Behaviour of termites: breeding and effects of repellents

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Introduction and Review of Literature

General biology of termite

Termites are social insects that live in colonies, which, in turn, function because the complementary roles played by the different caste. In United States, subterranean termites, Coptotermes spp. and Reticulitermes spp. are the most destructive and cause substantial economic damage to buildings/structures (Su & Scheffrahn 1990). A population of subterranean termites is comprised of distinct colonies. Colonies may occupy underground networks that link several foraging sites (Thorne & Breish 2001).

Termite foraging behavior

The nutritional ecology of termites has played a significant role in shaping their evolution and ecology organization (Thorne, 1999). To understand colony structure and the organization of foraging termites, it has been necessary to map both nest and feeding sites, frequently using behavioral assays of aggression to ascertain the colony affinity of a given nest or group of workers occupying a food source (Long & Thorne 2006). Also, dye indicators and mark release recapture studies have been developed to attempt to census colonies, determine colony boundaries, and plot the distribution of foragers and other spatial aspects of resource use (Su et al. 1984, Forschler & Townsend 1996, Thorne et al. 1996, Abdul Hafiz et al. 2007). Studies of termite foraging range generally offer statistic description of colony nest and satellite sub nest distribution and sometimes reveal the temporal dynamics of foraging range expansion and territorially, similar to what has been described in ants. Colony structure and the spatial

organization of foraging in termites are, nevertheless, poorly understood in comparison to other social insects.

The cryptic nesting and foraging habits of subterranean termites have hindered our understanding of many important features of their population biology, especially the distinctness of colonies and the breeding system within colonies. Their cryptic foraging and nesting habits, many features of subterranean termite biology have been difficult to study by using the traditional methods of field investigation. The main obstacle has been a lack of practical methods for delineating large numbers of colonies (Long & Thorne 2006).

Termite control

Chemical and physical barriers to prevent them from reaching wood construction are two basic methods that have been used to control termites. Currently, insecticides used for termite control can be categorized into three groups, organochlorine, cyclodiens, which are being phased out of use because of potential damage to the environment and human health; pyrethroids, which considered not effective because of their repellency and short residual life in soil; and new termiticides that are considered as non repellent and slow acting termiticide (Potter and Hillary 2003). Remedial control is extremely difficult with acutely toxic chemicals because termites avoid area with dead termites, preventing any further contact with the toxicant. For the Formosan subterranean termites, remedial control using slow-acting toxic baits is are more acceptable method than the use of acutely toxic insecticides. Sub lethal insecticide exposure may be expected to influence insect behavior because most insecticides attack the nervous

system resulting in detection by insects, distruption of physiological processes, and behavioral resistance (Haynes 1988, Silverman and Bieman 1993).

The purpose of applying insecticides to soil proximal to a structure's foundation of a continuous insecticide barrier in soils under and around the structure (Kamble, 1991). This barrier may be created by using either pre or post construction techniques. Post construction insecticide application technique include void treatments, soils trenching, sub slab injections, and soil rodding application. Chemical and physical barriers to prevent them from reaching wood construction are two basic methods that have been used to control termites (Abdul Hafiz et al. 2007). Remedial control is extremely difficult with acutely toxic chemicals because termites avoid area with dead termites, preventing any further contact with the toxicant. Sub lethal insecticide exposure may be expected to influence insect behavior because most insecticides attack the nervous system resulting in detection by insects, disruptions of physiological processes, and behavioral resistance (Haynes & Baker 1988, Silverman & Bieman 1993)

Non repellent termiticide

Termiticide that do not repel termites from penetrating into treated soil but rather successfully kill them were recently registered in the United States (Kard 2003) and other parts of the world. They have become popular alternatives to conventional repellent soil-poisoning agents (Shelton and Grace 2003). Nonrepellent termiticides have been shown to be effective for the elimination of termite infestations when applied around the exterior perimeter of the building (Potter & Hillary 2001)

New generations of termiticides which contain imidacloprid (Premise®), fipronil (Termidor®) and indoxacarb, chlorfenapyr (Phantom®), Chlorantraniliprole are used as soil-applied or by direct injection in the colony, posed lethal effects to subterranean termite (Osbrink & Lax 2003; Kamble & Davis, 2005; Hu et al., 2005; Spomer et al., 2009). The advantages of these new termiticides are slow-acting and non-repellent. Thus, termites cannot detect these termiticides when applied in to the soil or when used on filter paper in the laboratory. The transfer effect of imidacloprid, fipronil and indoxacarb among termite workers was studied by researchers and results showed that the termites were able to transfer the termiticides from the treated termites (donors) to the untreated termites (recipients) (Kard 2003, Thorne and Berisch 2001, Vargo & Parman 2004, Tomalski and Vargo 2005, Hu et al. 2005, Shelton & Grace 2006; Spomer et al., 2009).

Termite baiting

Baiting for suppression or elimination of subterranean termites is not a new idea. The ultimate goal of termite baits is to eliminate termites from structure (Su 1994, Su et al. 2000).

Recently, hexaflumuron baits have successfully been applied to control the field colonies of Coptotermes spp. and Reticulitermes spp. (Su 1994, Su et al. 2000). At the same time Nouviflumuron also have been widely used in termite baiting. In laboratory trials in which R. flavipes were fed radiolabeled noviflumuron or hexaflumuron, noviflumuron demonstrated significantly faster speed of action, greater potency, and nearly 4-fold slower clearance from termites compared with that of hexaflumuron (Sheets et al. 2000; Karr et al. 2004, Spomer & Kamble, 2006). Primer pheromones (Wilson & Bossert,

1963) are chemical messengers that can be passed among individuals, thereby triggering a physiological response in a recipient. In termites, JH is one such primer pheromone (Henderson 1996); it includes presoldiers differentiation from workers at excessively high titer. The differentiation of the soldier caste, a development end point, in respond to JH is indirect in maintaining immature features in all other non social insects (Henderson, 1996).

In the last decade there has been a dramatic increase in the research on and the development of bait systems for termite management. The increasing availability of bait systems for the control of active termite infestations is already significantly affecting termite management practices in many part of the world. There is even discussion of using such system as standalone measures for the long- term protection of a structure (Su et al. 2001; Grace et al. 1996). Even though these important and remarkable trends, baiting is developing technology, and many more changes to existing bait technology can be expected (Potter 1997). The bait matrices containing hexaflumuron, a chitin synthesis inhibitor, were most effective in reducing or eliminating foraging populations of Coptotermes formasanus Shiraki and Reticulitermes flavipes Kollar (Su & Scheffrahn, 1996, Su, 1994).

Molecular genetic marker

The application of molecular genetic markers to the field populations of subterranean termite provides a powerful way to discriminate among large numbers of colonies be identified and distinguished from each other. The application of molecular genetic marker to field populations of subterranean termites provides a powerful way to discriminate among large numbers of

conspecific colonies in a population as well as to determine colony breeding structure (Husseneder et. al. 2003)

Genetic markers provide the most powerful means for delineating the boundaries of colonies and for determining colony affiliation for groups of foraging workers (Thorne et al. 1999, Vargo 2003a, b). Microsatellite markers, with their co-dominant nature and high variability, are especially useful for investigation of colony distinctness (Vargo 2003b).

Genetic markers are also the most practical way to determine colony breeding structure (Thorne et al. 1999, Ross 2001), and there have been a growing number of genetic studies of breeding systems in termites (Luykx 1993, Husseneder et al. 1997, 1999, Husseneder & Grace 2001a, 2001b, Vargo et al. 2003), including some on Reticulitermes spp. (Cle´ment 1986, Reilly 1987, Jenkins et al. 2002, Bulmer et al. 2001, Vargo 2003b). The most detailed studies to date of the eastern subterranean termite, R. flavipes, are those of Reilly (1987) using allozymes, Bulmer et al. (2001) using allozymes and mitochondrial DNA haplotype data, and Vargo (2003a, b) using microsatellites and mitochondrial DNA sequence data. These studies have revealed variation in colony social organization in R. flavipes from very highly inbred colonies in Tennessee (Reilly 1987), to a mixture of approximately one third simple families and two thirds inbred colonies headed by many neotenic reproductives in Massachusetts (Bulmer et al. 2001), to three fourths simple families and one fourth inbred families with only a few neotenics in North Carolina (Vargo 2003b). The above studies were conducted in natural areas, and there are no comparable studies performed to date in urban areas around buildings. Colony social

organization in R. flavipes may vary in response to local ecological conditions (Bulmer et al. 2001), raising the possibility that for a given geographic area, colonies in urban habitats may differ in their social organization from those in natural habitats.

In a study of Reticulitermes Hesperus Banks in southern California, Haagsma and Rust (1995) found differences in colony size, foraging activity, and body weight between colonies in natural and urban habitats. In addition to providing a powerful way to determine colony distinctness and to infer colony breeding structure in subterranean termites, molecular genetic markers are useful for applied studies, such as tracking colonies over time after exposure to an insecticide treatment and determining whether termites that reappear after treatment are part of the originally treated colony or are from a neighboring untreated colony that has moved into the area (Husseneder et al. 2003). Such studies are critical in the evaluation of management practices that target septic colonies and aim to eliminate them or greatly suppress their populations.

Genetic/breeding structure population

Colony fusion

For social organisms, genetic structure assumes additional significant not only for the evaluation of social behavior, but also for the evolution of multiple queen societies, reproductive skew, sex ratio conflict, conflict over growth versus reproduction. Colony fusion has long been a suspected mechanism generating unusual colony genetic structure in termites. Colony genetic structure, reported a lack of genetic differentiation between colonies of Reticulitermes grasseiacross some regions of France and Spain. Together

with this genetic data, a lack of distinct nest mate discrimination in laboratory trials (Clement 1986) suggested that colony fusion was widespread in some populations. Although recent studies have failed to corroborate these earlier descriptions of widespread colony fusion in this species (DeHeer et al. 2005), Clements work raised considerable awareness about colony fusion for those working on other subterranean termites. In introduced populations of Coptotermes formosanus, inter-colony aggression is often found to be weak or variable (Husseneder and Grace 2001a, Cornelius & Osbrink, 2003) and one mark-release-recapture study described patterns consistent with a colony fusion event (Su and Scheffrahn, 1988). Nevertheless, genetic evidence for colony fusion in this species has remained elusive in spite of the relatively large numbers of colonies which have been assayed (Vargo et al., 2003, 2006; Husseneder et al., 2005).

For Reticulitermes flavipes many experimental results have suggested the possibility that colony boundaries were porous. Laboratory agonism studies (Grace 1996, Polizzi and Forschler 1998, 1999, Bulmer and Traniello 2002, Fisher and Gold, 2003) and field surveys of molecular diversity (Jenkins et al. 2002, Bulmer et al. 2001) both suggested that individuals originating from different colonies may not distinguish between nestmates and non-nestmates, and consequently share the same nests or tunnel systems. However, in contrast to other species of subterranean termites these findings in R. flavipes have been confirmed via more direct assessments of colony fusion (Fisher et al. 2004), including one molecular study that provided a time course of colony genotypes before and after merger (DeHeer

and Vargo 2004). Such fused colonies also appear to occur in other groups of termites.

The relatedness hypothesis indicates that fused colonies are preferentially formed between groups of related termites because any costs associated with fusion are reduced when relatedness remains high in these colonies. This argument necessarily assumes that such colonies experience some general benefits to increasing their size (Costa and Ross, 2003). The genetic diversity hypothesis posits that increased genetic diversity provides sufficient group-level benefits to offset the costs of decreased relatedness within colonies. The specific benefits of increased genetic diversity could include the same types of benefits hypothesized to occur elsewhere (Schmid-Hempel and Crozier, 1999), in addition to the potential benefit that could result from a reduction in inbreeding.

Simple family & extend family

Subterranean termite colony such as Coptotermes formosanus, generally begin as simple families headed by two primary (alate derived) reproductives that pair after mating (Tamashiro et al. 1987, Raina et al. 2003). As time goes by, the primary king and/or queen will be replaced by varying numbers of neotenics (non alate derived reproductives) from within colony; where this replacement leads to extended families with different degrees of inbreeding depending on the number of reproductives and number of generation of inbreeding (Thorne et. al 1999, Bulmer et al. 2001, Vargo et al. 2006, Husseneder et al. 2007)

Bioinformatics/ Computational Molecular Biology

Biological database is a large, organized body of persistent data, usually associated with computerized software designed to update, query, and retrieve components of the data stored within the system. A simple database can be a single file containing many records, each of which includes the same set of information (Lewis et al. 1995). For example, a record associated with a nucleotide sequence database typically contains information such as contact name, the input sequence with a description of the type of molecule, the scientific name of the source organism from which it was isolated, and often, literature citations associated with the sequence.

The term of " Bioinformatics" meaning the application of information technology to the field of molecular biology. Bioinformatics currently contains the creation and advancement of databases, algorithms, computational and statistical techniques, and theory to solve formal and practical problems arising from the management and analysis of biological data (Hogeweg1980). Over the past few decades rapid developments in genomic and other molecular research technologies and developments in information technologies have combined to produce a tremendous amount of information related to molecular biology. It is the name given to these mathematical and computing approaches used to glean understanding of biological processes. Common activities in bioinformatics include mapping and analyzing DNA and protein sequences, aligning different DNA and protein sequences to compare them and creating and viewing 3-D models of protein structures (Huang 2004)

Bioinformatics focus on developing and applying computationally intensive techniques (data mining, machine learning algorithms, and visualization) to achieve this goal. Major research efforts in the field include sequence alignment, gene finding, genome assembly, protein structure alignment, protein structure prediction, prediction of gene expression and protein-protein interactions, genome-wide association studies and the modeling of evolution (Huang 2004).

In the application of molecular ecology studies of termites, mitochondrial genes were used for taxonomy purpose in termites (Cameron & Whitting, 2007). Thus, the whole mitochondrial genome can be used to predict useful regions for further investigation in the multiple alignments methods application. Multiple alignments are the key starting point for prediction of protein secondary structure, residue accessibility, and the identification of residues important for specificity. Multiple alignments can provide the basis for the most sensitive sequence searching algorithms (Barton & Sternberg, 1990). Furthermore, methods in multiple alignments affective analysis of a well-constructed multiple alignments can provide important clues about which residues in the protein are important for stabilizing the second and tertiary structure of the protein (Altschul et al. 1997)

ClustalW will calculates the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen and will provide a better quality for sequences (Thomson et al. 1994). Meanwhile, T-Cofee application is better to overcome with some of the problems that standard hierarchical method s have in aligning sequences of very different length or that share only local region similarity (Notredame et al. 2000). The

program works by building a library of pairwise alignments for the sequence interest. Furthermore, T coffee has been adapted to include structural alignment and alignments from threading algorithms since it can work from pairwise alignments that originate from any source (Notredame et al. 2000). In addition, the program PSI-BLAST searches database with a single sequence, any high-scoring sequences that are found are built into a multiple alignment, and this multiple alignment is then used to derive a search " profile" for subsequent search of the database. This process is repeated until no new sequences are added to profile, or a specific number of iteration have been performed (Jones 1999).

Research Objectives

1. To test non-repellent termiticide model assay for their ability to induce detectable molecular changes in worker termites e. g., hemolymph proteins & their corresponding genes.
2. To infer the colony social organization of R. flavipes in urban habitat based on colony and population genetic structure and to compare this social organization in nearby natural habitat,
3. To track the foraging locations of a large number of Reticulitermes colonies from natural forest (Wilderness Park) over the course of three field seasons using previously developed microsatellites by Vargo (2000).
4. To analyze colony and population genetic structure/breeding system in natural and urban regions in order to determine the prevalence of neotenic reproduction and improve our understanding of the factors facilitating the spread of subterranean termites to new urban areas

5. To determine the termite colony either a simple colony or extended colony using molecular ecology methods and also to determine if the colony undergo any kind of colony fusion
6. To delineate the genetic structure of swarm aggregations of alates in order to infer potential mechanisms leading to inbreeding avoidance that do not require kin recognition.
7. To utilize bioinformatic/molecular computational biology application tools to analyzing data at molecular level using BLAST, Clustal X, TCofee for sequencing analysis, pairwise/multiple alignment to study colony breeding structure of R. flavipes

MATERIALS AND METHODS

Termite Collection & Sampling

1. Reticulitermes flavipes, the Eastern subterranean termite, will be collected from locations within Wilderness Park Recreational Area, Lincoln; NE. Twenty workers will be sampled in each collection point located every 20 m along two intersection transects.
2. The termites will be maintained on moistened corrugated cardboard and only 3-5th instar worker termites will be used for the study.

Detection of fused colonies

1. In order to detect colonies that had undergone fusion, collections of termites from large numbers of established colonies of R. flavipes will be collected between May 2009 and October 2011.
2. At each location, minimum of 20 adult workers in 95%ethanol from multiple feeding sites, and mapped the locations of these feeding sites relative to one another using compass and measuring tape (DeHeer

and Vargo, 2004). Samples will be stored in alcohol at 480C until DNAisolations could be performed.

Microsatellite genotyping (Fusion & Inbreeding)

1. Using the Puregene DNA purification kit (Gentra systems, Minnepolis MN, www. gentra. com), genomic DNA will be isolated from each of 20 whole worker bodies from each of the feeding sites.
2. At the location, minimum of 20 adult workers in 95% ethanol from multiple feeding sites will be collected, and mapped the locations of these feeding sites, and the locations of these feeding sites will be mapped relative to one another using compass and measuring tape.
3. Each genotype individual termites at six different microsatellite loci : Rf1-3, Rf21-1 and Rf24-2 from Vargo (2000) and Rs15, Rs76, and Rs78 from Dronnet et al. (2004).
4. PCR reactions will be setup in 96oC. Well plates in 5ul reaction mixtures containing 1X PCR buffer (Eppendorf), 2mM MgCl2, 0. 2 mM of each dNTP, 0. 2 U Taq polymerase (Eppendorf), 0. 4 pmol of forward primer. Forward primers will be labeled with IRD 700 fluorescent dye (LI-COR), and samples were run on Li-Cor 4200L automated sequencer.
5. Gel image will scored manually on Adobe Photoshop 7. 0 by comparison to a size ladder.

Colony affiliations

To determine colony affiliation of the collection points, methods of other studies will be followed.

1. Testing all pairs of collection points within each species for genotypic differentiation by means of a permutation test by using the program FSTAT (Goudet 2001).
2. Pairs of collection points that will be significantly differentiated will be grouped into the same colony

Morphology

1. Scanning electron microscopic pictures (coronal and profile views) will made of the heads for each species) and will be studied using a binocular microscope to evaluate the profile of the post clypeus.
2. Colour of the tibia of reproductives will be noted because this feature is an informative morphological indicator in association with the postclypeus profile.

Genetic Data Analysis/ Simple or Extend Colony (Breeding Structure)

1. The collection points will be analyzed to determine belonged to the same colony.
2. The genotypic frequencies between all pairs of collection points by means of a log-likehood (G) based test of differentiation using the program GENEPOP on the Web (Raymond & Rousset 1995; http://genepop. curtin. edu. au/). The overall significance will be determined via Fisher’s combined probability test.
3. G-test analysis will be performed with collection points over the large scale with multiple collection points within the extensively sampled sites and finally among the colonies from each population to verify genetic differences will be used.

4. Colonies will be classified as simple families when workers had genotypes consistent with being the direct offspring of one pair of reproductives and when the observed frequencies of the genotypes did not differ significantly from those expected under Mendelian segregation of alleles from two parents. Significance will be determines by a G-twst (P <0. 05).
5. Colonies will be classified as extended families when the genotype distributions within colonies were not consistent with being produced by a single pair of reproductives (e. g more than four genotypes at a locus or three or more homozygote genotypes) or genotypes frequencies deviated significantly from those expected in simple families.
6. Genetic relatedness among workers will be estimated for each colony and averaged over colonies for the same site will be using the computer program RELATEDNESS 5. 00 (Queller & Goodnight 1989)
7. Inbreeding-adjusted estimate of relatedness r* that better reflects the number of reproductive presents in each colony by using Pamilo (1985) formula R*= r- [2FIT /(1+FIT)]/ [1-2FIT/(1+FIT)] I, C, T representing different colonies
8. The breeding system and genetic differentiation among colonies will be investigated with hierarchial F – statistics, assuming the infinite allele model and with individuals nested in colonies.
9. The hierarchical analysis will be performed with F-stat program. It will be investigated whether there will be isolation by distance (a positive correlation between genetic differentiation and geographical distance) by calculating Fst between pairs of collection points within large

colonies using the programe FSTAT 2. 9. 3. 2 and by testing the significance of correlation between FST/(1-FST) and logarithm of geographical distances with MANTEL test (Mantel 1967). Computer program GENEPOP, with 10 000 permutations and the Mantel correlation coefficient r can be obtained with MANTEL (http://life. bio. sunysb. edu/morph/soft-mult. html)

Estimating Colony Foraging Area

1. Termite collected at a sampling point will considered to be affliated with a given colony if Fct, which measured genetic differentiation between collection points, will not significantly different from zero (i. e., 95% confidence interval did not overlap with zero.
2. Fct will be measured between all possible combinations of collection point’s pair at each site.
3. Variation in mitochondrial DNA markers will be provided additional support for colony designation (Bulmer et al. 2001). The maximum linear distance between collection points affiliated with the same colony will be used to estimate foraging range.
4. For colonies that will be composed of three or more collection points (n+4), will be estimated the area encompassed by non intersecting lines drawn between collection points and adjusted so it did not overlap with neighboring collection points affiliated with different colonies.

Swarming trap

The swarm aggregation of alates will be collected alive with light traps. The traps consisted of buckets with a battery operated black light attached to attract the alates.

Flight Observation

Sticky trap will be used to monitor the dispersal flights of these species, which, in hindsight, may help explain some of the differences in how they are genetically structured. In addition their emergence and landing location.

Sex ratio

Sex ratio in swarm aggregations were tested for significant deviation from 1: 1 using Binomial tests. Swarm aggregations were tested for significant genotypic differentiation with log likehood G Statistics using FSTAT 2. 9. 3. 2

Bioassay

Genetic mutation/change after being exposed to non repellent termiticide

1. The concentrations were 0. 001, 1. 00, 10, 100 and 1000 ppm for fipronil, imidacloprid and indoxacarb. In addition, distilled water was used as control for each termiticide.
2. The method used for treatment was described by Hu (2005). Five kilograms of soil were collected from the site of the subterranean termite C. gestroi colony.

The soil was then oven-dried. One kilogram for each termiticide was sieved and subsequently divided into six parts. The soil was placed in 100g increments into self-sealing plastic bags for treatment. Ten milliliters of the five concentrations of, fipronil, imidacloprid and indoxacarb will be added to

the 100 g bag of soil for testing. The bags were sealed and allowed to mix thoroughly.

The soil will be removed from the bags and placed in plastic bags (12 by 115 cm). The termiticide-treated soil was air-dried in a hood for ten days to allow solvents in the formulation to evaporate. Treatments were replicated three times using new stock solutions for each replicate.

Bioassay design:

1. Bioassay tubes designed by Su et al. (1995) will be used to investigate termite penetration in various thicknesses of termiticide-treated soil (Figure1).
2. At time of testing, dried treated soil will be removed from the hood, placed into plastic bags and 10 ml of water was pipetted into the bags to obtain 10% moisture content. Three (3 cm) wooden applicator sticks were placed at the bottom of the glass test tube (2 by 15 cm), and 7% non-nutrient agar was inserted into the test tube until it rested on the wooden sticks.
3. Oven-dried and sieved soil will be placed into plastic bags and moistened to concentrations of 10% (10ml /100g) to serve as untreated sand in the test designs. A 0. 0, 1. 0, 3. 0, 5. 0, or 10cm layer of termiticide-treated soil was centered between two layers of untreated soil (Figure. 1). A glass funnel will be used to place the soil in the test tube and a scintillation vial (10ml) attached to the shank of a screwdriver was used to lightly and level the layers of soil.

4. A 5 cm segment of termiticides-treated soil and a 5 cm segment of untreated soil served as control. Additionally, layers of untreated soil at various thicknesses in the bioassay tube will be served as control.
5. The soil segment will be capped with a 1. 0 cm core of agar and a 4cm by 4 cm portion of paper towel folded placed on the top of the tube. Eighty C. gestroi workers and five soldiers (20: 1) were placed in each test tube to simulate normal termite activity and prevent overcrowding. A piece of aluminum foil will be placed over the top and end of each tube, and will be covered with black plastic container to allow for darkness and prevent desiccation of the termites.
6. Bioassay tubes will be kept at 29±1°C. The experiment was a 6 (concentrations) x 3 (replicates) x 5 (soil thickness) factorial design with concentration and thickness as the main effects. Test tubes will be held vertically in test tube racks with termites at the top of the tube.
7. Observations will be made daily up to one week for the number of worker termite mortality, vertical distance of treated soil penetrated by termites and percentage of penetration into treated soil.
8. The percentage of termite mortality and soil penetration data were subjected to arc-sine transformation before analysis of variance and means were separated with Tukey HSD. Then percentage of penetration into treated soil and the percentage of mortality were analyzed with analysis of variances (ANOVA). Also the percentage of surviving termites located at the bottom of the bioassay unit was analyzed.
9. DNA will be isolated from dead termite to b