week chase period.

Treatment [b]	Treated Mean [a]	Control Mean [a]	p-value
*nC23	302.78 ± 11.29	6.09 ± 1.12	<0.0001
*nC24	179.11 ± 52.50	5.65 ± 1.09	0.0298
*nC25	220.26 ± 36.46	31.20 ± 5.28	0.0068
*nC27	217.94 ± 25.46	1.19 ± 0.37	0.0010

[a] Mean ± standard error of the mean; unit: µg hydrocarbon/gm termite (n=3)
[b] Asterisks (*) indicate significant difference ($p < 0.05$), based on two population t-test using comparisons of treatment mean values with control values

Table 4. Incorporation of dietary nC23, nC23, nC25, and nC27 into the internal hydrocarbon pool of *Reticulitermes flavipes* fed for 2 weeks followed by a 1 week chase period.

Treatment [b]	Treated Mean [a]	Control Mean [a]	p-value
*nC23	514.95 ± 113.61	56.26 ± 5.23	0.0157
*nC24	180.61 ± 29.84	50.14 ± 5.67	0.0127
*nC25	741.270 ± 97.80	304.12 ± 20.85	0.0068
*nC27	217.94 ± 25.462	38.22 ± 4.14	0.0022

[a] Mean ± standard error of the mean; unit: µg hydrocarbon/gm termite (n=3)
[b] Asterisks (*) indicate significant difference ($p < 0.05$), based on two population t-test using comparisons of treatment mean values with control values

Table 5. Effects of dietary hydrocarbons on total body water loss of *Reticulitermes flavipes*.

Time (hrs)	Treatment				
	nC17 [ab]	nC22 [ab]	nC26 [ab]	Z, Z-9,12-heptacosadiene, 3-methyl pentacosane, and nC25 [ab]	Control [ab]
2	6.4 ± 0.8	6.2 ± 0.4	8.0 ± 0.7	9.5* ± 0.5	7.1 ± 0.6
4	12.5 ± 1.3	12.7 ± 1.3	15.3 ± 1.3	13.8 ± 0.7	12.0 ± 1.9
6	18.8 ± 1.7	19.1 ± 1.7	20.5 ± 1.8	19.6 ± 1.0	16.1 ± 2.3
8	24.9± 2.1	24.2 ± 1.7	27.8* ± 2.3	25.5 ± 1.2	20.0 ± 2.5
10	31.7* ± 2.3	30.2 ± 1.8	37.4* ± 2.5	31.8* ± 1.5	23.7 ± 2.9
12	38.4* ± 2.5 [A]	36.1* ± 1.8	42.5* ± 2.6	39.8* ± 1.8	27.5 ± 2.9
24	63.3* ± 1.2	64.4* ± 1.0	72.1* ± 0.7	67.8* ± 0.6	55.3 ± 2.8

[a] Data represent Mean ± S.E.M.; units: % Total Body Water loss (TBW) per number of hours (n = 30)
[b] Means with astericks (*) indicate significant difference ($p < 0.05$), based on Dunnett's test that compares treated values with control values

Table 6. Effects of dietary hydrocarbons on mortality of *Reticulitermes flavipes* maintained at 0% relative humidity and 30˚C.

Time (hrs)	Treatment				
	nC17 [ab]	nC22 [ab]	nC26 [ab]	Z, Z-6, 9-heptacosadiene, 3-methyl pentacosane, and nC25 [ab]	Control [ab]
2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4	0.0 ± 0.0	3.3 ± 3.3	6.7 ± 4.6	0.0 ± 0.0	3.3 ± 3.3
6	3.3 ± 3.3	3.3 ± 3.3	6.7 ± 4.6	0.0 ± 0.0	6.7 ± 4.6
8	23.3 ± 7.9	23.3 ± 7.9	20.0 ± 7.4	6.7 ± 4.6	6.7 ± 4.6
10	43.3* ± 9.2	36.7* ± 8.9	33.3 ± 8.8	13.3 ± 6.3	10.0 ± 0.1
12	60.0* ± 9.1	60.0* ± 9.1	43.3* ± 9.2	36.7 ± 8.94	13.3 ± 6.3
24	100.0* ± 0.0	100.0* ± 0.0	100.0* ± 0.0	100.0* ± 0.0	53.3 ± 9.3

[a] Data represent Mean ± S.E.M.; units: % Mortality per number of hours (n = 30)
[b] Means with astericks (*) indicate significant difference ($p < 0.05$), based on Fisher's exact test that compares treated values with control values

Table 7. Effects of dietary hydrocarbons on cuticular permeability of *Reticulitermes flavipes*.

Treatment	Cuticular Permeability [ab]
nC17	$38.91^{AB} \pm 0.19$
nC22	$30.61^{BC} \pm 0.13$
nC26	$25.96^{CD} \pm 0.12$
Z, Z-9,12-heptacosadiene, 3-methyl pentacosane, nC25	$45.33^{A} \pm 0.14$
Control	$18.64^{D} \pm 0.11$

[a] Data represent Mean ± S.E.M.; units: expressed as μgH_20 lost$\cdot cm^{-2 \cdot h-1} \cdot mmHg^{-1}$ (n = 30)
[b] Means with the same letter indicate no significant differences ($p < 0.05$) using an ANOVA that utilizes a repeated measures model. Comparisons are pair-wise t-tests protected by contrast tests of the simple effect of treatment given time.

Table 8. Effects of dietary hydrocarbons on mortality of *Reticulitermes flavipes* maintained at 0% relative humidity and 25 ± 1˚C.

Time (day)	Treatment				
	nC17 [ab]	nC22 [ab]	nC26 [ab]	Z, Z-6, 9-heptacosadiene, 3-methyl pentacosane, and nC25 [ab]	Control [ab]
1	$83.3^{A} \pm 6.7$	$90.0^{A} \pm 5.8$	$90.0^{A} \pm 7.6$	$83.3^{A} \pm 3.3$	$60.0^{B} \pm 20.2$
2	$100.0^{A} \pm 0.0$	$100.0^{A} \pm 0.0$	$100.0^{A} \pm 0.0$	$100.0^{A} \pm 0.0$	$100.0^{A} \pm 0.0$

[a] Data represent Mean ± S.E.M.; units: % Mortality per number of days (n = 3)
[b] Means with the same letter indicate no significant differences ($p < 0.05$) using an ANOVA that utilizes a repeated measures model. Comparisons are pair-wise t-tests protected by contrast tests of the simple effect of treatment given time.

Table 9. Effects of dietary hydrocarbons on mortality of *Reticulitermes flavipes* maintained at 25% relative humidity and 25 ± 1˚C

Time (day)	Treatment				
	nC17 [ab]	nC22 [ab]	nC26 [ab]	Z, Z-6,9-heptacosadiene, 3-methyl pentacosane, and nC25 [ab]	Control [ab]
1	$45.0^{A} \pm 18.0$	$55.0^{A} \pm 24.7$	$55.0^{A} \pm 20.2$	$63.3^{A} \pm 13.6$	$51.7^{A} \pm 15.9$
2	$100.0^{A} \pm 0.0$	$100.0^{A} \pm 0.0$	$100.0^{A} \pm 0.0$	$100.0^{A} \pm 0.0$	$100.0^{A} \pm 0.0$

[a] Data represent Mean ± S.E.M.; units: % Mortality per number of days (n = 3)
[b] Means with the same letter indicate no significant differences ($p < 0.05$) using an ANOVA that utilizes a repeated measures model. Comparisons are pair-wise t-tests protected by contrast tests of the simple effect of treatment given time.

Table 10. Effects of dietary hydrocarbons on mortality of *Reticulitermes flavipes* maintained at 55% humidity and 25 ± 1˚C

Time (day)	Treatment				
	nC17 [ab]	nC22 [ab]	nC26 [ab]	Z, Z-6, 9-heptacosadiene, 3-methyl pentacosane, and nC25 [ab]	Control [ab]
1	$0.0^{A} \pm 3.61$	$13.3^{A} \pm 10.9$	$8.3^{A} \pm 1.7$	$5.0^{A} \pm 2.9$	$8.3^{A} \pm 1.7$
2	$40.0^{B} \pm 13.2$	$70.0^{A} \pm 10.4$	$68.3^{A} \pm 21.7$	$45.0^{B} \pm 10.4$	$63.3^{AB} \pm 1.7$
3	$81.7^{B} \pm 7.3$	$93.3^{A} \pm 6.7$	$96.7^{A} \pm 3.3$	$91.7^{A} \pm 8.3$	$91.7^{AB} \pm 4.4$
4	$100.0^{A} \pm 0.0$	$100.0^{A} \pm 0.0$	$100.0^{A} \pm 0.0$	$100.0^{A} \pm 0.0$	$100.0^{A} \pm 0.0$

[a] Data represent Mean ± S.E.M.; units: % Mortality per number of days (n = 3)
[b] Means with the same letter indicate no significant differences ($p < 0.05$) using an ANOVA that utilizes a repeated measures model. Comparisons are pair-wise t-tests protected by contrast tests of the simple effect of treatment given time.

Table 11. Effects of dietary hydrocarbons on mortality of *Reticulitermes flavipes* maintained at 75% relative humidity and 25 ± 1˚C

Time (day)	Treatment				
	nC17 [ab]	nC22 [ab]	nC26 [ab]	Z, Z-6, 9-heptacosadiene, 3-methyl pentacosane, and nC25 [ab]	Control [ab]
1	$15.0^{A} \pm 12.6$	$23.3^{A} \pm 8.8$	$48.3^{A} \pm 24.6$	$20.0^{A} \pm 11.6$	$23.33^{A} \pm 8.8$
2	$66.7^{B} \pm 26.2$	$96.7^{A} \pm 1.7$	$88.3^{A} \pm 11.7$	$65.0^{B} \pm 32.5$	$93.3^{A} \pm 1.7$
3	$98.3^{A} \pm 1.7$	$100.0^{A} \pm 0.0$	$100.0^{A} \pm 0.0$	$93.3^{A} \pm 6.7$	$100.0^{A} \pm 0.0$
4	$100.0^{A} \pm 0.0$	$100.0^{A} \pm 0.0$	$100.0^{A} \pm 0.0$	$100.0^{A} \pm 0.0$	$100.0^{A} \pm 0.0$

[a] Data represent Mean ± S.E.M.; units: % Mortality per number of days (n = 3)
[b] Means with the same letter indicate no significant differences ($p < 0.05$) using an ANOVA that utilizes a repeated measures model. Comparisons are pair-wise t-tests protected by contrast tests of the simple effect of treatment given time.

Table 12. Effects of dietary hydrocarbons on mortality of *Reticulitermes flavipes* maintained at 100% relative humidity and 25 ± 1˚C

Time (day)	Treatment				
	nC17 [ab]	nC22 [ab]	nC26 [ab]	Z, Z-6, 9 - heptacosadiene, 3-methyl pentacosane, and nC25 [ab]	Control [ab]
1	$0.0^{A} \pm 0.0$	$0.0^{A} \pm 0.0$	$0.0^{A} \pm 0.0$	$1.7^{A} \pm 1.67$	$0.0^{A} \pm 0.0$
2	$0.0^{A} \pm 0.0$	$1.7^{A} \pm 1.67$	$1.7^{A} \pm 1.67$	$1.7^{A} \pm 1.67$	$0.0^{A} \pm 0.0$
3	$0.0^{A} \pm 0.0$	$1.7^{A} \pm 1.67$	$1.7^{A} \pm 1.67$	$1.7^{A} \pm 1.67$	$0.0^{A} \pm 0.0$
4	$0.0^{A} \pm 0.0$	$1.7^{A} \pm 1.67$	$1.7^{A} \pm 1.67$	$1.7^{A} \pm 1.67$	$0.0^{A} \pm 0.0$
5	$0.0^{A} \pm 0.0$	$1.7^{A} \pm 1.67$	$1.7^{A} \pm 1.67$	$1.7^{A} \pm 1.67$	$0.0^{A} \pm 0.0$

[a] Data represent Mean ± S.E.M.; units: % Mortality per number of days (n = 3)
[b] Means with the same letter indicate no significant differences ($p < 0.05$) using an ANOVA that utilizes a repeated measures model. Comparisons are pair-wise t-tests protected by contrast tests of the simple effect of treatment given time.

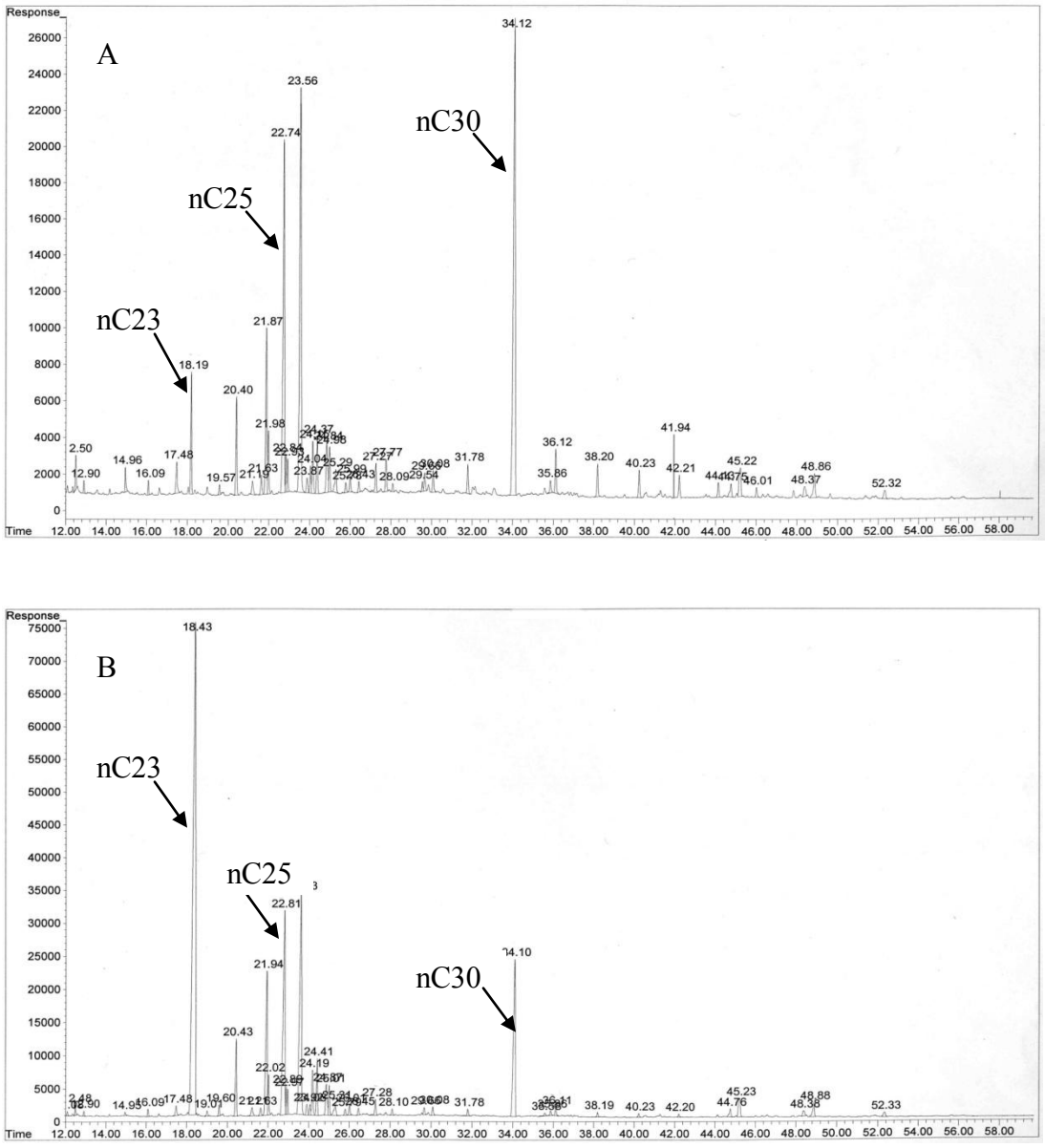

Fig. 1. Chromatograms of cuticular hydrocarbons from *Reticulitermes flavipes*.
A. Termites that were fed on non-treated filter paper for 2 weeks followed by a 1 week chase period (period during which termites were fed on non-treated filter paper) (control group). B. Termites that were fed with nC23 treated filter paper for two weeks followed by a 1 week chase period.

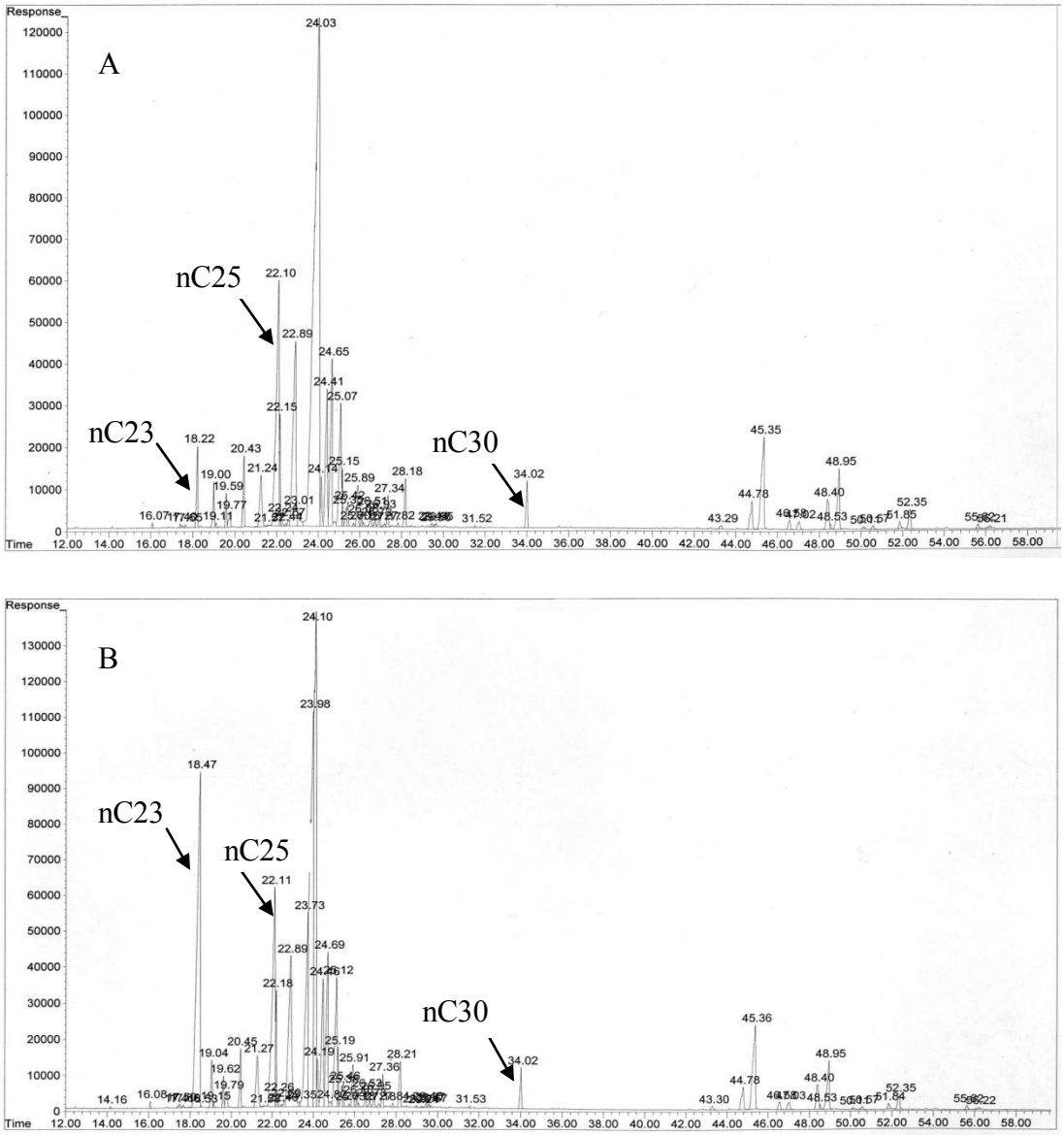

Fig. 2. Chromatograms of internal hydrocarbons from *Reticulitermes flavipes*.
A. Termites that were fed on non-treated filter paper for 2 weeks followed by a 1 week chase period (control group). B. Termites that were fed with nC23 treated filter paper for 2 weeks followed by a 1 week chase period.

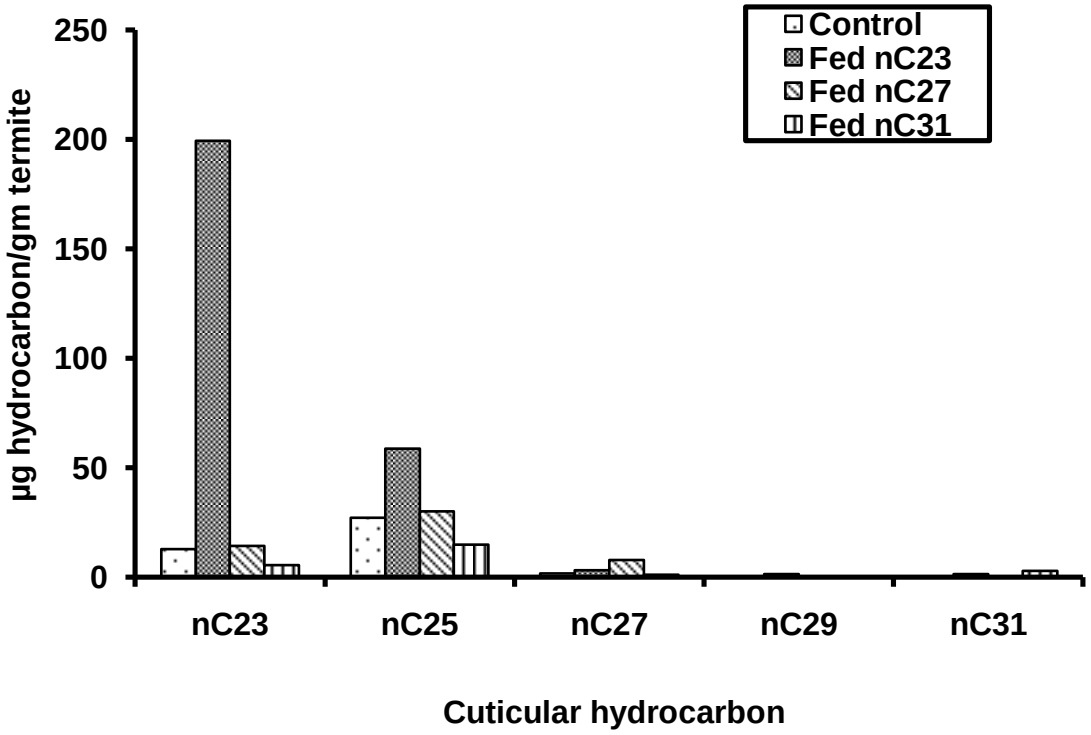

Fig. 3. Cuticular hydrocarbon content of *Reticulitermes flavipes* fed different n-alkanes. One hundred termites were fed filter paper impregnated with 5.0 mg. of nC23, nC27, or nC31 for 2 weeks with a 1 week chase period. Cuticular hydrocarbons (nC23, nC25, nC27, nC29, and nC31) were analyzed and expressed as µg of hydrocarbon per gram of termite.

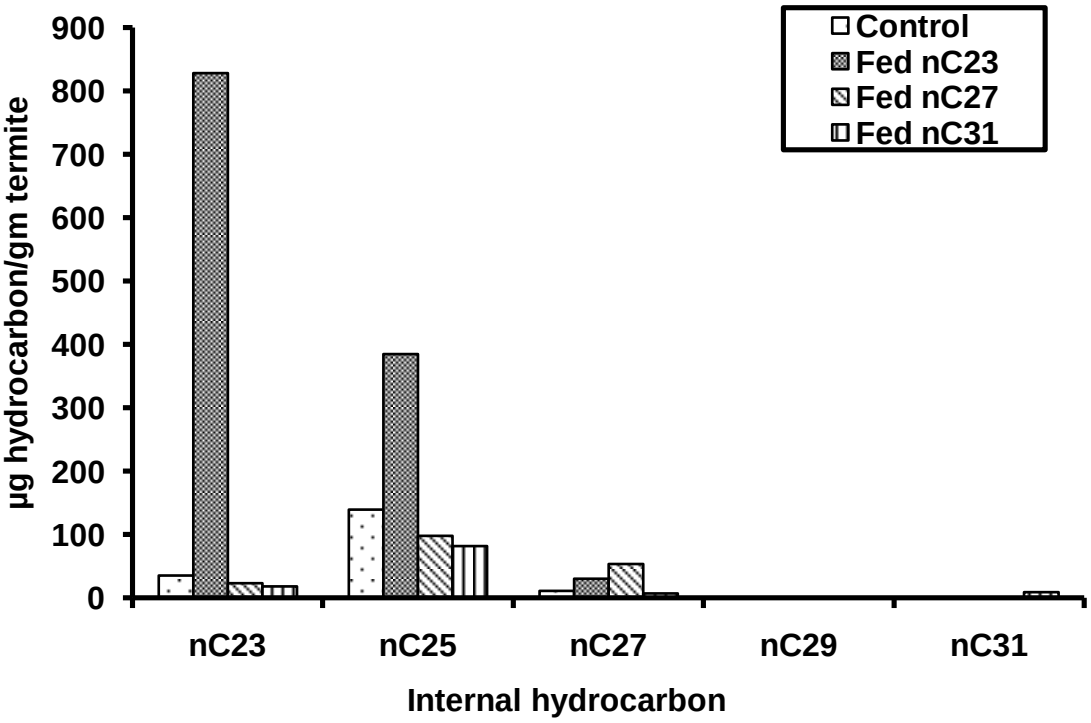

Fig. 4. Internal hydrocarbon content of *Reticulitermes flavipes* fed different n-alkanes. One hundred termites were fed filter paper impregnanted with 5.0 mg. of nC23, nC27, or nC31 for 2 weeks with a 1 week chase period. Internal hydrocarbons (nC23, nC25, nC27, nC29, and nC31) were analyzed and expressed as µg of hydrocarbon per gram of termite.

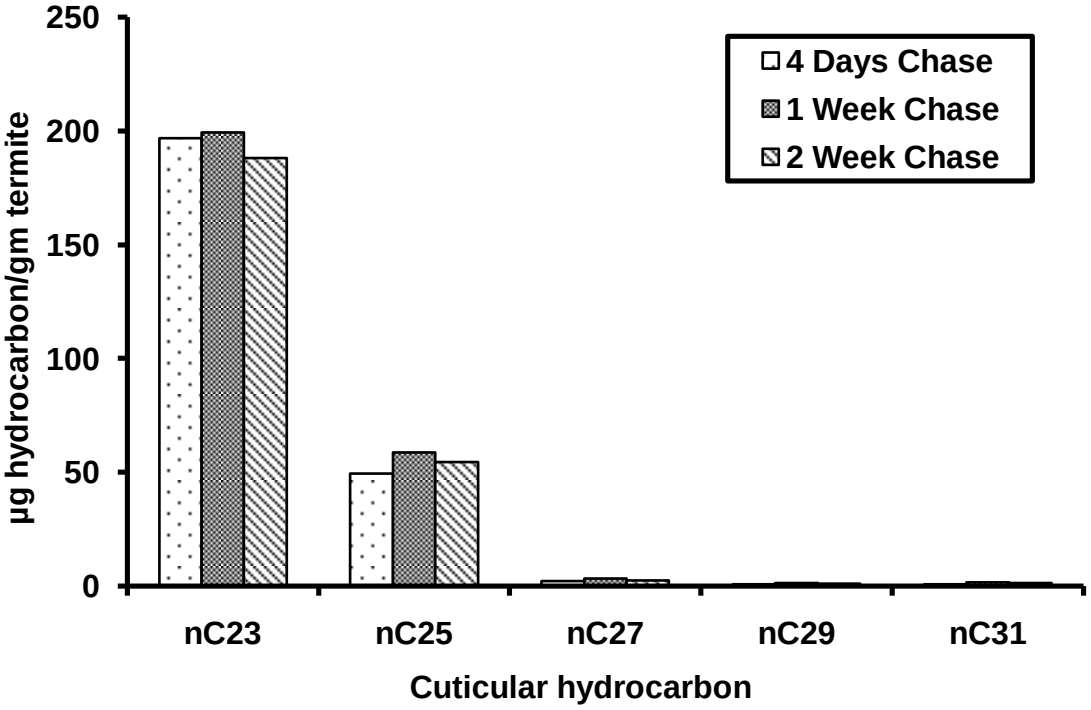

Fig. 5. Cuticular hydrocarbon content of *Reticulitermes flavipes* fed with nC23 for 2 weeks followed by different length chase periods. One hundred termites were fed filter paper impregnated with 5 mg. of nC23 followed by 4 day, 1 week or 2 week chase periods. Cuticular hydrocarbons were analyzed and expressed as µg of hydrocarbon per gram of termite.

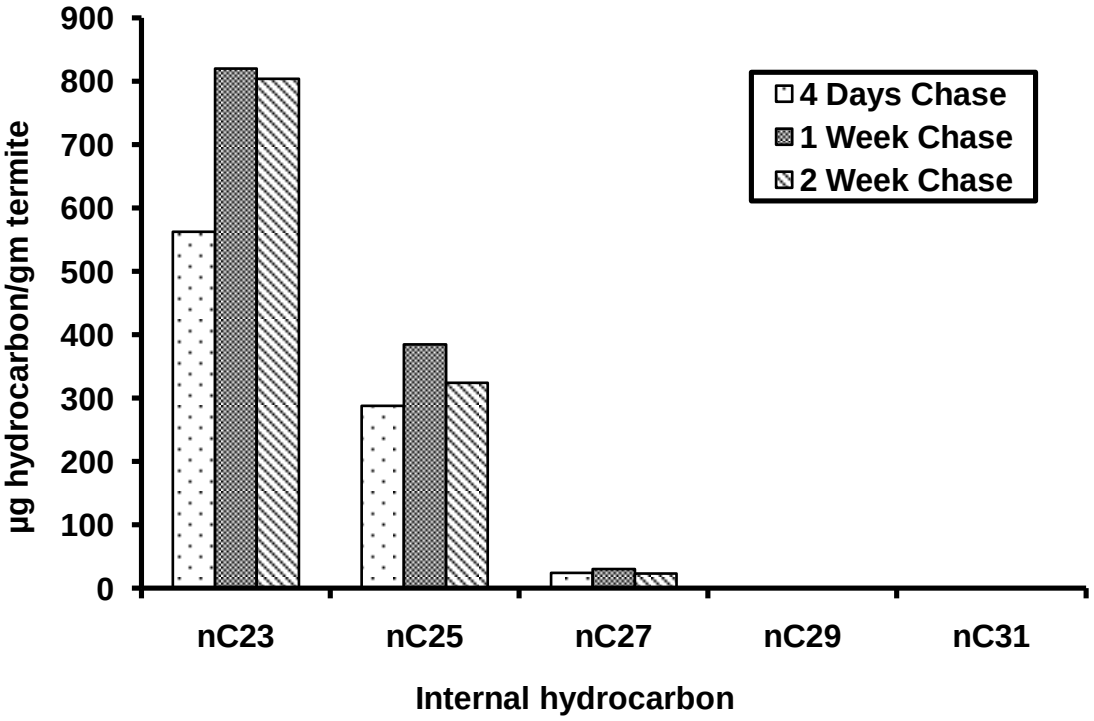

Fig. 6. Internal hydrocarbon content of *Reticulitermes flavipes* fed with nC23 for 2 weeks followed by different length chase periods. One hundred termites were fed filter paper impregnated with 5 mg of nC23 followed by 4 day, 1 week or 2 week chase periods. Internal hydrocarbons were analyzed and expressed as µg of hydrocarbon per gram of termite.

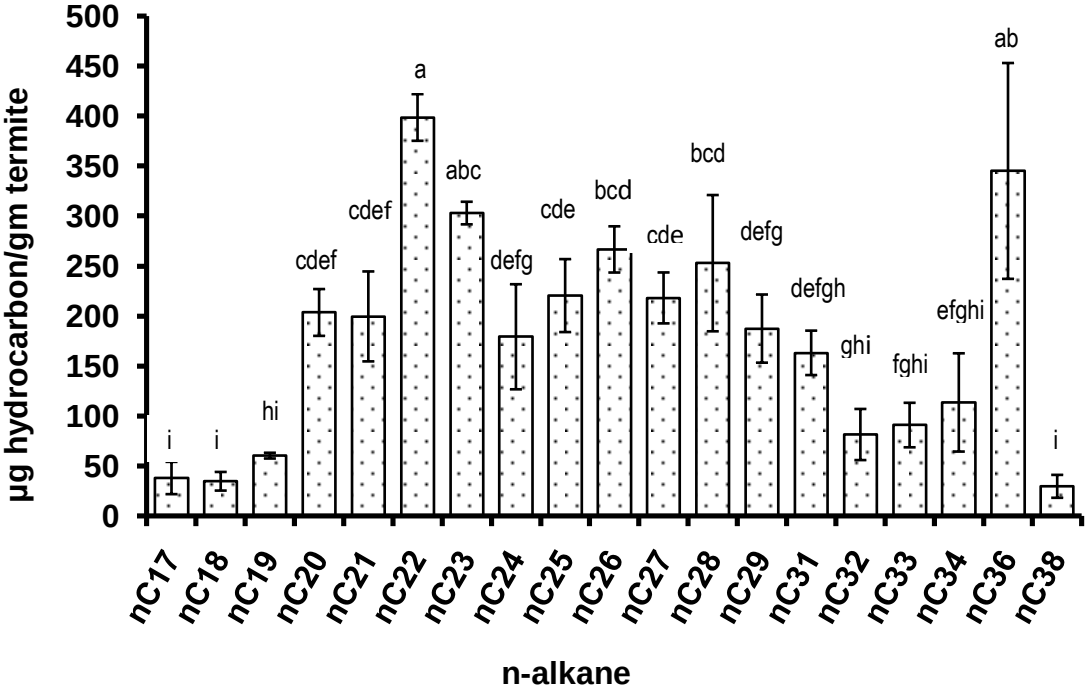

Fig.7. Incorporation of different dietary n-alkanes into the cuticular hydrocarbons of *Reticulitermes flavipes*. Termites were fed filter impregnated with 15.4 µmoles of individual n-alkanes for 2 weeks followed by a 1 week chase period. Cuticular hydrocarbons were analyzed and expressed as µg of hydrocarbon per gram of termite. Bars represent mean ± standard error of the mean (n=3). Means with the same letter indicate no significant differences ($p < 0.05$) using an ANOVA that utilizes a repeated measures model. Comparisons are pair-wise t-tests protected by contrast tests of the simple effect of treatment given time.

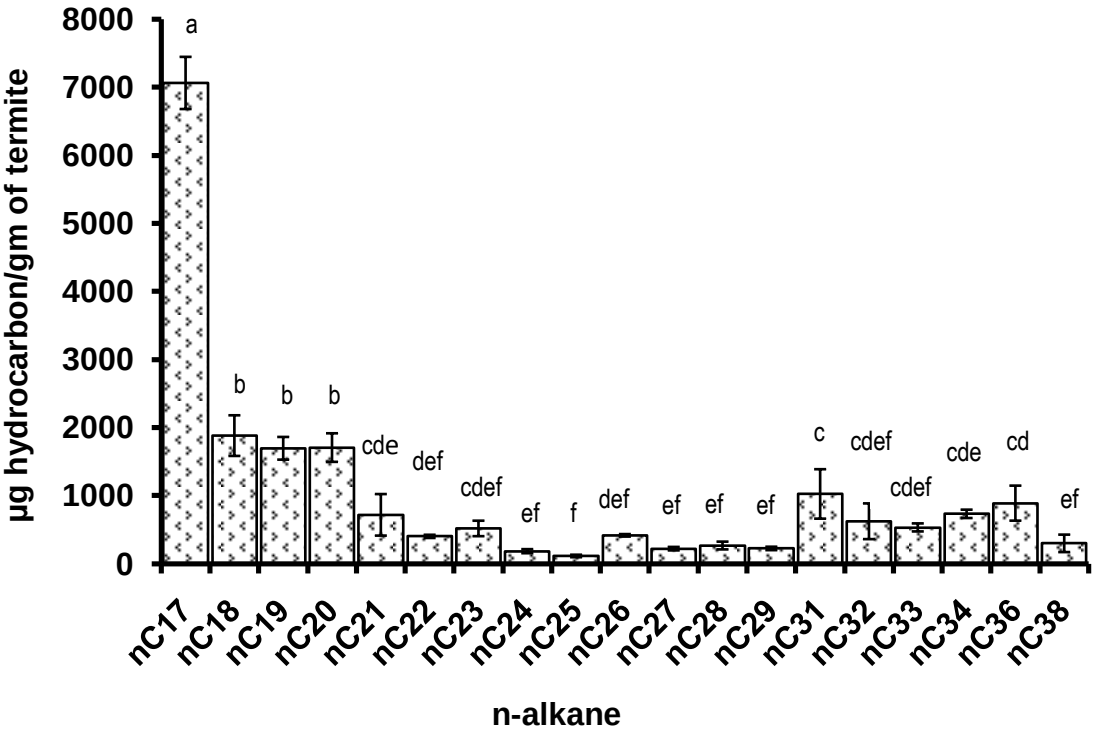

Fig. 8. Incorporation of dietary n-alkanes into the internal hydrocarbons of *Reticulitermes flavipes*. Termites were fed filter impregnated with 15.4 µmoles of individual n-alkanes for 2 weeks followed by a 1 week chase period. Internal hydrocarbons were analyzed and expressed as µg of hydrocarbon per gram of termite. Bars represent mean ± standard error of the mean (n=3). Means with the same letter indicate no significant differences ($p < 0.05$) using an ANOVA that utilizes a repeated measures model. Comparisons are pair-wise t-tests protected by contrast tests of the simple effect of treatment given time.

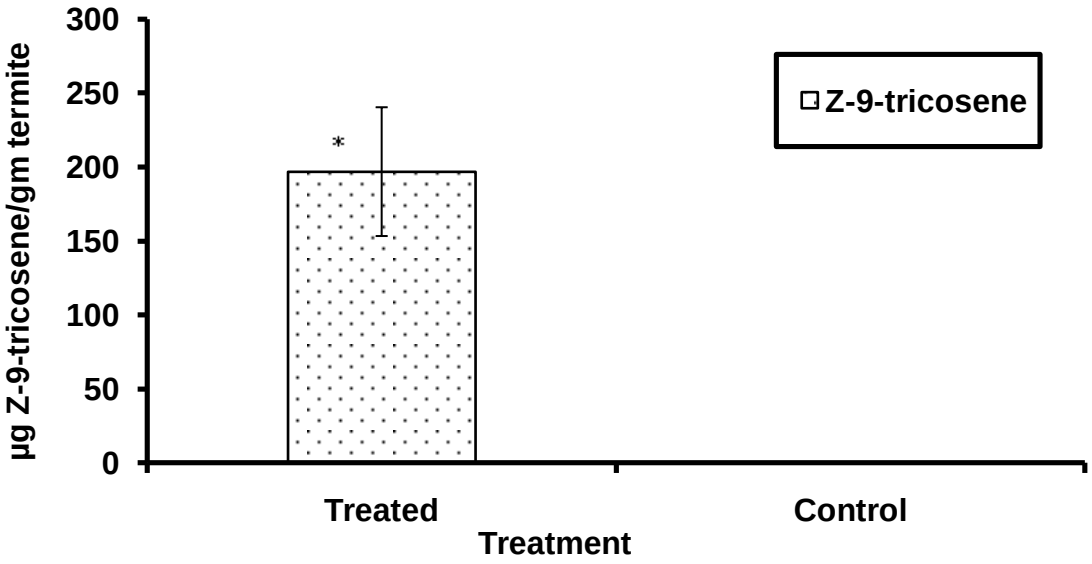

Fig.9. Incorporation of dietary Z-9-tricosene into the cuticular hydrocarbons of *Reticulitermes flavipes*. Fifty termites were fed filter impregnated with 15.4 µmoles of Z-9-tricosene for 2 weeks followed by a 1 week chase period. Cuticular hydrocarbons were analyzed and expressed as µg of Z-9-tricosene per gram of termite. Bars represent mean ± standard error of the mean (n=3). Asterisks (*) indicate significant difference ($p < 0.05$), based on one population sample t-test.

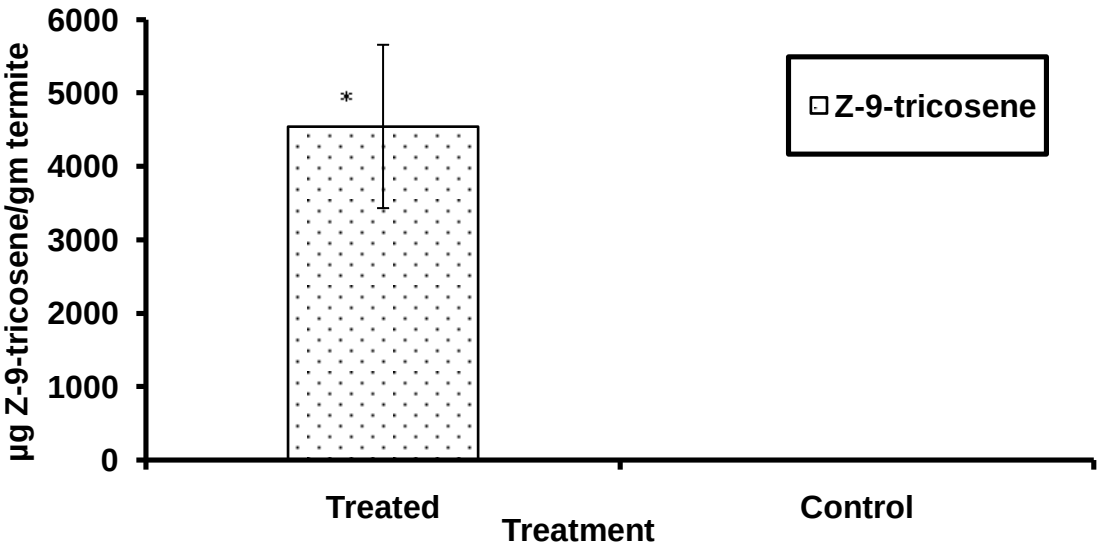

Fig.10. Incorporation of dietary Z-9-tricosene into the internal hydrocarbon pool of *Reticulitermes flavipes*. Fifty termites were fed filter impregnated with 15.4 µmoles of Z-9-tricosene for 2 weeks followed by a 1 week chase period. Internal hydrocarbons were analyzed and expressed as µg of Z-9-tricosene per gram of termite. Bars represent mean ± standard error of the mean (n=3). Asterisks (*) indicate significant difference ($p < 0.05$) based on one population sample t-test.

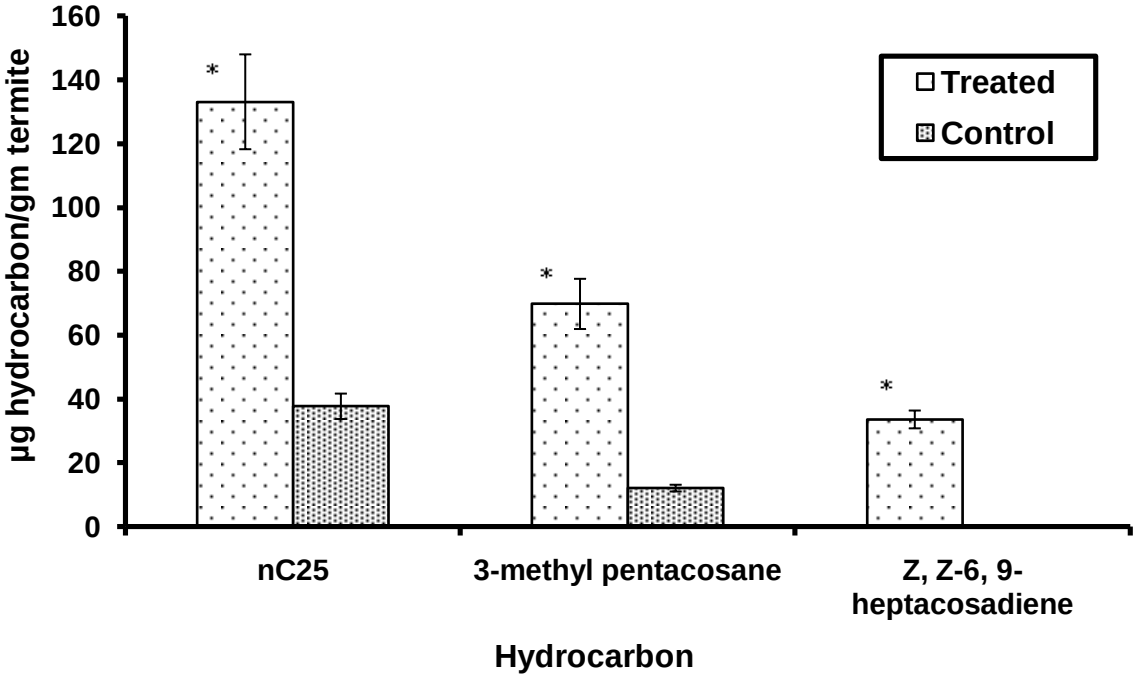

Fig.11. Incorporation of dietary nC25, Z, Z-9, 12-heptacosadiene, and 3-methyl pentacosane into the cuticular hydrocarbons of *Reticulitermes flavipes*. Fifty termites were fed filter impregnated with a combined total of 15.4 µmoles of nC25, Z, Z-heptacosadiene, and 3-methyl pentacosane for 2 weeks followed by a 1 week chase period. Cuticular hydrocarbons were analyzed and expressed as µg hydrocarbon per gram of termite. Bars represent mean ± standard error of the mean (n=3). Asterisks (*) indicate significant difference ($p < 0.05$) between treated sample values and control values, based on two population sample t-test.

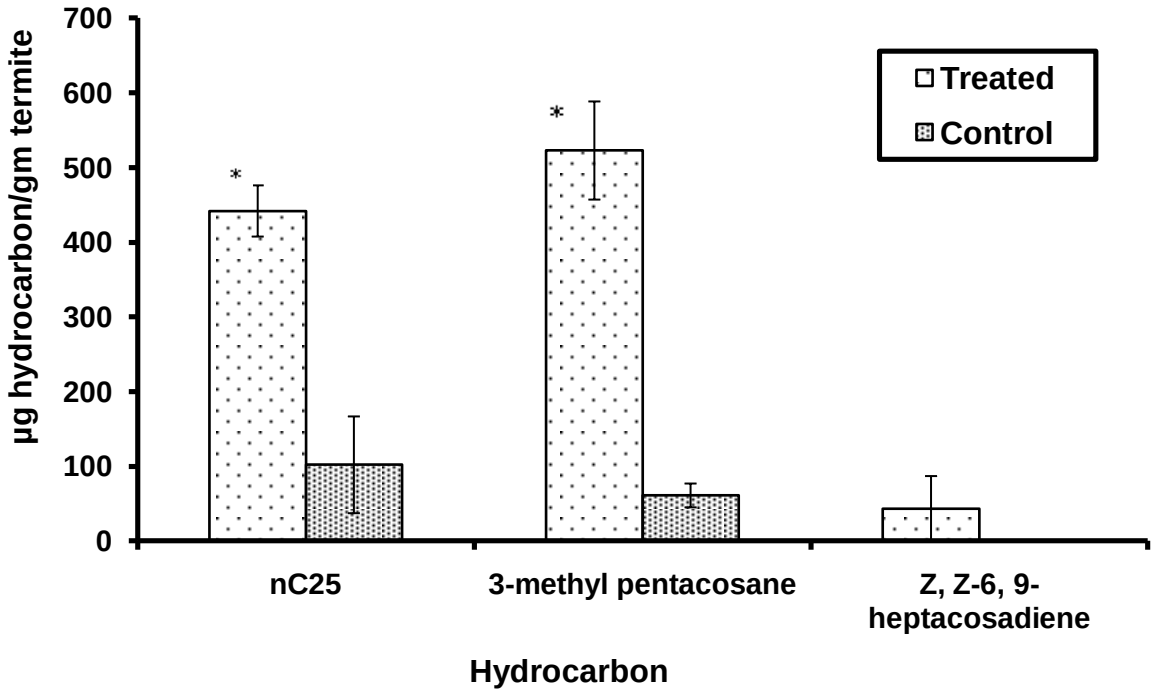

Fig.12. Incorporation of dietary nC25, Z, Z-9, 12-heptacosadiene, and 3-methyl pentacosane into the internal hydrocarbon pool of *Reticulitermes flavipes*. Fifty termites were fed filter paper impregnated with a combined total of 15.4 µmoles of nC25, Z, Z-heptacosadiene, and 3-methyl pentacosane for 2 weeks followed by a 1 week chase period. Internal hydrocarbons were analyzed and expressed as µg hydrocarbon per gram of termite. Bars represent mean ± standard error of the mean (n=3). Asterisks (*) indicate significant difference ($p < 0.05$) between treated sample values and control values, based on two population sample t-test.

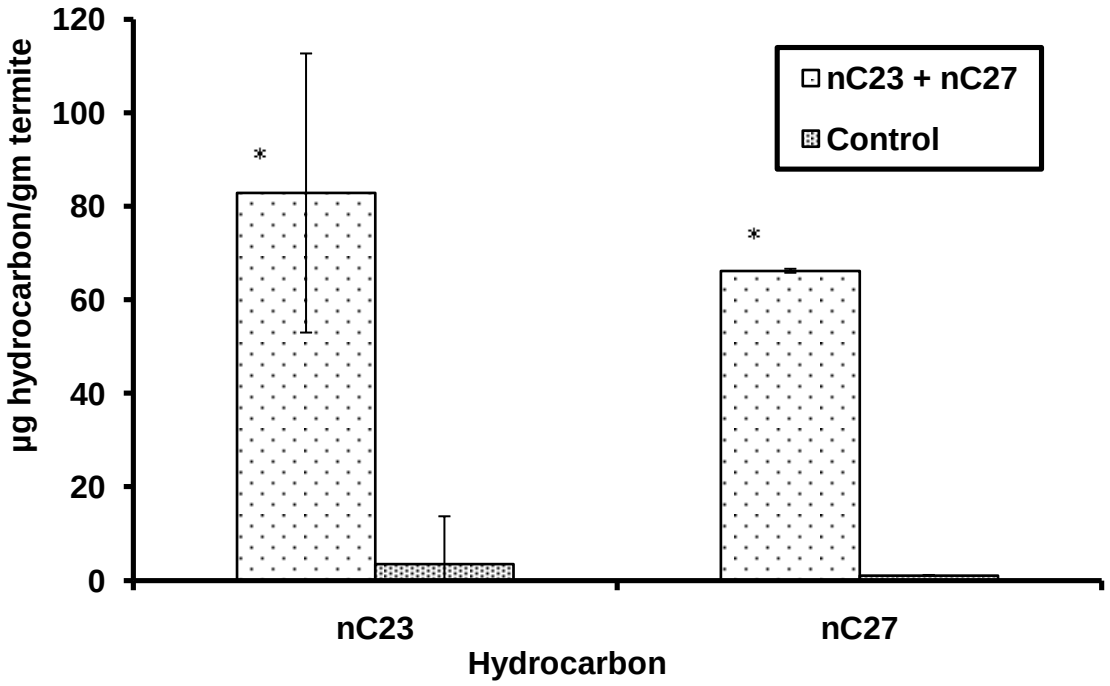

Fig.13. Incorporation of dietary nC23 and nC27 into the cuticular hydrocarbons of *Reticulitermes flavipes*. Fifty termites were fed filter paper impregnated with 15.4 µmoles each of nC23 and nC27 for 2 weeks followed by a 1 week chase period. Cuticular hydrocarbons were analyzed and expressed as µg hydrocarbon per gram of termite. Bars represent mean ± standard error of the mean (n=3). Asterisks (*) indicate significant difference ($p < 0.05$) between treated sample value and control values, based on two population sample t-test.

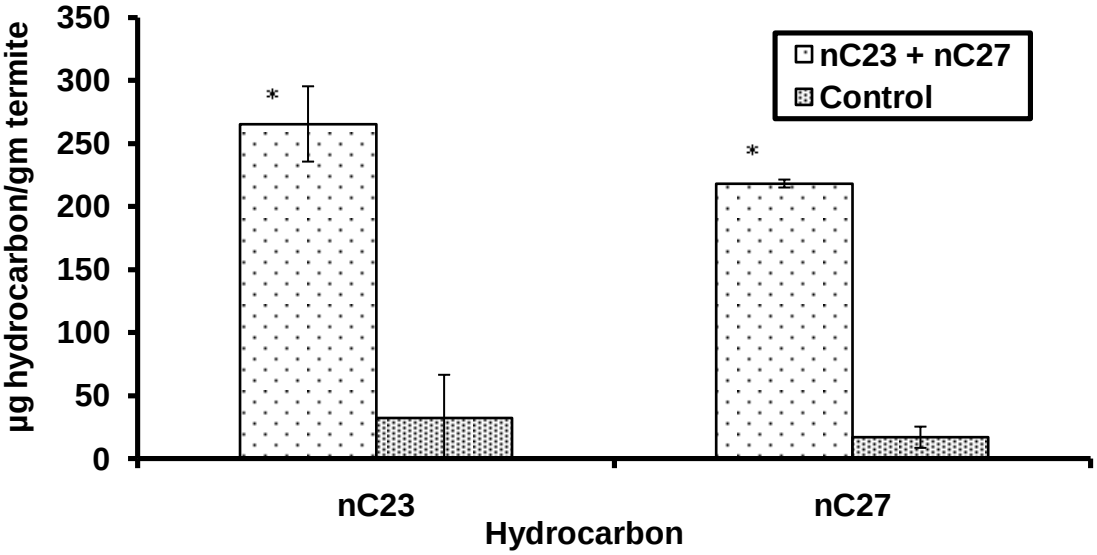

Fig.14. Incorporation of dietary nC23 and nC27 into the internal hydrocarbon pool of *Reticulitermes flavipes*. Fifty termites were fed filter paper impregnated with 15.4 µmoles each of nC23 and nC27 for 2 weeks followed by a 1 week chase period. Internal hydrocarbons were analyzed and expressed as µg hydrocarbon per gram of termite. Bars represent mean ± standard error of the mean (n=3). Asterisks (*) indicate significant difference ($p < 0.05$) between treated sample values and control values, based on two population sample t-test..

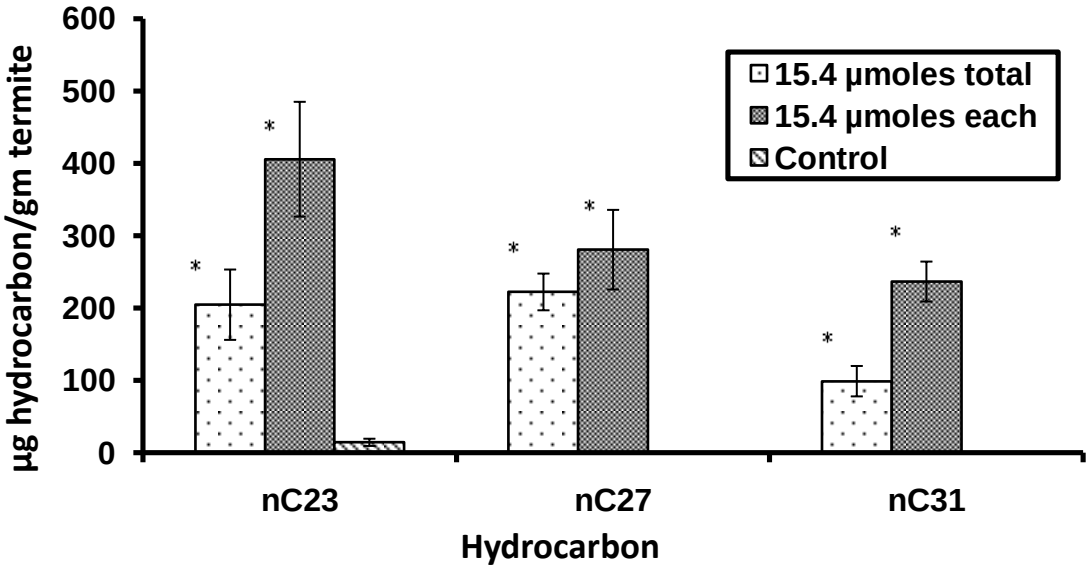

Fig.15. Incorporation of dietary nC23, nC27, and nC31 into the cuticular hydrocarbons of *Reticulitermes flavipes*. Fifty termites were fed filter paper impregnated with either a combined total of 15.4 µmoles of nC23, nC27, and nC31 or 15.4 µmoles each of nC23, nC27, and nC31for 2 weeks followed by a 1 week chase period. Cuticular hydrocarbons were analyzed and expressed as µg hydrocarbon per gram of termite. Bars represent mean ± standard error of the mean (n=3). Asterisks (*) indicate significant difference ($p < 0.05$) between treated sample values and control values, based on two population sample t-test.

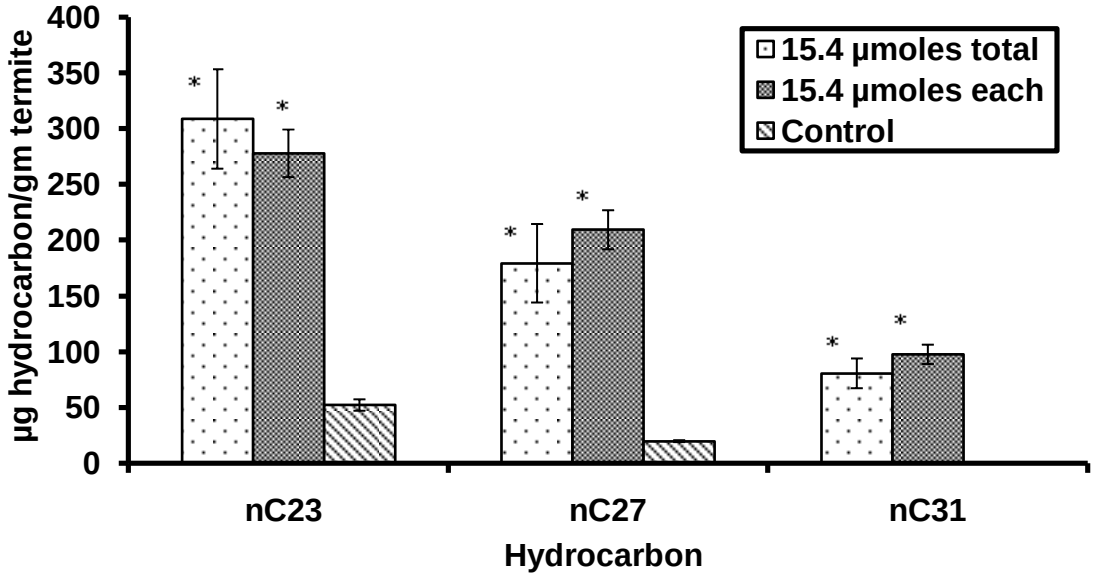

Fig.16. Incorporation of dietary nC23, nC27, and nC31 into the internal hydrocarbon pool of *Reticulitermes flavipes*. Fifty termites were fed paper filter impregnated with either a combined total of 15.4 µmoles of nC23, nC27, and nC31 or 15.4 µmoles each of nC23, nC27, and nC31for 2 weeks followed by a 1 week chase period. Internal hydrocarbons were analyzed and expressed as µg hydrocarbon per gram of termite. Bars represent mean ± standard error of the mean (n=3). Asterisks (*) indicate significant difference ($p < 0.05$) between treated sample values and control values, based on two population sample t-test.

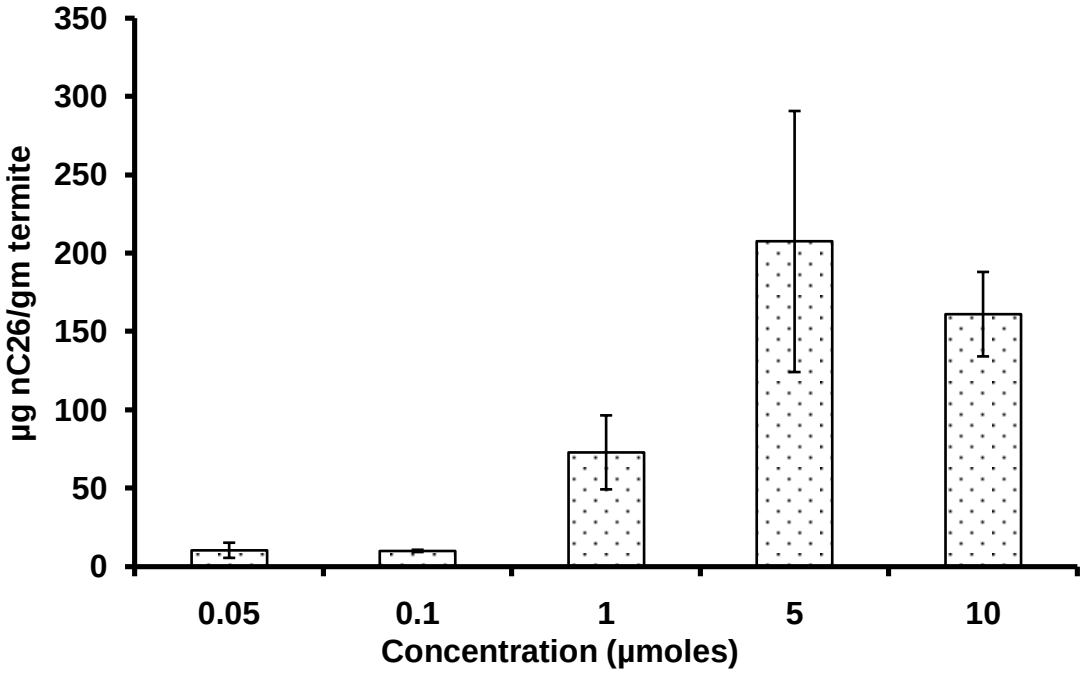

Fig. 17. Amount of nC26 in the cuticle of *Reticulitermes flavipes* fed different amounts of nC26. Fifty termites were fed varying amounts of nC26 for 2 weeks with a 1 week chase period to determine the effect of dietary hydrocarbon dose on its incorporation into the cuticular hydrocarbons. Cuticular hydrocarbon were analyzed for nC26 content which was expressed as µg nC26 per gram of termite. Bars represent mean ± standard error of the mean (n=3).

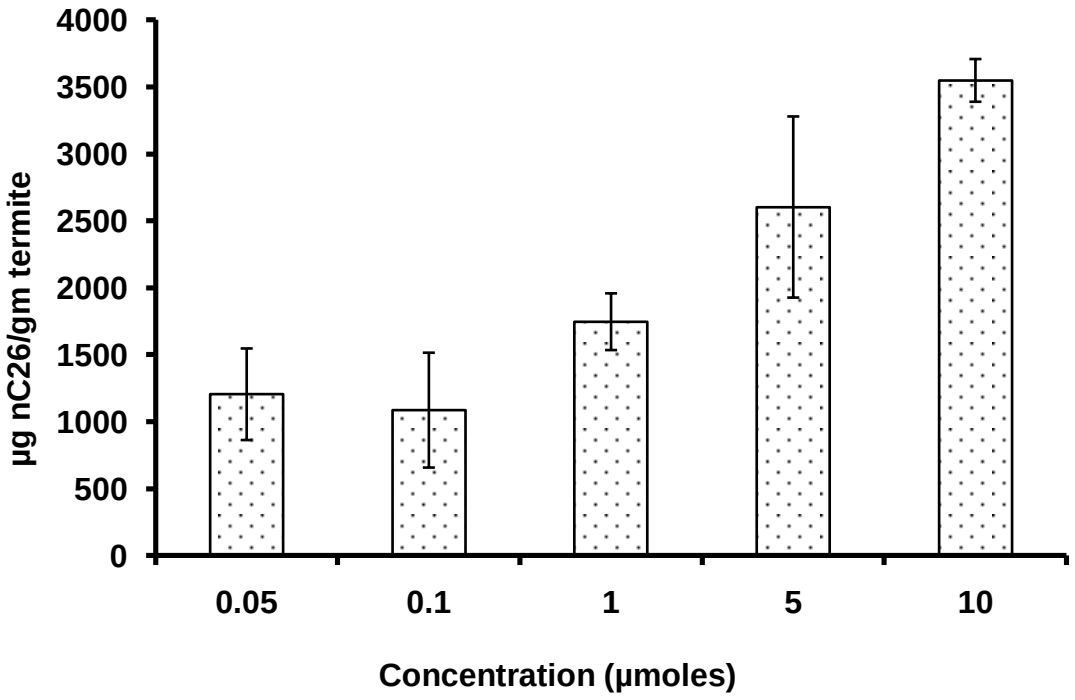

Fig. 18. Amount of nC26 in the internal hydrocarbons of *Reticulitermes flavipes* fed different amounts of nC26. Fifty termites were fed varying amounts of nC26 for 2 weeks followed by a 1 week chase period to determine the effect of dietary hydrocarbon dose on its incorporation into the internal hydrocarbon pool. Internal hydrocarbon were analyzed for nC26 content which is expressed as µg nC26 per gram of termite. Bars represent mean ± standard error of the mean (n=3).

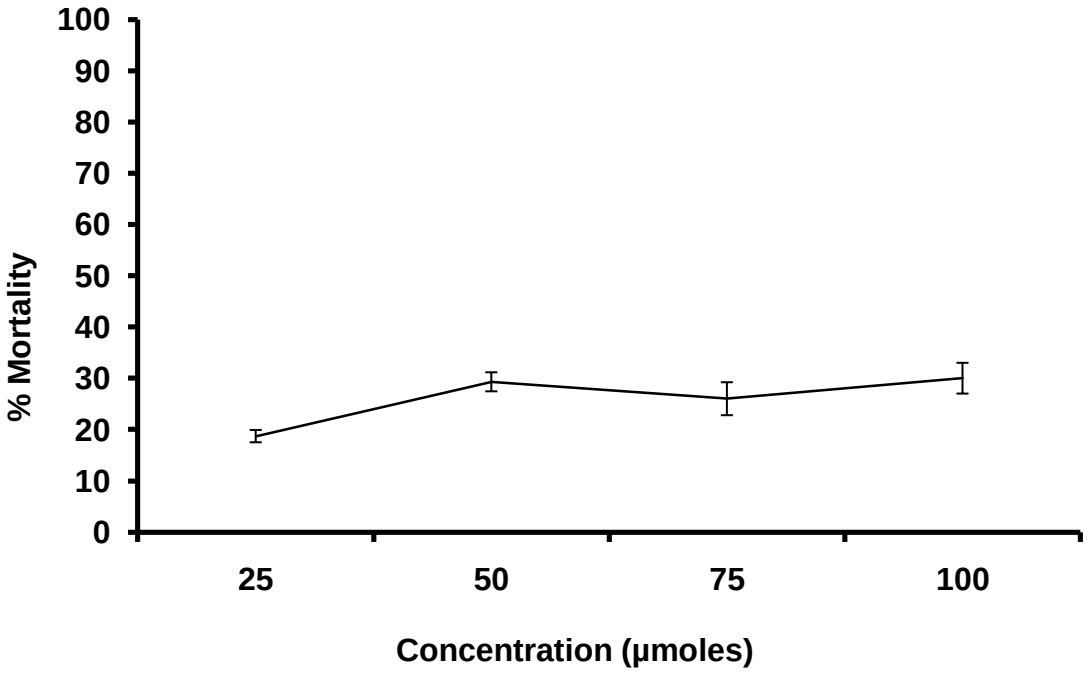

Fig. 19. Effects of high concentrations of dietary nC26 on mortality of *Reticulitermes flavipes*. Fifty termites were fed varying concentrations of nC26 for 2 weeks followed by a 1 week chase period. Termites were removed after the chase period and counted to determine mortality. Bars represent mean ± standard error of the mean (n=3).

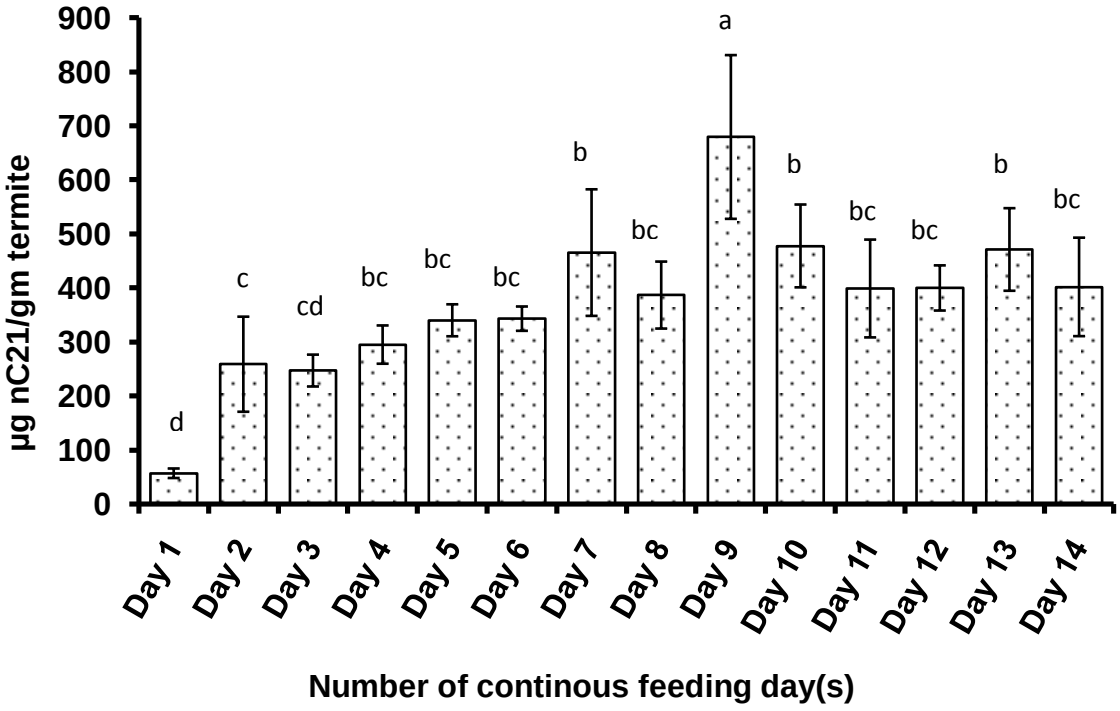

Fig. 20. Daily incorporation of nC21 into *Reticulitermes flavipes*'s cuticular hydrocarbons during 2 weeks of feeding. Three hundred termites were fed filter paper impregnated with 15.4 µmoles of nC21. Twenty termites were removed every 24 hours for 14 days and cuticular hydrocarbons were analyzed for nC21 content, which is expressed as µg of nC21 per gram of termite. Bars represent mean ± standard error of the mean (n=3). Means with the same letter indicate no significant differences ($p < 0.05$) using an ANOVA that utilizes a repeated measures model. Comparisons are pair-wise t-tests protected by contrast tests of the simple effect of treatment given time.

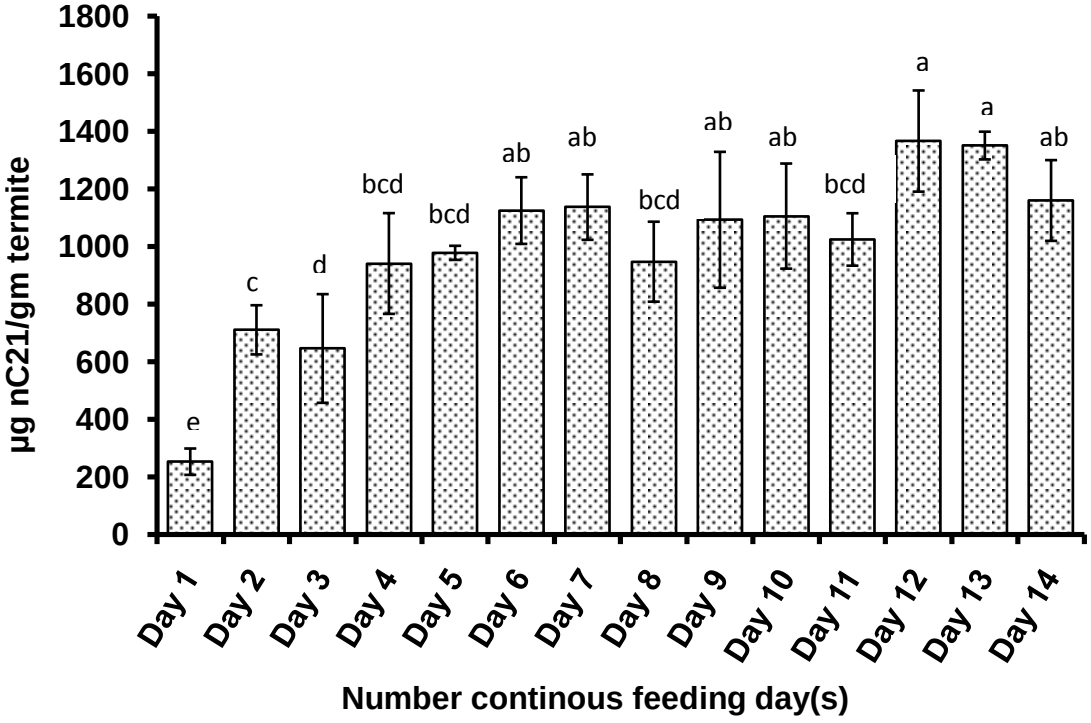

Fig. 21. Daily incorporation of nC21 onto *Reticulitermes flavipes*'s internal hydrocarbon pool during 2 weeks of feeding. Three hundred termites were fed 15.4 µmoles of nC21 impregnated filter paper. Twenty termites were removed every 24 hours for 14 days and internal hydrocarbons were analyzed for nC21 content, which is expressed as µg of nC21 per gram of termite. Bars represent mean ± standard error of the mean (n=3). Means with the letter indicate no significant differences ($p < 0.05$) using an ANOVA that utilizes a repeated measures model. Comparisons are pair-wise t-tests protected by contrast tests of the simple effect of treatment given time.

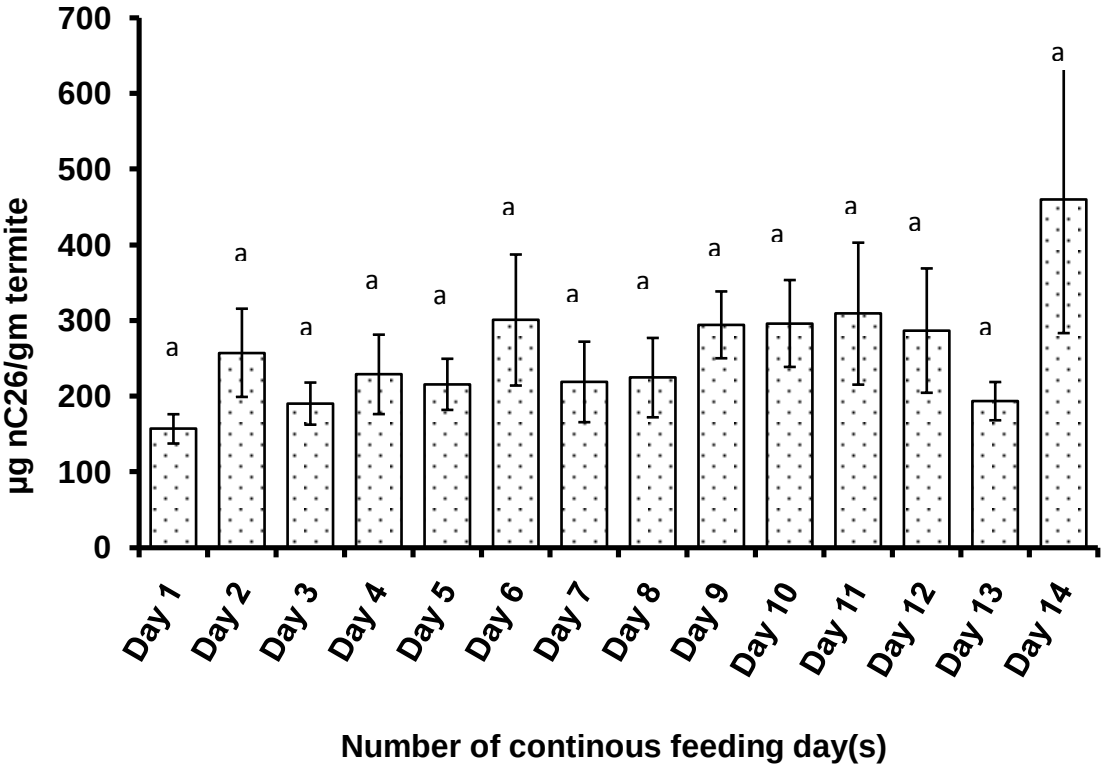

Fig. 22. Daily incorporation of nC26 into *Reticulitermes flavipes*'s cuticular hydrocarbons during 2 weeks of feeding. Three hundred termites were fed filter paper impregnated with 15.4 µmoles of nC26. Twenty termites were removed every 24 hours for 14 days and cuticular hydrocarbons were analyzed for nC26 content, which is expressed as µg of nC26 per gram of termite. Bars represent mean ± standard error of the mean (n=3). Means with the same letter indicate no significant differences ($p < 0.05$) using an ANOVA that utilizes a repeated measures model. Comparisons are pair-wise t-tests protected by contrast tests of the simple effect of treatment given time.

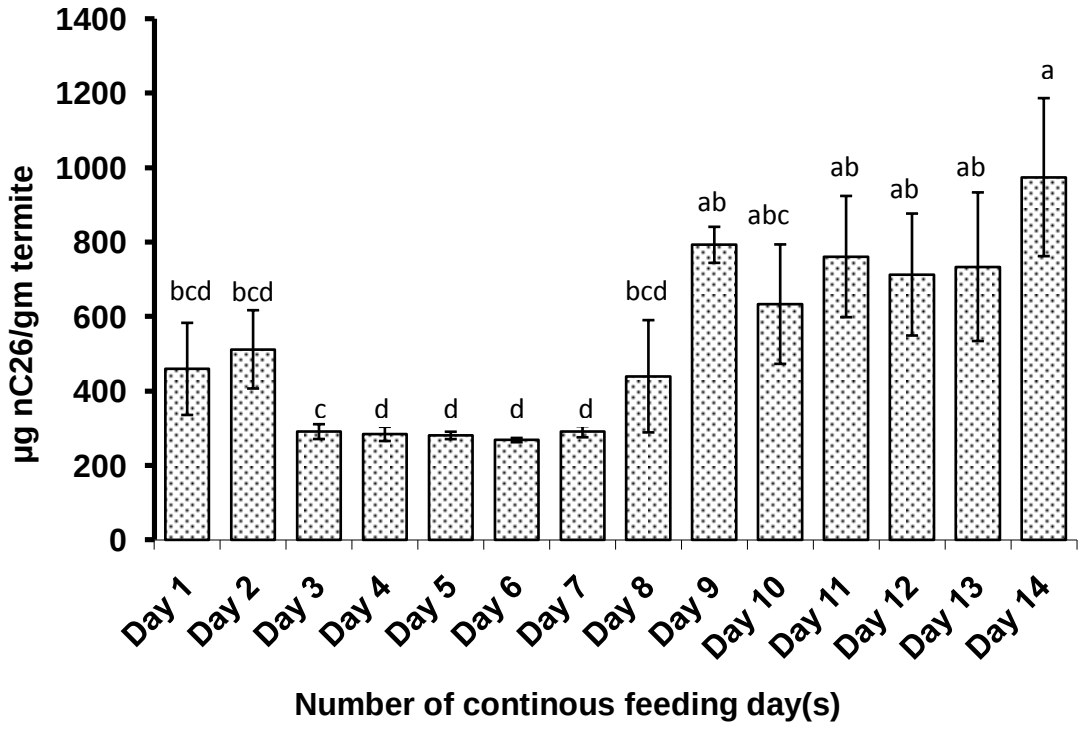

Fig. 23. Daily incorporation of nC26 onto *Reticulitermes flavipes*'s internal hydrocarbon pool for a period of 2 weeks. Three hundred termites were fed 15.4 µmoles of nC26 impregnated filter paper. Twenty termites were removed every 24 hours for 14 days and internal hydrocarbons were analyzed for nC26 content, which is expressed as µg of nC26 per gram of termite. Bars represent mean ± standard error of the mean (n=3). Means with the same letter indicate no significant differences ($p < 0.05$) using an ANOVA that utilizes a repeated measures model. Comparisons are pair-wise t-tests protected by contrast tests of the simple effect of treatment given time.

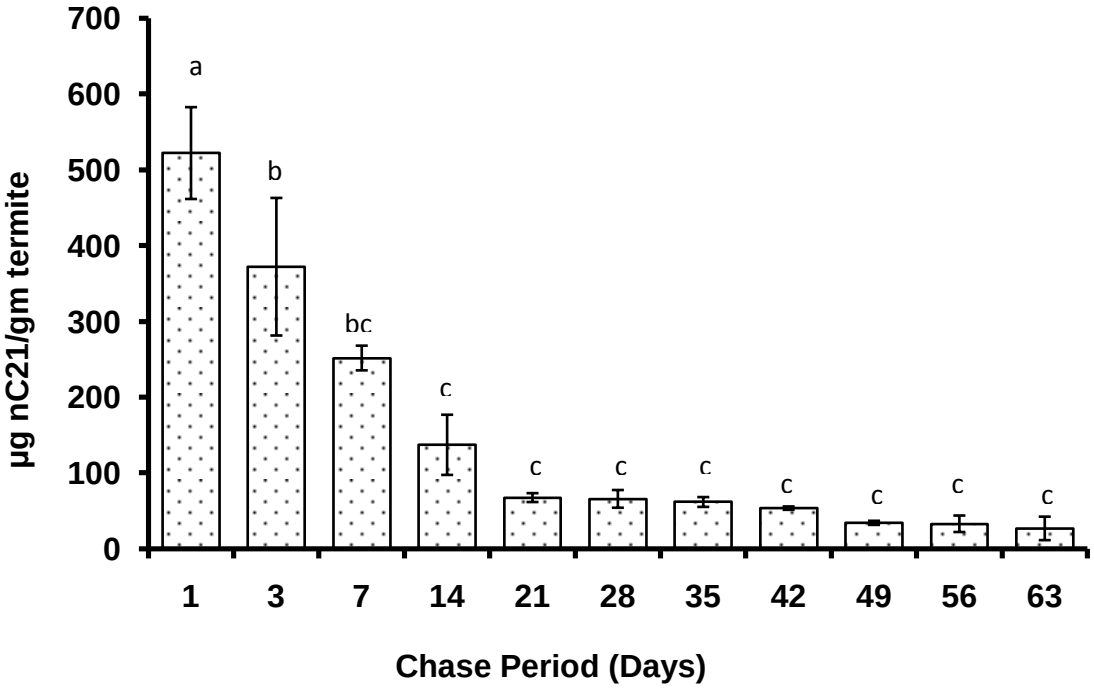

Fig. 24. Time dependent loss of dietary nC21 from *Reticulitermes flavipes*'s cuticular hydrocarbons. Termites were fed filter paper impregnated with 15.4 µmoles of nC21 for 2 weeks and then moved to non-treated filter paper. Twenty termites were removed at each time interval during the chase period and the cuticular hydrocarbons analyzed for nC21 content, which is expressed as µg nC21 per gram of termite. Bars represent mean ± standard error of the mean (n=3). Means with the same letter indicate no significant differences ($p < 0.05$) using an ANOVA that utilizes a repeated measures model. Comparisons are pair-wise t-tests protected by contrast tests of the simple effect of treatment given time.

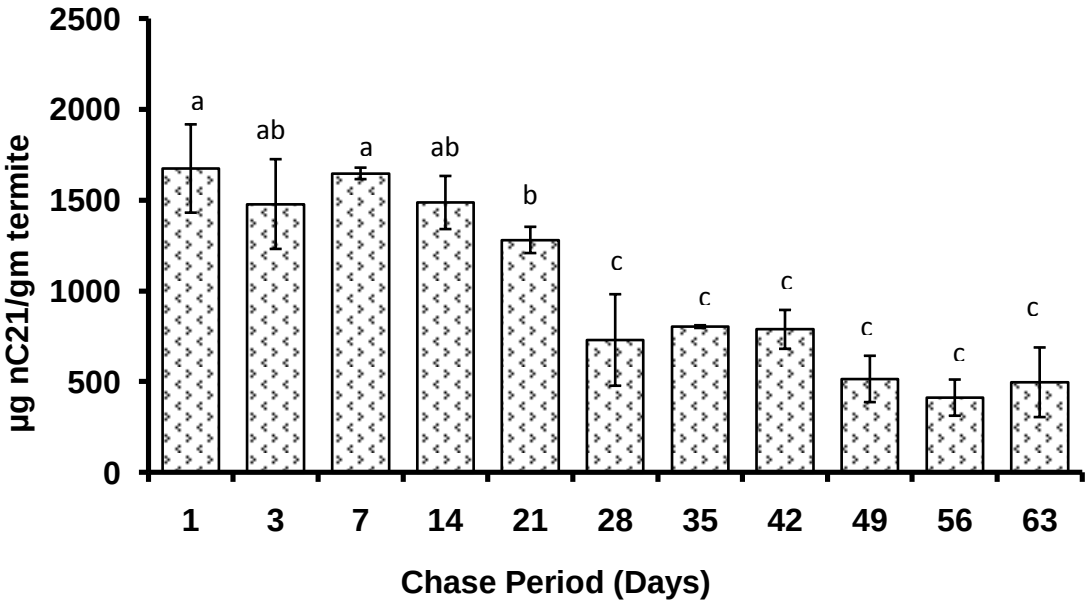

Fig. 25. Time dependent loss of dietary nC21 from *Reticulitermes flavipes*'s internal hydrocarbon pool. Termites were fed on filter paper impregnated with 15.4 µmoles of nC21 for 2 weeks and then moved to non-treated filter paper. Twenty termites were removed at each time interval during the chase period and analyzed for nC21 content, which is expressed as µg nC21 per gram of termite. Bars represent mean ± standard error of the mean (n=3). Means with the same letter indicate no significant differences ($p < 0.05$) using an ANOVA that utilizes a repeated measures model. Comparisons are pair-wise t-tests protected by contrast tests of the simple effect of treatment given time.

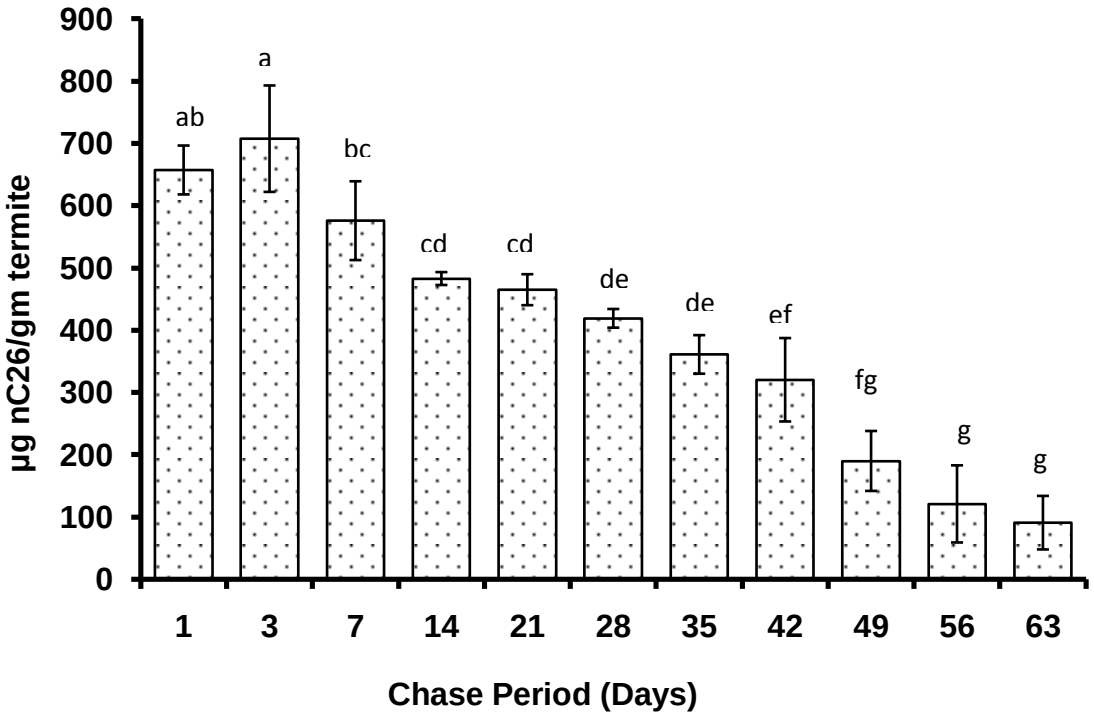

Fig. 26. Time dependent loss of dietary nC26 from *Reticulitermes flavipes*'s cuticular hydrocarbons. Termites were fed on filter paper impregnated with 15.4 µmoles of nC26 for 2 weeks and then moved to non-treated filter paper. Twenty termites were removed at each time interval during the chase period and the cuticular hydrocarbons analyzed for nC26 content, which is expressed as µg nC26 per gram of termite. Bars represent mean ± standard error of the mean (n=3). Means with the same letter indicate no significant differences ($p < 0.05$) using an ANOVA that utilizes a repeated measures model. Comparisons are pair-wise t-tests protected by contrast tests of the simple effect of treatment given time.

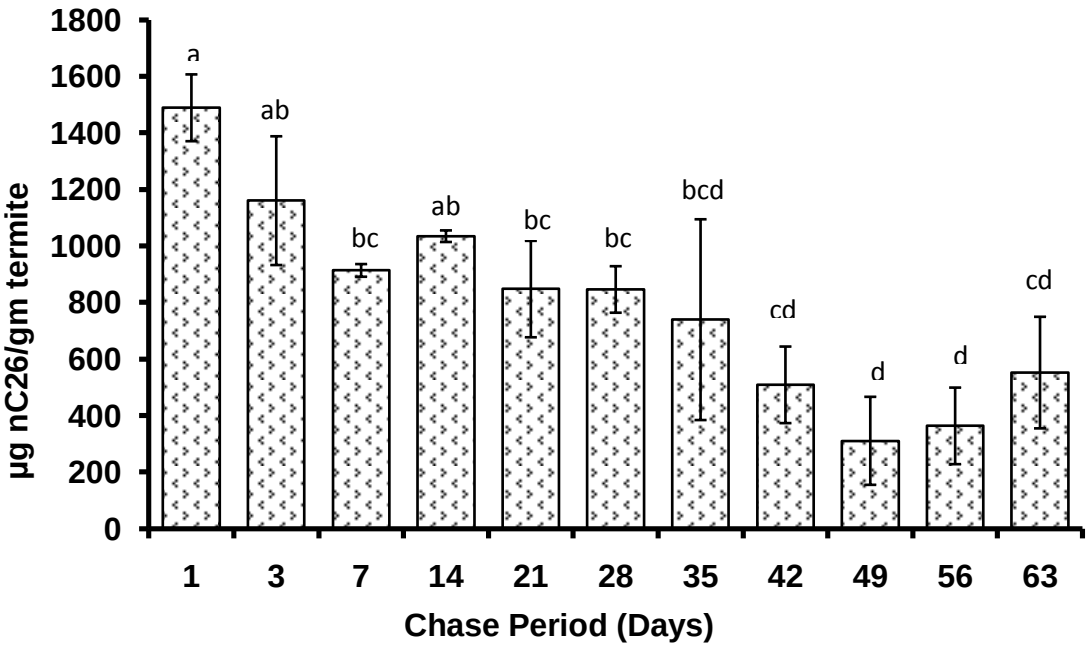

Fig. 27. Time dependent loss of dietary nC26 from *Reticulitermes flavipes*'s internal hydrocarbon pool. Termites were fed on filter paper impregnated with 15.4 µmoles of nC26 for 2 weeks and then moved to non-treated filter paper. Twenty termites were removed at each time interval during the chase period and analyzed for nC26 content, which is expressed as µg nC26 per gram of termite. Bars represent mean ± standard error of the mean (n=3). Means with the same letter indicate no significant differences ($p < 0.05$) using an ANOVA that utilizes a repeated measures model. Comparisons are pair-wise t-tests protected by contrast tests of the simple effect of treatment given time.

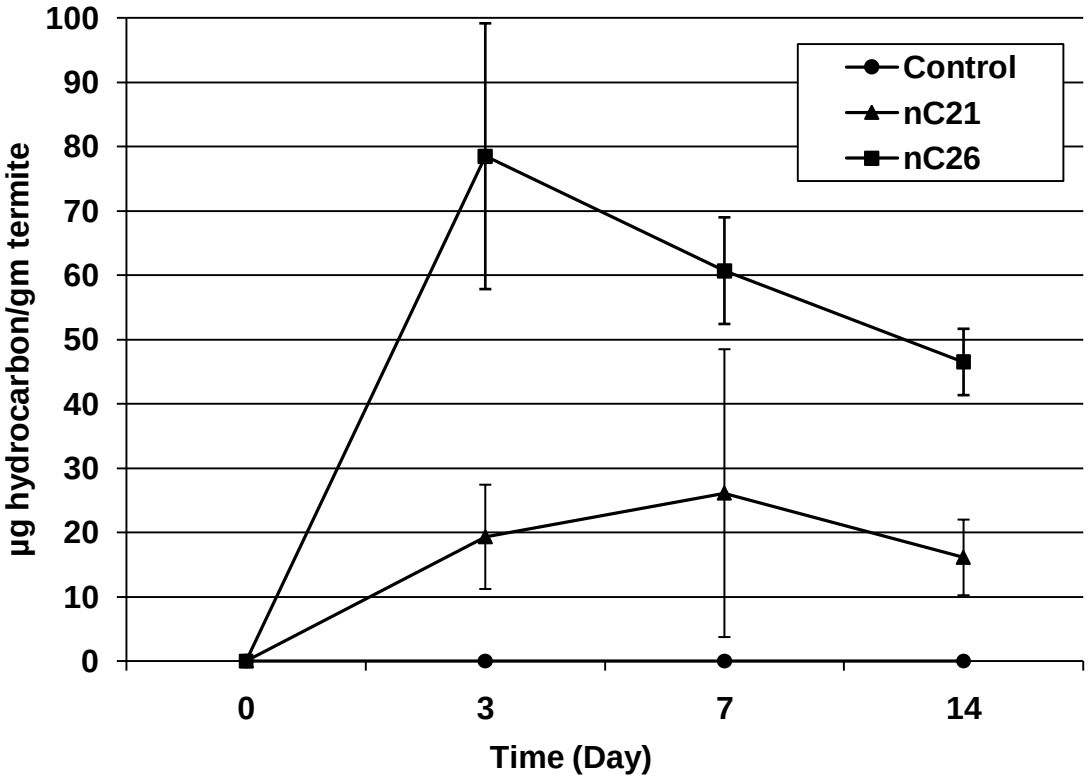

Fig. 28. Transfer of nC21 and nC26 through social interactions from treated *Reticulitermes flavipes* to their non-treated nestmates' cuticle. One hundred fifty termites were fed filter paper dyed and impregnated with 15.4 µmoles of nC21 or nC26 for 2 weeks. After 2 weeks, treated termites were placed with 150 non-dyed, non-treated termites, all then fed on non-treated filter paper. Twenty non-treated termites were removed after 3 days, 7 days, and 14 days and cuticular hydrocarbons analyzed for nC21 and nC26 content, which is expressed as µg hydrocarbon per gram of termite. Bars represent mean ± standard error of the mean (n=3).

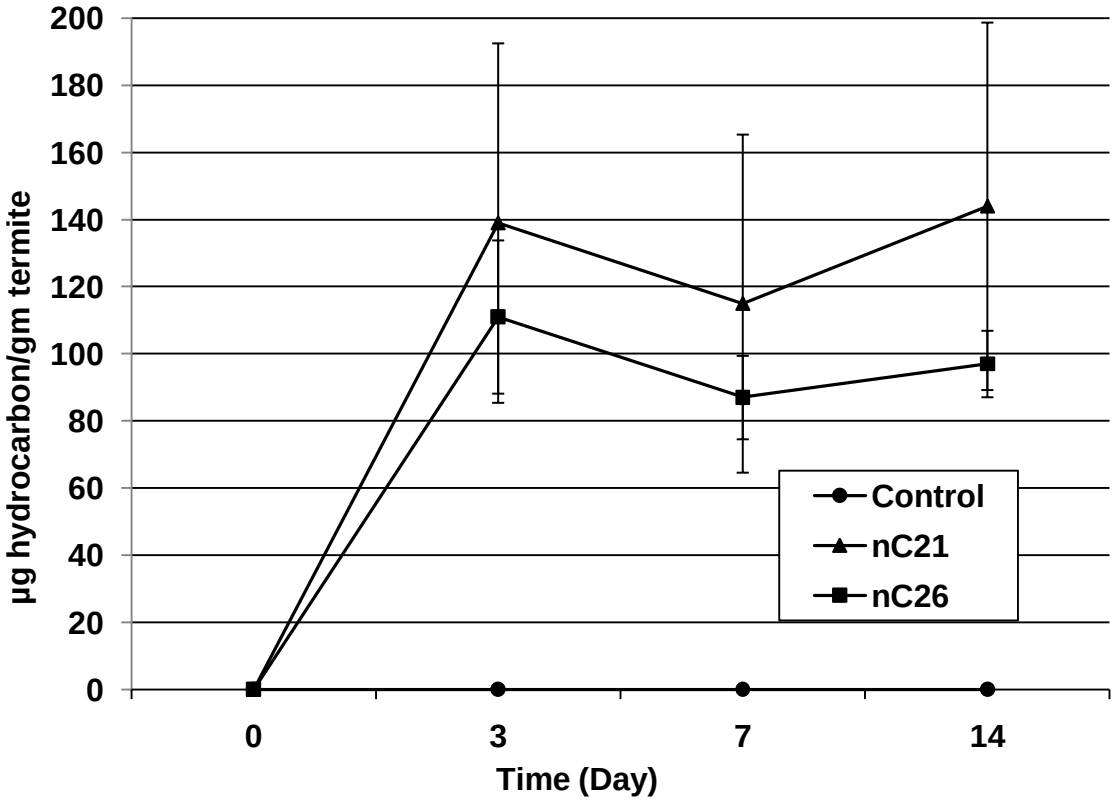

Fig 29. Transfer of nC21 and nC26 through social interactions from treated *Reticulitermes flavipes* to their non-treated nestmates' internal hydrocarbon pool. One hundred fifty termites were fed filter paper dyed and impregnated with 15.4 µmoles of nC21 or nC26 for 2 weeks. After 2 weeks, treated termites were mixed with 150 non-dyed, non-treated termites and fed on non-treated filter paper. Twenty non-treated termites were removed after 3 days, 7 days, and 14 days and internal hydrocarbons analyzed for nC21and nC26 content which is expressed as µg hydrocarbon per gram of termite. Bars represent mean ± standard error of the mean (n=3).

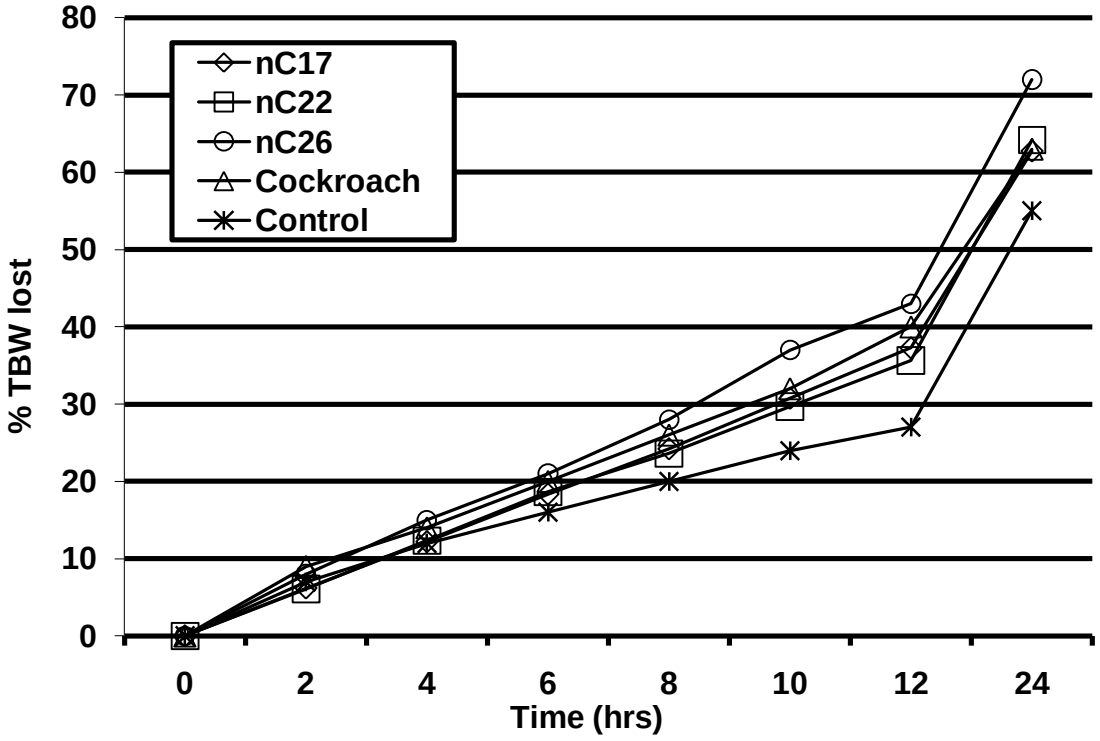

Fig. 30. Effects of dietary hydrocarbons on total body water loss of *Reticulitermes flavipes*. Twenty termites were fed filter paper impregnated with 15.4 µmoles of nC17, nC22, nC26, or a combination of nC25, Z, Z-6, 9-heptacosadiene, and 3-methyl pentacosane (American cockroach extract) for 2 weeks. Following feeding, ten termites were removed and individually weighed to the nearest 0.01 mg. Termites were placed in individual vials in a desiccator at 0% relative humidity and 30˚C. Termites were removed at each time interval and weighed to calculate percent total body water (TBW) loss per hour (n=30).

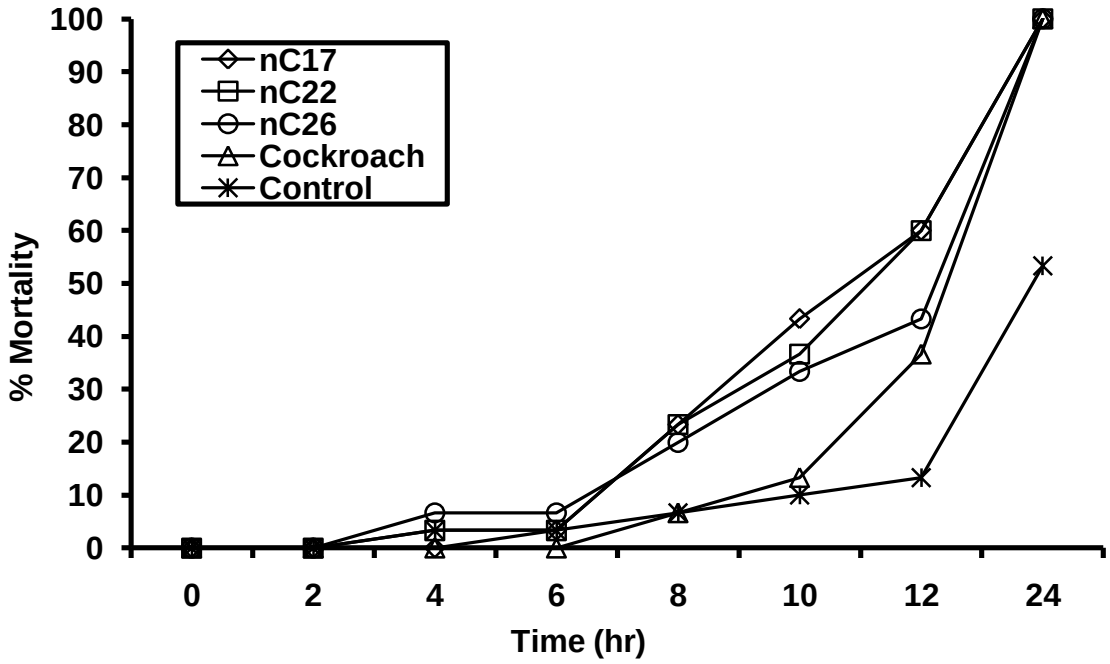

Fig. 31. Effects of dietary hydrocarbons on mortality of *Reticulitermes flavipes* maintained at 0% relative humidity and 30˚C. Twenty termites were fed filter paper impregnated with 15.4 µmoles of nC17, nC22, nC26, or a combination of n-C25, Z, Z-6, 9-heptacosadiene, and 3-methyl pentacosane (American cockroach extract) for 2 weeks. Following feeding, ten termites were placed in individual vials in a desiccator at 0% relative humidity and 30˚C. Termites were observed at each time interval to determine if mortality occurred to calculate % mortality per hour (n=30).

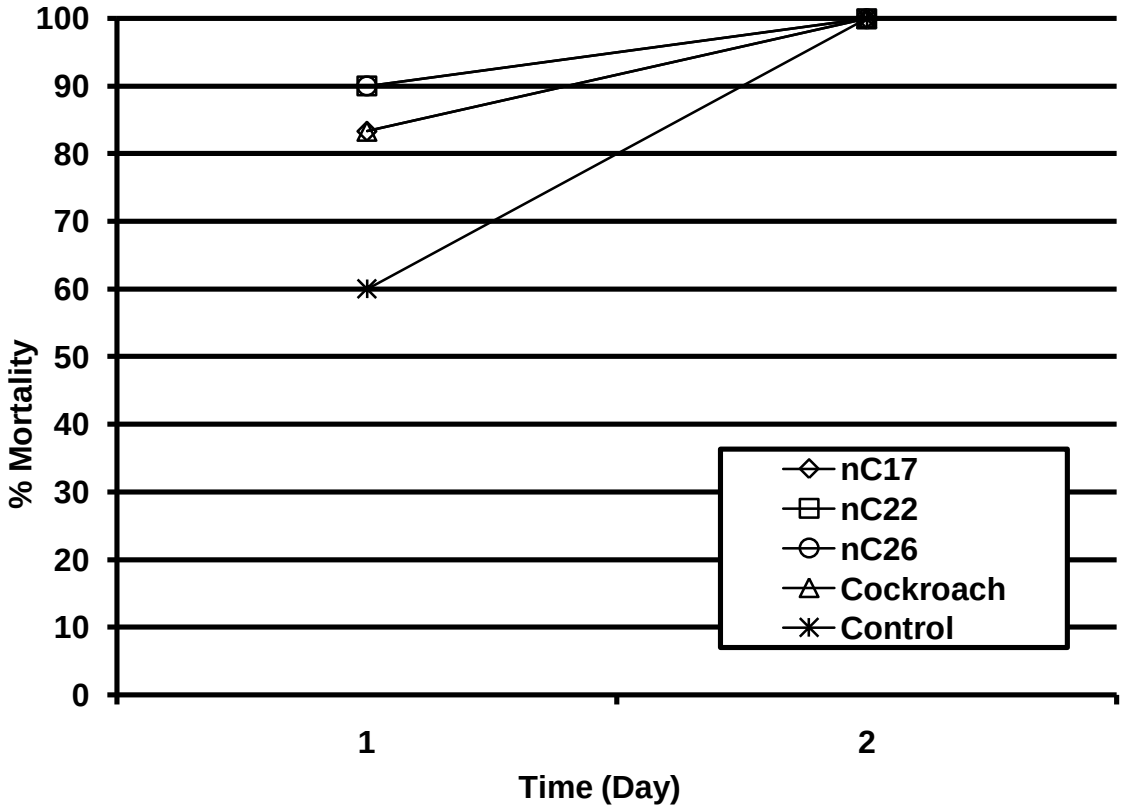

Fig. 32. Effects of dietary hydrocarbons on mortality of *Reticulitermes flavipes* maintained at 0% relative humidity and 25 ± 1˚C. Twenty-five termites were fed filter paper impregnated with15.4 µmoles of nC17, nC22, nC26, or a combination of n-pentacosane, Z, Z-6, 9-heptacosadiene, and 3-methyl pentacosane (American cockroach extract) for 2 weeks. Twenty termites from each treatment were maintained at 0% relative humidity and 25 ± 1˚C and mortality determined every 24 hours to calculate % mortality per day (n=3).

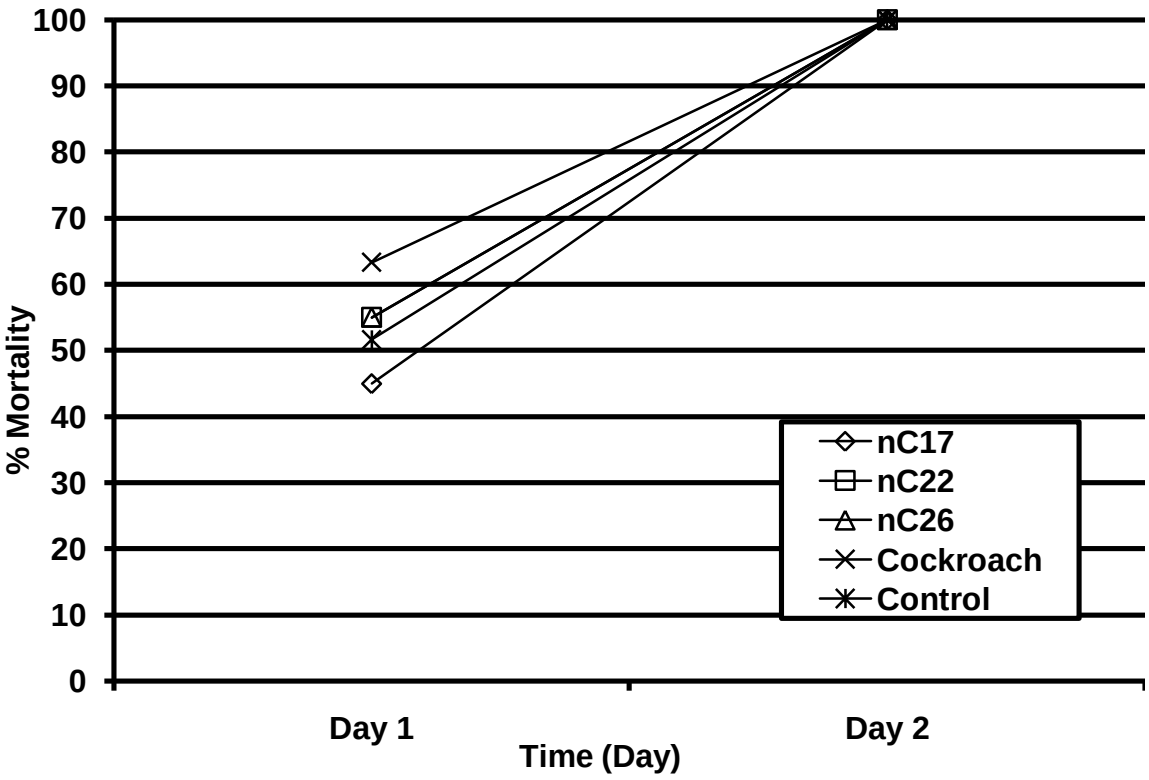

Fig. 35. Effects of dietary hydrocarbons on mortality of *Reticulitermes flavipes* maintained at 25% relative humidity and 25 ± 1˚C. Twenty-five termites were fed filter paper impregnated with 15.4 µmoles of nC17, nC22, nC26, or a combination of n-pentacosane, Z, Z-6, 9-heptacosadiene, and 3-methyl pentacosane (American cockroach extract) for 2 weeks. Twenty termites from each treatment were maintained at 25% relative humidity and 25 ± 1˚C and mortality determined every 24 hours to calculate % mortality per day (n=3).

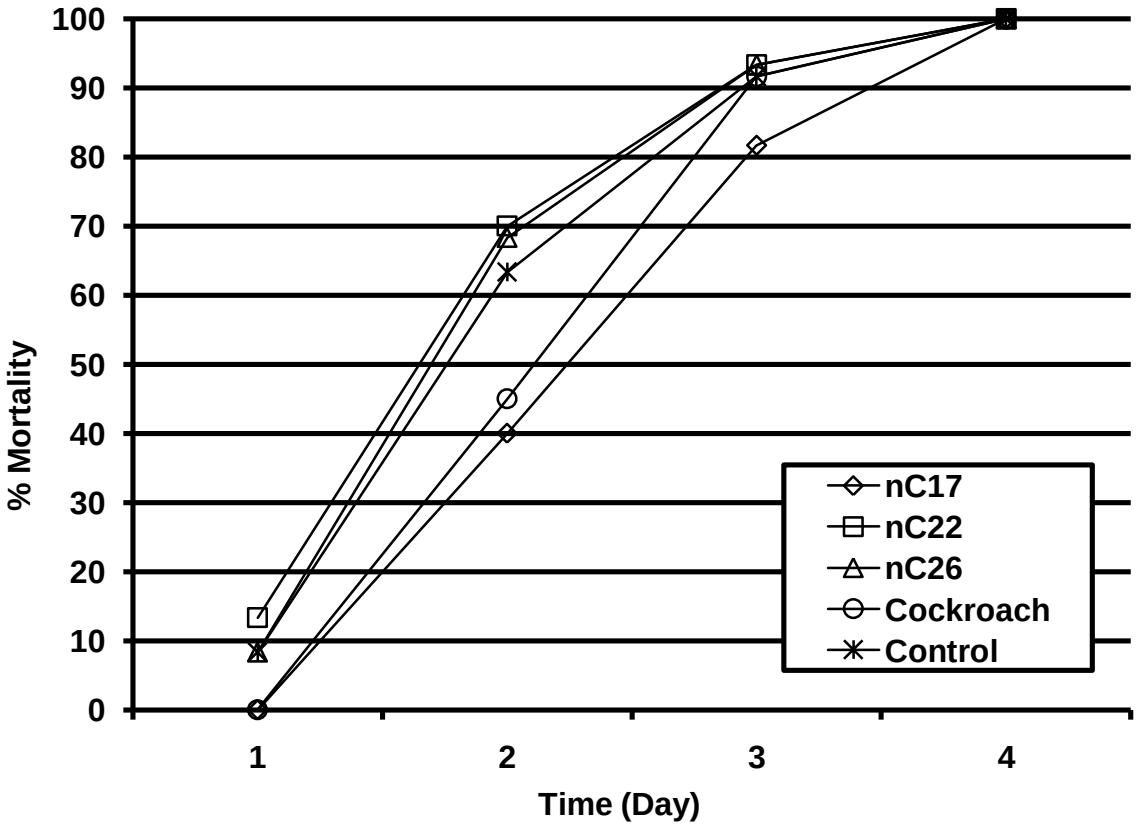

Fig. 36. Effects of dietary hydrocarbons on mortality of *Reticulitermes flavipes* maintained at 55% relative humidity and 25 ± 1˚C. Twenty-five termites were fed filter paper impregnated with 15.4 µmoles of nC17, nC22, nC26, or a combination of n-pentacosane, Z, Z-6, 9-heptacosadiene, and 3-methyl pentacosane (American cockroach extract) for 2 weeks. Twenty termites from each treatment were maintained at 55% relative humidity and 25 ± 1˚C and mortality determined every 24 hours to calculate % mortality per day (n=3).

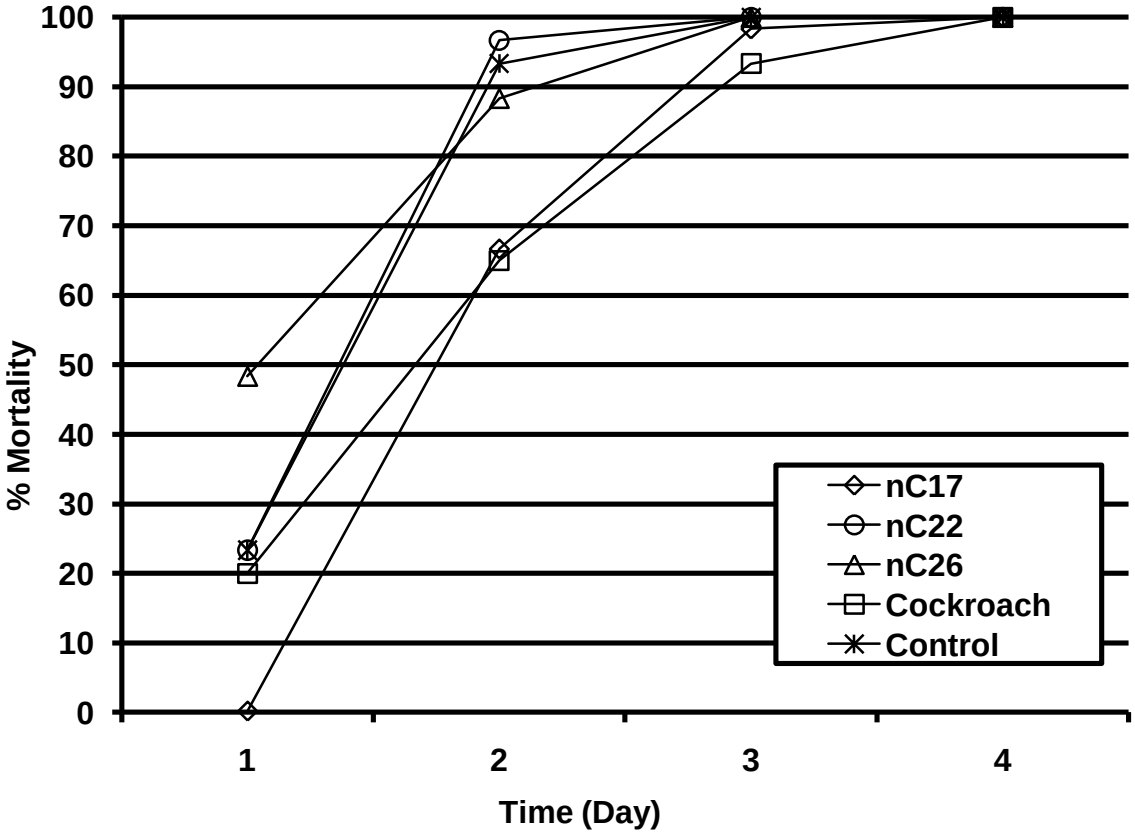

Fig. 37. Effects of dietary hydrocarbons on mortality of *Reticulitermes flavipes* maintained at 75% relative humidity and 25 ± 1˚C. Twenty-five termites were fed filter paper impregnated with 15.4 µmoles of nC17, nC22, nC26, or a combination of n-pentacosane, Z, Z-6, 9-heptacosadiene, and 3-methyl pentacosane (American cockroach extract) for 2 weeks. Twenty termites from each treatment were maintained at 75% relative humidity and 25 ± 1˚C and mortality determined every 24 hours to calculate % mortality per day (n=3).

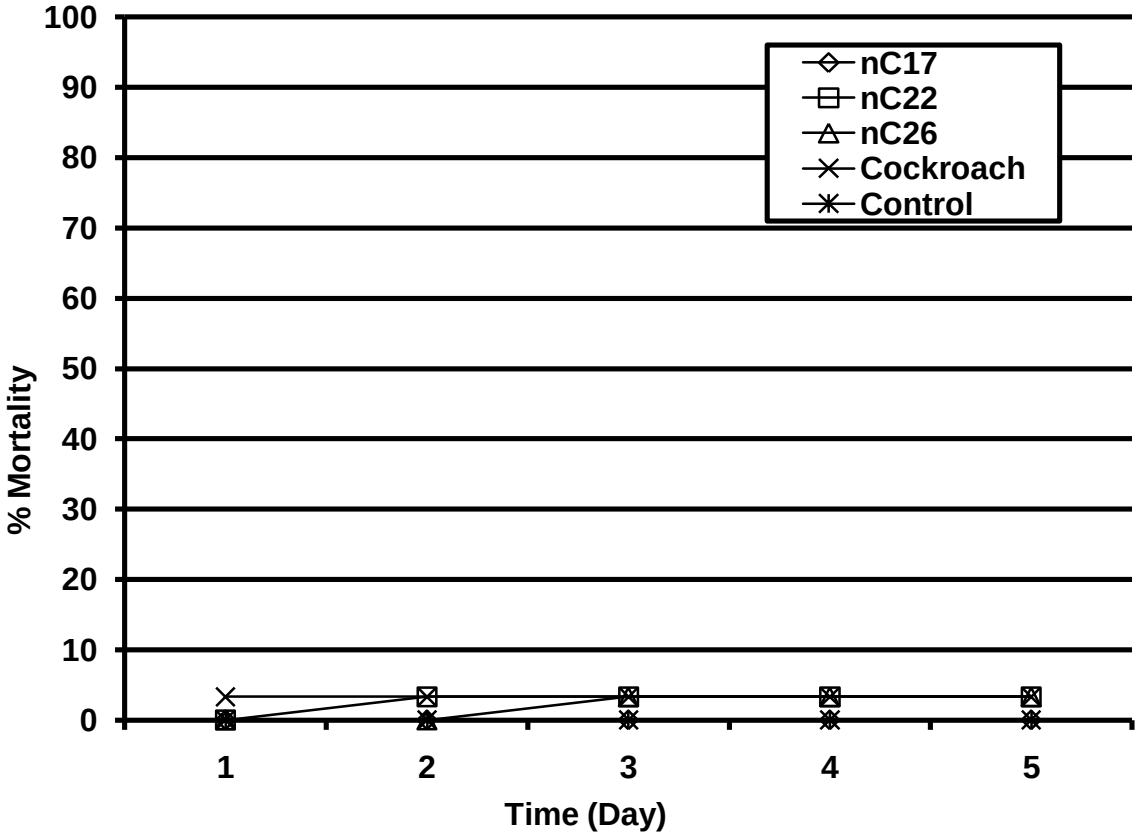

Fig. 38. Effects of dietary hydrocarbons on mortality of *Reticulitermes flavipes* maintained at 100% relative humidity and 25 ± 1˚C. Twenty-five termites were fed filter paper impregnated with 15.4 µmoles of nC17, nC22, nC26, or a combination of n-pentacosane, Z, Z-6, 9-heptacosadiene, and 3-methyl pentacosane (American cockroach extract) for 2 weeks. Twenty termites from each treatment were maintained at 100% relative humidity and 25 ± 1˚C and mortality determined every 24 hours to calculate % mortality per day (n=3).

VITA

Ngan Thanh Thi Nguyen Rawlings

Candidate for the Degree of

Doctor of Philosophy

Dissertation: DIETARY MANIPULATION OF CUTICULAR HYDROCARBONS IN THE SUBTERRANEAN TERMITE, *RETICULITERMES FLAVIPES* (KOLLAR)

Major Field: Entomology

Biographical:

Personal Data: Born in Wichita Falls, Texas on May 8, 1980

Education:

Completed the requirements for the Doctor of Philosophy in Entomology at Oklahoma State University, Stillwater, Oklahoma in December 2010.

Completed the requirements for the Master of Science in Biology at Midwestern State University, Wichita Falls, Texas in May 2005.

Completed the requirements for the Bachelor of Science in Biology at Midwestern State University, Wichita Falls, Texas in May 2002.

Experience: General Zoology teaching assistant as an undergraduate. Lab coordinator (Anatomy and Physiology I & II and Comparative Vertebrate Anatomy) and teaching assistant while working on my master's degree at MSU. Teaching/graduate assistant (Insects and Society and Horticultural Insects) while working on my doctoral degree at OSU. My academic and professional background includes entomology and organismal biology.

Professional Memberships: Entomological Society of America, Beta Beta Beta, Phi Eta Sigma, Alpha Lambda Delta

Name: Ngan Thanh Thi Nguyen Rawlings Date of Degree: December, 2010

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: DIETARY MANIPULATION OF CUTICULAR HYDROCARBONS IN THE SUBTERRANEAN TERMITE, *RETICULITERMES FLAVIPES* (KOLLAR)

Pages in Study: 112 Candidate for the Degree of Doctor of Philosophy

Major Field: Entomology

Scope and Method of Study: The purpose of this study was to examine the effects of dietary hydrocarbon in *Reticulitermes flavipes*. Pure hydrocarbons were added to filter paper and fed to *R. flavipes* for two weeks followed by a chase period. Cuticular and internal hydrocarbons were extracted and isolated. Cuticular and internal hydrocarbons were analyzed using a HP 5890 gas chromatograph. Hydrocarbons were identified using internal standard and Agilent ChemStation software.

Findings and Conclusions: Cuticular and internal hydrocarbon profiles were altered with dietary hydrocarbons. Dietary hydrocarbons that were used included n-alkanes, alkenes, and methyl-branched hydrocarbons and were applied as a single hydrocarbon, a combination of hydrocarbons, or at different concentrations. Dietary hydrocarbons were incorporated into both hydrocarbon pools within the first 24 hours of feeding and were still in the termite system at 63 days. Results show that dietary hydrocarbons can be transferred from treated termites to their non-treated nestmates through their social behaviors. Altered cuticular hydrocarbon profiles affected cuticular water balance in desiccation experiments. Treated termites had a significantly higher total body water loss than the control termites and suffered 100% mortality after 24 hours. Control termites only had 50% mortality after 24 hours in the desiccation experiments. Cuticular permeability (CP) values indicated that adding a short-chain hydrocarbon or alkene and methyl-branched hydrocarbon to *R. flavipes's* diet caused a greater cuticular water loss compared with the control termites in the first 2 hours. Altered cuticular hydrocarbon profiles did not have an effect at a moderate relative humidity (55˚C) but did confirm results from the desiccation experiment where 100% mortality was observed after 24 hours. Further studies need to be conducted to examine how altered cuticular hydrocarbons affect termite behavior and water balance in field conditions, and whether dietary hydrocarbons can be implemented for termite management.

ADVISER'S APPROVAL: Dr. Jack Dillwith