BIOLOGY AND BEHAVIOR OF OKLAHOMA SUBTERRANEAN TERMITES (ISOPTERA: RHINOTERMITIDAE)

By

KENNETH S. BROWN

Bachelor of Science
Missouri Southern State College
Joplin, Missouri
2000

Master of Science
Oklahoma State University
Stillwater, Oklahoma
2002

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 2005

BIOLOGY AND BEHAVIOR OF OKLAHOMA

SUBTERRANEAN TERMITES

(ISOPTERA: RHINOTERMITIDAE)

Thesis Approved:

Bradford M. Kard
Thesis Advisor

Jack W. Dillwith

Phillip G. Mulder Jr.

Jim T. Criswell

Mark E. Payton

A. Gordon Emslie
Dean of the Graduate College

PREFACE

This dissertation is organized into six chapters. Chapter I is a general introduction including an introduction to three research projects, the main objectives of each, and an explanation of the dissertation format. Chapter II is a literature review focusing on taxonomy and ecology of subterranean termites, with special attention to the genus *Reticulitermes* (Isoptera: Rhinotermitidae). Chapters III, IV, and V are formal manuscripts of the research conducted during my Ph.D. program and are written in compliance with the publication policies and guidelines of the specific journals to which each will be submitted. Chapter VI is a general summary and concluding remarks.

I have gained much since I moved to Stillwater five years ago to begin graduate school at OSU, and have many people to thank. This campus is where I met my wife Katie. We shared a class together in Willard Hall and held our wedding reception in the same building on June 16th 2001. Katie gave birth to our son Reece at Stillwater Medical Center on August 7th 2003. I have also gained the friendship of numerous fellow graduate students as well as departmental faculty and staff members. I thank the Lord for bringing me to Stillwater and blessing me greatly during my time here. I would like to express my sincere appreciation to Dr. Brad Kard for his guidance and friendship while serving as my major professor for both of my graduate degrees. I would also like to thank Drs. Jim Criswell, Phil Mulder, Jack Dillwith, and Mark Payton for their valuable advice and assistance while serving as members of my committee. Finally, I would like to thank my family for their love and support.

TABLE OF CONTENTS

Chapter **Page**

LIST OF TABLES

Table **Page**

CHAPTER III

CHAPTER IV

CHAPTER V

LIST OF FIGURES

Figure **Page**

CHAPTER III

CHAPTER IV

CHAPTER V

CHAPTER I. GENERAL INTRODUCTION

Termites are the most important structural pest in the United States and are capable of causing extensive damage to houses and other wooden structures. Costs for control, prevention, and repair of termite damage can reach $2 billion annually. The distribution of termite infestation in the state of Oklahoma is categorized as moderate to heavy, with the extreme southeastern corner of the state considered very heavy (Jones 2000; Suiter et al. 2002). Despite the economic threat these structural pests pose to homeowners and business owners within the state, only limited information on the biology, behavior, and ecology of the subterranean termites of Oklahoma can be found.

The study of subterranean termites is difficult due to their cryptic lifestyle. Understanding the nature of subterranean termite colonies may yield clues that can be used for improved management and control strategies. Characterizing subterranean colonies includes such parameters as foraging territory delineation, caste ratio determination, and foraging population estimation. Such characterizations have been completed for colonies in urban, wildland, and even desert landscapes, with drastically contrasting results. Results of colony characterization studies have been used to evaluate the efficacy of baits and baiting systems, to develop a more complete understanding of the expanse of the gallery systems and earthen nests that comprise a termite colony, and in understanding the role subterranean termites play in natural systems. To date, no such characterization has been done on termites in undisturbed native tall-grass prairie habitat.

Subterranean termites utilize a combination of soil and frass to construct the earthen nests and shelter tubes in which they live. Termites are known to transport soil long distances to exploit a food source. Termites are also known to move throughout the

soil strata and therefore play a major role in soil turnover and nutrient cycling. However, the degree to which termites are capable of moving soil is not well understood.

A majority of the termites occurring in Oklahoma are *Reticulitermes* species. Reliable identification of species within this genus is problematic. Castes used for identification are not always common within the colony. For this reason, identification methods other than soldier and alate morphology are desirable. Over the past twenty-five years, cuticular hydrocarbons have been evaluated as an additionaltool for identification. Rather than simplifying species identification; however, results of recent hydrocarbon studies have indicated that the taxonomy of the genus *Reticulitermes* may be more complex than previously thought. It is possible that variations in methods of collection and preservation of specimens used for cuticular hydrocarbon analyses in these studies could account for some of the variations in the resulting hydrocarbon profiles. Further investigations into cuticular hydrocarbon preparation methodologies for chemotaxonomic identification of species within the genus *Reticulitermes* are warranted.

The overall objectives of this research are to elucidate the biology and behavior of Oklahoma subterranean termites, including colony characteristics, soil movement capabilities, and effects of specimen preparation methodologies on the accuracy of cuticular hydrocarbon analyses.

Objectives

I. Characterize colonies of indigenous Oklahoma termites on a native tall-grass prairie, including foraging population estimates, foraging territory delineations, and caste ratio determinations.

II. Evaluate the soil movement capabilities of *Reticulitermes flavipes* (Kollar).

III. Investigate variables affecting cuticular hydrocarbon profiles in termites (Isoptera: Rhinotermitidae).

Explanation of Dissertation Format

The general introduction is followed by a literature review (Chapter II). Chapters III, IV, and V are devoted to individual papers to be published. Chapter VI is a general summary, followed by appendices. References are provided for citations in the literature review and papers to be published. The first paper (Chapter III) is a characterization of three colonies of subterranean termites in native tall-grass prairie habitat. This includes caste ratio determinations, foraging territory delineations, and foraging population estimates. The second paper (Chapter IV) evaluates the soil movement capabilities of *Reticulitermes flavipes*, the most commonly occurring termite in Oklahoma. Chapters III and IV follow the general publication policies and guidelines for submission to Environmental Entomology. In the final paper (Chapter V), variables such as collection technique and preservation method are evaluated for possible influences on resulting cuticular hydrocarbon profiles of *Reticulitermes flavipes*. Chapter V follows the general publication policies and guidelines for submission to the Journal of Chemical Ecology

CHAPTER II. LITERATURE REVIEW

Background

General

Termites are social insects that function as primary decomposers of wood and cellulose materials in many of areas of the United States (U.S.) and throughout the world. Despite their essential role in the detritus cycle, termites are most often recognized for damage caused to wooden structures. Estimates of annual costs for prevention, control, and repair of termite damage in the United States range from $1-to-11 billion (Potter 1997; Jones 2000; Su 2002).

It is generally accepted that seven families exist within the order Isoptera: Mastotermitidae (primitive), Serritermitidae, Kalotermitidae, Hodotermitidae, Termopsidae, Rhinotermitidae, and Termitidae (higher termites) (Kambhampati and Eggleton 2000). Termites are also commonly classified as subterranean, drywood, or dampwood based on their ecology and preferred habitats. Such terms; however, do not always correspond to scientific classification, as termites within the same family may prefer different habitats. For example, most species in the Rhinotermitidae are considered subterranean, however, one species in this family, *Prorhinotermes simplex* (Hagen), is considered a dampwood termite.

Five termite families (Kalotermitidae, Hodotermitidae, Termopsidae, Rhinotermitidae, and Termitidae) are found in the United States (Gleason and Koehler 1980). Over a third of the fifty species of termites found in the U.S. are structural pests (Thorne 1998). However, nearly ninety percent of damage and control costs can be attributed to a few species of subterranean termites within the genus *Reticulitermes*, and the species *Coptotermes formosanus* Shiraki (Haverty et al. 1999b).

Control - Chemical

For the past fifty years, termite control has relied primarily on the use of broad-spectrum chemical insecticides. Chlorinated hydrocarbons, which had long lasting residual effects, were used until the mid 1980's when they were replaced by organophosphates and pyrethroids. Recently, boric acid was evaluated as a soil termiticide for controling subterranean termites with limited results (Kard 2001). These insecticides are often applied to foundation soil as a pre-treatment (prior to the pouring of the concrete slab) on new construction projects. On existing structures, insecticides are applied around the inside and outside perimeter of foundations as well as under the slabs along expansion joints, settlement cracks, around utility penetrations, and behind veneers via drilling.

The goal of this type of termite control is to create a continuous chemical barrier between termite colonies and wooden components of the structure. Techniques for inspecting and treating many types of construction, including difficult control situations, are described by Bennett et al. (1988). These techniques include the mechanical alteration of structures to eliminate conditions which are conducive to termite invasion (e.g., reduction of moisture near or in the structure; eliminating wood-to-soil contact), proper termiticide treatments to soil and foundations (including concrete slab, basement, and crawl space foundations), and preservative treatment of wood used in construction.

Recently, several factors have been identified that influence the effectiveness of chemical treatments to soil. For example, termites can exploit gaps in chemical barriers as small as 3-4 cm (Kuriachan and Gold 1998). Osbrink et al. (2001) showed that economically important termites such as *C. formosanus* and *Reticulitermes virginicus*

(Banks) exhibit inter-colony and intra-colony variation in susceptibility to a range of commonly used termiticides. Their study suggests that the development of somewhat resistant surviving workers into supplementary reproductives in colonies exposed to termiticides could result in rapid development of insecticide resistant colonies.

Effectiveness of a termiticide treatment can also be influenced by soil composition in the area being treated. A study by Ramakrishnan et al. (2000) showed that imidacloprid, a commonly used soil termiticide, was most effective in reducing feeding of *Reticulitermes flavipes* (Kollar) in treated sand and least effective in treated silty clay-loam soils (a soil type commonly found in Oklahoma). Finally, numbers of termites exposed to a chemical was shown to affect tolerances to chemical insecticides. DeSouza et al. (2001) attributed increased survivorship among larger groups of termites exposed to chlorpyrifos to the phenomenon of "social facilitation" which they define as the ordinary patterns of behavior that are initiated or increased in pace or frequency by the presence or actions of other animals. Factors such as these, coupled with growing concerns from homeowners about the use of chemical insecticides in the last decade, have renewed interest in alternative methods of termite control.

Control – Alternatives

Numerous termite control alternatives have been suggested, ranging from changes in the application techniques of liquid termiticides to the use of biological control agents. Potter and Hillery (2002) showed that an exterior perimeter-only treatment of liquid termiticides (fipronil or imidacloprid) provided control without the need for invasive drilling through brick veneers or into foundations. Physical barriers using materials such as stainless steel mesh, crushed basalt, and sized sand particles have been used to exclude

termites from structures. The effectiveness and use of such materials; however, is limited by the requirement for species-specific effective particle size ranges and rigid quality control needed during installations (Su and Scheffrahn 1992; Yates et al. 2000). Another alternative to synthetic chemical insecticides that showed promising results in laboratory experiments is the use of natural plant extracts (Blaske and Hertel 2001). This study showed that formulated isoborneol, cedarwood oil, and two constituents of coconut oil were each repellent (choice tests) and toxic (no-choice tests) to foraging subterranean termites, and could possibly be effective as a soil treatment although field studies have not been conducted.

Although biological control of termites is a goal termite researchers have sought for nearly seventy years (Snyder 1935; Lee and Wood 1971), this area of termite control has experienced only limited success. Reviews by Logan et al. (1990), Grace (1997), Culliney and Grace (2000), and Rath (2000), on biological control of termites indicate that the most promising biocontrol agents are two entomopathogenic fungi in the class Deuteromycetes, *Beauveria bassiana* (Balsamo) and *Metarrhizium anisopliae* (Metschnikoff) Sorokin. Laboratory experiments have shown that termites are highly susceptible to these fungi. However, little commercial use and lack of successful field trials were attributed to a number of limiting factors, including avoidance of fungal conidia by termites, behavioral mechanisms such as the removal and isolation of infected nestmates, and defensive fungistatic secretions. Other factors limiting adoption of these materials include dificulty in directly introducing large quantities of conidia to foraging termites and maintaining living fungal cultures in baits. Finally, although biological agents often undergo streamlined EPA registration procedures, constraints placed on

research in this area by environmental protection laws that limit importation of insect pathogens, and lack of public acceptance hinder commercial developement. A study by Ramakrishnan et al. (1999) demonstrated an increased susceptibility of *R. flavipes* to *M. anisopliae* when the termites were also exposed to imidacloprid. It is likely that future biological control efforts on termites rests in integrated pest management strategies similar to these mentioned.

Baits and Baiting Systems

One termite control alternative with considerable success and attention is the use of baits. Baiting involves the placement of stations below or above-ground, each containing cellulose material (i.e., wooden stakes, paper products), around the perimeter of a structure. In one baiting system, these stations are initially used to monitor for termite activity. Once activity is detected, a chemical bait is placed in the station. Bait is then consumed and passed to other members of the colony via trophallaxis. In other systems, the chemical bait is placed in the station when it is first installed, eliminating the monitoring period. Several chemicals have been evaluated for use in such baiting schemes. These chemicals fall into three general categories; slow-acting toxicants, juvenile hormone analogs, and chitin synthesis inhibitors (Su 1994).

In the category of slow-acting toxicants, Esenther and Beal (1974, 1978) showed that mirex (dechlorane) suppressed activity of *Reticulitermes* spp. Su et al. (1991) demonstrated that suppression of foraging populations of *C. formosanus* could be achieved using A-9248 (diiodomethyl para-tolyl sulfone). A study by Kard (2001) showed that boric acid (BA) is a non-repellent toxicant, and suggests that future evaluations of borates of lower water solubility than BA may identify boron compounds

that can be effectively used in baiting systems. Other slow-acting toxicants proposed for use in baiting systems include hydramethylnon, avermectin B_1, and sulfluramid (Su et al. 1982; Su et al. 1987; Su and Scheffrahn 1991).

According to different authors, as summarized by Hrdy et al. (2001), juvenile hormone analogs (JHAs) induce detrimental physiological effects thatcause termite mortality, including disruption of ecdysis, loss of intestinal symbionts, and, most commonly, excessive soldier formation. Fenoxycarb, a JHA used in early bait tests, effectively suppressed foraging activity in field colonies of *Reticulitermes* sp. (Jones 1989). Further, a carbamate derivative of 2-(4-hydroxybenzyl)-1-cyclohexanone, commonly referred to as W-328 (a JHA), demonstrated detrimental affects against *Reticulitermes* sp. and *C. formosanus* (Hrdy et al. 2001).

Chitin synthesis inhibitors (CHIs) interfere with molting processes of many insects, including termites. CHIs such as diflubenzeron, hexaflumuron, and chlorfluazuron have been evaluated as baits against termites (Rojas and Morales-Ramos 2001). Commercially, hexaflumuron achieved early success as the active ingredient in DowElanco's Sentricon™ Termite Colony Elimination System. Laboratory studies have shown that hexaflumuron is readily distributed, slowly metabolized, and slowly cleared from termite populations (Sheets et al. 2000). This system has since incorporated the active ingredient noviflumuron, thathas shown improved efficacy in both laboratory and field studies (DeMark et al. 2004; Eger 2004). The Sentricon system has been successfully used throughout the U.S. and several other countries against a number of termite species including *R. flavipes* and *Coptotermes sp.* (Forschler and Ryder 1996a; Haagsma and Bean 1998; Getty et al. 2000; Prabakaran 2001; Lee 2002). Sentricon was

also effective in difficult control sites where previous chemical control methods failed (Kistner and Sbragia 2001), and was shown by Grace and Su (2001) to be an effective long-term termite control option. This product is also being evaluated for use as an agricultural termite control method for citrus crops in Florida (Stansly et al. 2001).

Use of baits and baiting systems, despite their apparent success, is not devoid of enigmas. The specific methodology used in evaluating the efficacy of baits is controversial, largely due to the difficulty associated with assessing what effects and level of control these products exhibit on cryptic nests of subterranean termites (Su 1994; Forschler and Ryder 1996b; Thorne and Forschler 2000; Evans 2001; Rojas and Morales-Ramos 2001). Non-target species, especially some ant species, that may exclude termites from a station, are commonly found within in-ground monitoring and bait stations (Gulmahamad 1998; Scharf et al. 2002). Because molting is progressively inhibited as ambient temperatures decrease, effects of insect growth regulators like JHAs and CHIs are also significantly influenced by temperature (van den Meiracker et al. 2001). Further, consistent monitoring of bait stations is necessary and time-consuming for pest control operators. To reduce time on site, Su (2001b) demonstrated that a computerized remote monitoring system, that detects breakage of a circuit within a bait station, can be used to reduce the time required to conduct labor-intensive visits and to increase the accuracy of termite detection.

Another problem associated with baiting systems is that foraging termites find a low percentage of installed bait stations. Henderson et al. (1998) showed that directed placement of baits in areas conducive to termites around structures increased the chance that specific stations would become active. This study is contrasted by the findings of

Potter et al. (2001) who found no significant difference in rates of attack on monitors placed in areas thought to be conducive to termite foraging. They suggest that pest control operators would most likely be unable to predict "with any degree of reliability" the locations of termite foraging and subsequent preferential bait placements. A recent study by Reinhard et al. (2002) identified the potential for using the natural phagostimulant hydroquinone to attract termites to in-ground stations.

As use of new, directed termite control methods increase so too does the need for a better understanding of basic termite biology. Accurate species identification, colony size, composition, and foraging capabilities, are all important features to understand and areas where more research is needed.

Taxonomy

Species Identification

The literature contains several identification keys to the termites of the U.S. (Banks and Snyder 1920; Banks 1946; Snyder 1954; Gleason and Koehler 1980; Nutting 1990; Scheffrahn and Su 1994). However, the reliability of species identification within the genus *Reticulitermes* using any of these published keys has been called into question (Thorne 1998; Jones 2000). Weesner's (1970) call for revision of this genus, "certainly this genus is woefully in need of a critical taxonomic study", has recently been echoed by various authors (Haverty and Nelson 1997; Thorne 1998; Haverty et al. 1999a; Jones 2000).

Identifications are based on morphological characteristics of soldier and alate castes. The most reliable way to identify species is to collect both castes from the same colony. This can be difficult because soldiers make up a low percentage of the caste ratio and

alates are not always present in termite populations of a given colony. Identification is further complicated by the possibility of hybridization between species within a genus, specifically between *Reticulitermes tibialis* Banks and *Reticulitermes hesperus* Banks (Pickens 1934), and between *R. flavipes* and *R. virginicus* (Banks 1946; Howard et al. 1981). In addition, recent taxonomic studies have revealed that specimens from different colonies of *Reticulitermes* currently identified as the same species may have multiple cuticular hydrocarbon phenotypes. Because cuticular hydrocarbon phenotypes are thought to be species specific among termites, the authors of these studies suggest that a number of undescribed taxa within the genus exist in various locations throughout the U.S. (Haverty et al. 1991; Haverty et al. 1996b; Haverty and Nelson 1997; Haverty et al. 1999b; Jenkins et al. 2000; Nelson et al. 2001).

Cuticular Hydrocarbon Analysis

Hydrocarbons are important components of the insect epicuticle and serve multiple physiological functions. One such function is decreasing cuticular permeability. This is important both in preventing desiccation and hindering absorption of insecticides (Howard and Blomquist 1982, Blomquist et al. 1987). Hydrocarbons also serve semiochemical roles such as sex attractants and aphrodisiacs, as well as territory markers, recruitment stimulators, and alarm pheromones (defense secretions, or as kairomones) (Howard and Blomquist 1982).

Initial investigations into the cuticular hydrocarbons (CH) of termites in the late 1970's and early 1980's offered another possible function, that of species and caste recognition. Evidence supporting this hypothesis was found in qualitative and quantitative differences between CH profiles of morphologically similar species such as

R. flavipes (Howard et al. 1978) and *R. virginicus* (Howard et al. 1982) and *Nasutitermes corniger* (Motschulsky) and *N. ephratae* (Holmgren) (Howard et al. 1988) and in qualitative differences between castes of the same species (Howard et al. 1982). The species recognition function of CHs in termites is further supported by the work of Bagneres et al. (1991). Through a series of agonism studies, these researchers not only showed that aggression towards conspecifics could be eliminated by removing the cuticular "signature" of the foreign individuals but that the extract could then be transferred to "lures" which once again elicited aggression. The possibility of utilizing CH profiles as a taxonomic tool is desirable because of the difficulty of identifications based solely on morphology.

Since these initial investigations, many studies have evaluated the possibility of using CH profiles for termite identification. Variability in these profiles has indicated undescribed species in many termite genera, including *Zootermopsis* (Haverty et al. 1988), *Heterotermes* (Watson et al. 1989), and *Coptotermes* (Brown et al. 1990). Within *Reticulitermes* of the United States, CH profiles have indicated two or more undescribed taxa in northern California (Haverty et al. 1991; Haverty and Nelson 1997), ten to twelve undescribed taxa in Georgia (Haverty et al. 1996b, 1999b), one new taxon from New Mexico, three or four new taxa from Arizona, and one new taxon from Nevada (Haverty et al. 1999b).

To lend support to undescribed species claims, these diverse CH phenotypes have been correlated with other factors such as agonism (Haverty and Thorne 1989; Haverty et al. 1999a) and soldier defense secretions (Nelson et al. 2001). However, aggression between soldiers from different colonies of the same species of *Reticulitermes* is common

and therefore not necessarily an indication of speciation. Few CH studies have included morphological descriptions (Watson et al. 1989). Because complete morphological descriptions have historically been required to validate new species identifications, many of these claims remain unsubstantiated.

Another tool that has been recently employed for termite taxonomy is the evaluation of genetic relatedness based on mitochondrial DNA and protein electrophoresis (Korman et al. 1991; Jenkins et al. 2000, 2001; Austin et al. 2002; Ye et al. 2004). Contrasting the extreme variations in CH profiles, results of these genetic studies have, with few exceptions, supported current termite taxonomy based on morphological differences. Haverty and Thorne (1989) indicated that alates of *Zootermopsis*, with distinctly different hydrocarbon phenotypes, were capable of mating and producing viable offspring. A subsequent study (Korman et al. 1991) on genetic relatedness based on protein electrophoresis supported the accepted taxonomy of *Zootermopsis* and not the variation seen within their CH profiles. In fact, DNA sequences of *R. santonensis* De Feytaud (the most destructive species of subterranean termite in Europe), *R. flavipes*, and *R. arenicola* in the United States, indicate that all three are possibly conspecific (Jenkins et al. 2001; Ye et al. 2004).

So, why then are there such drastic differences between results of termite taxonomic studies based on morphology and genetic relatedness compared with those based on CH profiles? Many possible explanations could account for variations seen in termite hydrocarbon profiles. One possible explanation is that CH profiles are not species specific in termites. This possibility was expressed by Haverty et al. (1990) referring to species of *Nasutitermes* "…hydrocarbon profiles may not show exact qualitative and/or

quantitative species specificity, especially across a broad geographic range." In some insects (i.e., *Drosophila, Musca*), a greater variability in the cuticular substances between males and females of the same species has been described than between those of different species (Nelson et al. 1981, Luyten 1982). Three distinct species of Australian termites have qualitatively similar hydrocarbon profiles (Brown et al. 1996). Finally, Bagneres et al. (1990) suggest thatCH profiles of *R. santonensis* and *R. flavipes* may be identical.

Environmental factors may also explain variations seen in termite CH profiles. Factors such as temperature and relative humidity (Woodrow et al. 2000), and season of the year (Haverty et al. 1996c) have been shown to affect CH profiles of termites. Further, Woodrow et al. (2000) showed that termites are capable of modifying their CH mixtures in response to these environmental factors. However, these factors often result in minor quantitative, not qualitative differences in profiles. Because species determinations are based on qualitative differences these environmental factors may not explain all of the variability.

An additional explanation that may account for the variation seen in termite CH profiles is actually an artifact of analytical techniques. Minor variations in analytical technique can result in vastly different CH profiles. Suggestions for standardization of sampling methodologies can be found in Blomquist et al. (1987) and Haverty et al. (1996a). One particular problem associated with CH sampling is the possibility of extracting contaminatinginternal lipids. Blomquist et al. (1987) warn "…hydrocarbons, as well as other surface lipid components, are also found in the hemolymph and internal tissues…the type of components found internally may differ from that found on the surface…care must be taken when extracting surface lipids that the extraction conditions

do not result in theextraction of internal lipids." Many specimens used in recent studies were desiccated prior to CH extraction, a method thatdoes not result in the extraction of internal hydrocarbons according to Haverty et al. (1996a). In fact, some specimens have been intentionally decapitated in order to expedite desiccation (Haverty et al. 1997). Resulting profiles of *R. flavipes* using desiccated specimens differ markedly from profiles established by Howard et al. (1978) who used fresh specimens, particularly with respect to those hydrocarbons that would elute after *n*-C26. Haverty et al. (1996b) suggest that earlier studies missed late eluting components due to the use of packed columns rather than capillary columns "which provide superior sensitivity and resolution of cuticular hydrocarbons." Another possible explanation is that these late eluting components are actually being extracted from hemolymph or internal tissues.

These possibilities indicate that multiple identification tactics such as morphology and genetic relatedness need to be used in conjunction with CH analyses. This also illustrates the need for further studies investigating environmental and analytical factors that may affect termite CH profiles.

Colony Characteristics/Foraging

Colony Structure

Subterranean termite colonies exhibit social polymorphism with an organized caste system. Castes include immatures (larvae), nymphs (intermediates), soldiers, workers, and reproductives (primary king and queen; neotenics). It has been the general assumption that these colonies are "closed" systems in which all individuals are the progeny of a single founding king and queen, and that the primary mode of dispersal

among termites is the result of seasonal flights when primary reproductives establish new colonies.

Recent genetic and behavioral studies; however, have shown that colonies are more dynamic than previously thought and are often established by budding, in which a number of secondary reproductives, workers, and soldiers become isolated from the main colony, producing an entirely new colony without a mating flight (Thorne et al. 1999). Studies of mitochondrial DNA by Jenkins et al. (1999) demonstrated that individuals of the same termite colony may have different maternal lineages, indicating the possibility of colony fusion (two separate colonies merging into one) in *Reticulitermes*. Colony fusion was also demonstrated by Matsuura and Nishida (2001) by assessing agonistic behaviors among laboratory colonies of *Reticulitermes speratus* Kolbe. In addition, a genetic study by Bulmer et al. (2001) revealed that colonies of *R. flavipes* can have a variety of modes of reproductive organization ranging from colonies headed by a single pair of primary reproductives, to colonies containing multiple secondary reproductives, to large colonies containing offspring of multiple unrelated queens. These studies demonstrate the variability in caste structure associated with subterranean termite colonies.

Foraging Behavior

The cryptic nature of subterranean termites makes gathering specific information on their foraging behavior difficult. Previous studies have estimated thata single subterranean termite colony can cover a foraging territory encompassing several hundred to a few thousand square meters (Jones 1990; Su et al. 1993; Su 2001a), and contain up to five-to-seven million foraging termites (Jones 1988; Su et al. 1993).

Foraging behavior of subterranean termites is influenced by a number of abiotic factors including soil type and moisture content, soil temperature, and season. Foraging is most intense during summer months with daily peaks in late morning and afternoon, but occurrs around noon in winter (Nutting et al. 1975; Haverty et al. 1999c; Evans and Gleeson 2001). Temperature and moisture preferences are somewhat species specific, with *R. tibialis* and *R. flavipes* preferring forage habitats with cool temperatures and high moisture. Others, such as *Heterotermes aureus* Snyder and *R. hageni*, forage during periods of extended heat and relatively low moisture (Nutting et al. 1975; Jones 1988; Haagsma and Rust 1995; Houseman et al. 2001). Foraging is also affected by soil type. Subterranean termites are more prevalent in sandy soils than in clay-loams (Jones 1988). Puche and Su (2001) demonstrated that greater termite densities result in increased foraging activity. Termites follow artificial guidelines and tunnels in soil that may help them find food sources more readily (Pitts-Singer and Forschler 2000; Brown 2002). Furthermore, a study by Haagsma and Rust (1995) suggested thatin the presence of irrigation, temperature-controlled buildings, and increased cellulose resources, urban settings may be more conducive to termite foraging than their native habitats.

Flight Times

Seasonal dispersal flights of primary reproductives result in founding of new colonies. Termites are considered relatively weak fliers but reports of alate collections at altitudes of 1000 – 3000 feet demonstrate the capability of long distance dispersal (Light 1934; Snyder 1935). It has been suggested that many factors influence the timing of these nuptial flights, including species differences, season, geographic location, temperature, soil moisture, changing light intensity, barometric pressure, and atmospheric electricity

(Snyder 1935; Nutting 1969; Thorne 1998). Most flights occur on warm days during the spring and summer following rain, although smaller fall flights are common. In areas where these specific conditions are not met, such as the northern range limits of *R. flavipes*, flights may be rare and dispersal may depend entirely on the phenomenon of budding (Thorne 1998). Despite these many variables, predictable peak flight dates have been established for many species across their distributions. *R. flavipes* peak flights occur from February through May, flights of *R. virginicus* peak from March to June, whereas peaks of *R. hageni* occur later in the year around August (Snyder 1954; Weesner 1970; Jones 2000). Flights of *R. tibialis* are more variable and occur over a wide range depending on the geographic location (Weesner 1970). Seasonal flight data offer further clues for correct species identification, both by comparison with known peak flight times for specific species and by providing winged reproductives thatare readily identified. These data can also indicate the relative age and approximate establishment time of a specific colony, as nuptial flights normally do not occur from young colonies (Nutting 1969).

Wood Preferences

It is useful to determine any preferences that subterranean termites may have for specific species of wood, both for well-informed construction practices and improved control techniques. A study by Smythe and Carter (1970) elucidatedwood preferences exhibited by three species of subterranean termites, *C. formosanus*, *R. flavipes*, and *R. virginicus*. In choice tests, these three species preferred sugar maple, loblolly pine, and slash pine compared with redwood and ponderosa pine. This and similar studies often use wood consumption rates to indicate feeding preferences (Ripa et al. 2002). Several

authors, as reviewed by Thorne (1998), identified various factors affecting wood consumption rates by termites, including ambient temperature in the laboratory or field, caste ratios of the colonies being tested, termite mortality during the study period, and size of wood resource used in the study. Getty and Haverty (1998) showed that termites consumed more wood that contained some level of fungal decay compared with sound wood. These same authors also proved that the type and stage of fungal decay also had a significant impact on wood consumption by termites. Furthermore, in choice-feeding tests *C. formosanus* was shown to prefer wood with previous feeding damage compared with undamaged wood (Delaplane and LaFage 1989). These confounding factors make comparisons between studies and comparisons between laboratory and field conditions seemingly ambiguous (Thorne 1998). Despite inherent problems associated with comparisons of wood consumption, it is likely that in the future wood consumption rates will continue to be a valuable tool in well-designed laboratory and field studies.

Literature Cited

Austin, J. W., A. L. Szalanski, P. Uva, A.-G. Bagneres, and A. Kence. 2002. A comparative genetic analysis of the subterranean termite genus *Reticulitermes* (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 95: 753-760.

Bagneres, A. G., J. L. Clement, M. S. Blum, R. F. Severson, C. Joulie, and C. Lange. 1990. Cuticular hydrocarbons and defensive compounds of *Reticulitermes flavipes* (Kollar) and *R. santonensis* (Feytaud): polymorphism and chemotaxonomy. J. Chem. Ecol. 16: 3213-3244.

Bagneres, A.-G., A. Killian, J.-L. Clement, and C. Lange. 1991. Interspecific recognition among termites of the genus *Reticulitermes*: evidence for a role for the cuticular hydrocarbons. J. Chem. Ecol. 17: 2397-2420.

Banks, F. A. 1946. Species distinction in *Reticulitermes*. Dept. of Zoology. 27 Pp. University of Chicago, Chicago, IL.

Banks, N., and T. E. Snyder. 1920. Revision of the Nearctic termites. U.S. Natl. Mus. Bull. 108. 228 Pp.

Bennett, G. W., J. M. Owens, and R. M. Corrigan. 1988. Truman's Scientific Guide to Pest Control Operations. Edgell Communications, Duluth, MN.

Blaske, V.-U., and H. Hertel. 2001. Repellent and toxic effects of plant extracts on subterranean termites (Isoptera: Rhinotermitidae). J. of Econ. Entomol. 94: 1200-1208.

Blomquist, G. J., D. R. Nelson, and M. de Renobales. 1987. Chemistry, biochemistry, and physiology of insect cuticular lipids. Arch. Insect Biochem. Physiol. 6: 227-265.

Brown, K. S. 2002. Survey of the termites (Iosptera) of Oklahoma and influence of artificial guidelines in soil on channeling foraging subterranean termites. M.S. Thesis. Dept. of Entomology and Plant Pathology. Oklahoma State University, Stillwater, OK. 72 Pp.

Brown, W. V., J. A. L. Watson, F. L. Carter, M. J. Lacey, R. A. Barrett, and C. A. McDaniel. 1990. Preliminary examination of cuticular hydrocarbons of worker termites as chemotaxonomic characters for some Australian species of *Coptotermes* (Isoptera: Rhinotermitidae). Sociobiol. 16: 305-328.

Brown, W. V., J. A. L. Watson, and M. J. Lacey. 1996. A chemotaxonomic survey using cuticular hydrocarbons of some species of the Australian harvester termite genus *Drepanotermes* (Isoptera: Termitidae). Sociobiol. 27: 199-221.

Bulmer, M. S., E. S. Adams, and J. F. A. Traniello. 2001. Variation in colony structure in the subterranean termite *Reticulitermes flavipes*. Behav. Ecol. Sociobiol. 49: 236-243.

Culliney, T. W., and J. K. Grace. 2000. Prospects for the biological control of subterranean termites (Isoptera: Rhinotermitidae), with special reference to *Coptotermes formosanus*. Bull. Entomol. Res. 90: 9-21.

Delaplane, K. S., and J. P. LaFage. 1989. Preference of the Formosan subterranean termite (Isoptera: Rhinotermitidae) for wood damaged by conspecifics. J. Econ. Entomol. 82: 1363-1366.

DeMark, J. J., J. E. King, A. J. Griffin. 2004. Laboratory characterization of Recruit® IV termite bait (0.5% noviflumuron) with preferred textured cellulose (PTC), an improved bait matrix for subterranean termites. Proc. 52nd Annual Meeting Entomol. Soc. Am., Salt Lake City, UT.

DeSouza, O., O. Miramontes, C. A. Santos, and D. L. Bernardo. 2001. Social facilitation affecting tolerance to poisoning in termites (Insecta, Isoptera). Insectes Sociaux 48: 21-24.

Eger, J. 2004. Field effectiveness of Recruit IV® termite bait (0.5% noviflumuron) with preferred textured cellulose (PTC), an improved bait matrix for subterranean termites. Proc. 52nd Annual Meeting Entomol. Soc. Am., Salt Lake City, UT.

Esenther, G. R., and R. H. Beal. 1974. Attractant-mirex bait suppresses activity of *Reticulitermes* spp. J. Econ. Entomol. 67: 85-88.

Esenther, G. R., and R. H. Beal. 1978. Insecticidal baits on field plot perimeters suppress *Reticulitermes*. J. Econ. Entomol. 71: 604-607.

Evans, T. A. 2001. Estimating relative decline in populations of subterranean termites (Isoptera: Rhinotermitidae) due to baiting. J. Econ. Entomol. 94: 1602-1609.

Evans, T. A., and P. V. Gleeson. 2001. Seasonal and daily activity patterns of subterranean, wood-eating termite foragers. Australian J. Zool. 49: 311-321.

Forschler, B. T., and J. C. Ryder, Jr. 1996a. Subterranean termite colony response to baiting with hexaflumuron in Georgia. Down to Earth 15: 30-35.

Forschler, B. T., and J. C. Ryder, Jr. 1996b. Subterranean termite, *Reticulitermes* spp. (Isoptera: Rhinotermitidae), colony response to baiting with hexaflumuron using a prototype commercial termite baiting system. J. Entomol. Sci. 31: 143-151.

Getty, G. M., and M. I. Haverty. 1998. Consumption of sound and decayed ponderosa pine and Douglas-fir by *Reticulitermes* spp. (Isoptera: Rhinotermitidae) from Northern California. J. Econ. Entomol. 91: 650-654.

Getty, G. M., M. I. Haverty, K. A. Copren, and V. R. Lewis. 2000. Response of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) in Northern California to baiting with hexaflumuron with Sentricon Termite Colony Elimination System. J. Econ. Entomol. 93: 1498-1507.

Gleason, R. W., and P. G. Koehler. 1980. Termites of the Eastern and Southeastern United States: pictorial keys to soldiers and winged reproductives. Florida Coop. Ext. Serv. Bull. 192. 14 Pp.

Grace, J. K. 1997. Biological control strategies for suppression of termites. J. Agric. Entomol. 14: 281-289.

Grace, J. K., and N.-Y. Su. 2001. Evidence supporting the use of termite baiting systems for long-term structural protection (Isoptera). Sociobiol. 37: 301-310.

Gulmahamad, H. 1998. Fauna associated with in-ground subterranean termite monitoring and bait stations in Southern California. Pan-Pacific Entomol. 74: 134-139.

Haagsma, K., and J. Bean. 1998. Evaluation of a hexaflumuron-based bait to control subterranean termites in Southern California (Isoptera: Rhinotermitidae). Sociobiol. 31: 363-369.

Haagsma, K. A., and M. K. Rust. 1995. Colony size estimates, foraging trends, and physiological characteristics of the western subterranean termite (Isoptera: Rhinotermitidae). Environ. Entomol. 24: 1520-1528.

Haverty, M. I., and B. L. Thorne. 1989. Agonistic behavior correlated with hydrocarbon phenotypes in dampwood termites, *Zootermopsis* (Isoptera: Termopsidae). J. Insect Behav. 2: 523-543.

Haverty, M. I., and L. J. Nelson. 1997. Cuticular hydrocarbons of *Reticulitermes* (Isoptera: Rhinotermitidae) from Northern California indicate undescribed species. Comp. Biochem. Physiol. 118B: 869-880.

Haverty, M. I., M. Page, L. J. Nelson, and G. J. Blomquist. 1988. Cuticular hydrocarbons of dampwood termites, *Zootermopsis*: intra- and intercolony variation and potential as taxonomic characters. J. Chem. Ecol. 14: 1035-1058.

Haverty, M. I., B. L. Thorne, and M. Page. 1990. Surface hydrocarbon components of two species of *Nasutitermes* from Trinidad. J. Chem Ecol. 16: 2441-2450.

Haverty, M. I., L. J. Nelson, and M. Page. 1991. Preliminary investigations of the cuticular hydrocarbons from North American *Reticulitermes* and tropical and subtropical *Coptotermes* (Isoptera: Rhinotermitidae) for chemotaxonomic studies. Sociobiol. 19: 51-76.

Haverty, M. I., B. L. Thorne, and L. J. Nelson. 1996a. Hydrocarbons of *Nasutitermes acajutlae* and comparison of methodologies for sampling cuticular hydrocarbons of Caribbean termites for taxonomic and ecological studies. J. Chem. Ecol. 22: 2081-2109.

Haverty, M. I., B. T. Forschler, and L. J. Nelson. 1996b. An assessment of the taxonomy of *Reticulitermes* (Isoptera: Rhinotermitidae) from the Southeastern United States based on cuticular hydrocarbons. Sociobiol. 28: 287-318.

Haverty, M. I., J. K. Grace, L. J. Nelson, and R. T. Yamamoto. 1996c. Intercaste, intercolony, and temporal variation in cuticular hydrocarbons of *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). J. Chem. Ecol. 22: 1813-1834.

Haverty, M. I., M. S. Collins, L. J. Nelson, and B. L. Thorne. 1997. Cuticular hydrocarbons of termites of the British Virgin Islands. J. Chem. Ecol. 23: 927-964.

Haverty, M. I., K. A. Copren, G. M. Getty, and V. R. Lewis. 1999a. Agonistic behavior and cuticular hydrocarbon phenotypes of colonies of *Reticulitermes* (Isoptera: Rhinotermitidae) from northern California. Ann. Entomol. Soc. Am. 92: 269-277.

Haverty, M. I., L. J. Nelson, and B. T. Forschler. 1999b. New cuticular hydrocarbon phenotypes of *Reticulitermes* (Isoptera: Rhinotermitidae) from the United States. Sociobiol. 34: 1-21.

Haverty, M. I., G. M. Getty, K. A. Copren, and V. R. Lewis. 1999c. Seasonal foraging and feeding behavior of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) in a wildland and a residential location in Northern California. Environ. Entomol. 28: 1077-1084.

Henderson, G., K. Sharpe-McCollum, and C. Dunaway. 1998. Subterranean termite (Isoptera: Rhinotermitidae) attack on ground monitors around an apartment complex in fixed pattern placements versus conducive placements. Florida Entomol. 81: 461-464.

Houseman, R. M., R. E. Gold, and B. M. Pawson. 2001. Resource partitioning in two sympatric species of subterranean termites, *Reticulitermes flavipes* and *Reticulitermes hageni* (Isoptera: Rhinotermitidae). Environ. Entomol. 30: 673-685.

Howard, R. W., C. A. McDaniel, and G. J. Blomquist. 1978. Cuticular hydrocarbons of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae). J. Chem. Ecol. 4: 233-245.

Howard, R. W., E. J. Mallette, M. I. Haverty, and R. V. Smythe. 1981. Laboratory evaluation of within-species, between-species, and parthenogenetic reproduction in *Reticulitermes flavipes* and *Reticulitermes virginicus*. Psyche 88: 75-87.

Howard, R. W., G. J. Blomquist. 1982. Chemical ecology and biochemistry of insect hydrocarbons. Ann. Rev. Entomol. 27: 149-172.

Howard, R. W., C. A. McDaniel, D. R. Nelson, G. J. Blomquist, L. T. Gelbaum, and L. H. Zalkow. 1982. Cuticular hydrocarbons of *Reticulitermes virginicus* (Banks) and their role as potential species- and caste-recognition cues. J. Chem. Ecol. 8: 1227-1239.

Howard, R. W., B. L. Thorne, S. C. Levings, and C. A. McDaniel. 1988. Cuticular hydrocarbons as chemotaxonomic characters for *Nasutitermes coringer* (Motshulsky) and *N. ephratae* (Holmgren) (Isoptera: Termitidae). Ann. Entomol. Soc. Am. 81: 395-399.

Hrdy, I., J. Kuldova, and Z. Wimmer. 2001. A juvenile hormone analogue with potential for termite control: laboratory tests with *Reticulitermes santonensis*, *Reticulitermes flaviceps* and *Coptotermes formosanus* (Isopt., Rhinotermitidae). J. Appl. Entomol. 125: 403-411.

Jenkins, T. M., C. J. Basten, S. Kresovich, and B. T. Forschler. 1999. Mitochondrial gene sequence questions *Reticulitermes* sp. social structure (Isoptera: Rhinotermitidae). Sociobiol. 34: 161-172.

Jenkins, T. M., M. I. Haverty, C. J. Basten, L. J. Nelson, M. Page, and B. T. Forschler. 2000. Correlation of mitochondrial haplotypes with cuticular hydrocarbon phenotypes of sympatric *Reticulitermes* species from the Southeastern United States. J. Chem. Ecol. 26: 1525-1542.

Jenkins, T. M., R. E. Dean, R. Verkerk, and B. T. Forschler. 2001. Phylogenetic analyses of two mitochondrial genes and one nuclear intron region illuminate European subterranean termite (Isoptera: Rhinotermitidae) gene flow, taxonomy, and introduction dynamics. Mol. Phylogenet. Evol. 20: 286-293.

Jones, S. C. 1988. Foraging and distribution of subterranean termites. *In* P. A. Zungoli [ed.], National Conference on Urban Entomology. pp. 23-32. University of Maryland, College Park, MD.

Jones, S. C. 1989. Field evaluation of fenoxycarb as a bait toxicant for subterranean termite control. Sociobiol. 15: 33-41.

Jones, S. C. 1990. Delineation of *Heterotermes aureus* (Isoptera: Rhinotermitidae) foraging territories in a Sonoran Desert grassland. Environ. Entomol. 19: 1047-1054.

Jones, S. C. 2000. Subterranean termites. *In* R. E. Gold and S. C. Jones [eds.], Handbook of Household and Structural Insect Pests. pp. 119-124. Entomol. Soc. Amer., Lanham, MD.

Kambhampati, S., and P. Eggleton. 2000. Taxonomy and phylogeny of termites. *In* T. Abe, D. E. Bignell and M. Higashi [eds.], Termites: Evolution, Sociality, Symbioses, Ecology. pp. 1-23. Kluwer Academic Publishers, Dordrecht / Boston / London.

Kard, B. M. 2001. Detrimental effects of boric-acid-treated soil against foraging subterranean termites (Isoptera: Rhinotermitidae). Sociobiol. 37: 363-378.

Kistner, D. H., and R. J. Sbragia. 2001. The use of the Sentricon™ *Termite Colony Elimination System* for controlling termites in difficult control sites in Northern California. Sociobiol. 37: 265-280.

Korman, A. K., D. P. Pashley, M. I. Haverty, and J. P. La Fage. 1991. Allozymic relationships among cuticular hydrocarbon phenotypes of *Zootermopsis* species (Isoptera: Termopsidae). Ann. Entomol. Soc. Am. 84: 1-9.

Kuriachan, I., and R. E. Gold. 1998. Evaluation of the ability of *Reticulitermes flavipes* Kollar, a subterranean termite (Isoptera: Rhinotermitidae), to differentiate between termiticide treated and untreated soils in laboratory tests. Sociobiol. 32: 151-166.

Lee, C.-Y. 2002. Control of foraging colonies of subterranean termites, *Coptotermes travians* (Isoptera: Rhinotermitidae) in Malaysia using hexaflumuron baits. Sociobiol. 39: 411-416.

Lee, K. E., and T. G. Wood. 1971. Termites and Soils Academic Press Inc., New York.

Light, S. F. 1934. The constitution and development of the termite colony. *In* C. A. Kofoid [ed.], Termites and Termite Control. pp. 22-41. University of California Press, Berkeley, CA.

Logan, J. W. M., R. H. Cowie, and T. G. Wood. 1990. Termite (Isoptera) control in agriculture and forestry by non-chemical methods: a review. Bull. of Entomol. Res. 80: 309-330.

Luyten, I. 1982. Variations intraspecifique et interspecifique des hydrocarbures cuticularies chez *Drosophila simulans* et deseseces affines. C. R. Acad. Sci. Paris 295: 733-736.

Matsuura, K., and T. Nishida. 2001. Colony fusion in a termite: what makes the society "open"? Insectes Sociaux 48: 378-383.

Nelson, D. R., J. W. Dillwith, and G. J. Blomquist. 1981. Cuticular hydrocarbons of the house fly *Musca domestica*. Insect Biochem. 11: 187-197.

Nelson, L. J., L. G. Cool, B. T. Forschler, and M. I. Haverty. 2001. Correspondence of soldier defense secretion mixtures with cuticular hydrocarbon phenotypes for chemotaxonomy of the termite genus *Reticulitermes* in North America. J. of Chem. Ecol. 27: 1449-1479.

Nutting, W. L. 1969. Flight and colony foundation. *In* K. Krishna and F. Weesner [eds.], Biology of Termites. pp. 233-282. Academic Press, New York.

Nutting, W. L. 1990. Insecta: Isoptera. *In* D. L. Dindal [ed.], Soil Biology Guide. pp. 997-1032. John Wiley & Sons, New York.

Nutting, W. L., M. I. Haverty, and J. P. LaFage. 1975. Demography of termite colonies as related to various environmental factors: population dynamics and role in the detritus cycle. Utah State Univ., Logan, US/IBP Desert Biome Res. Memo. 75-31. 26 Pp.

Osbrink, W. L. A., A. R. Lax, and R. J. Brenner. 2001. Insecticide susceptibility in *Coptotermes formosanus* and *Reticulitermes virginicus* (Isoptera: Rhinotermitidae). J. Econ. Entomol. 94: 1217-1228.

Pickens, A. L. 1934. The barren-lands subterranean termite *Reticulitermes tibialis*. *In* C. A. Kofoid [ed.], Termites and Termite Control, 2nd ed. pp. 184-186. Univ. of California Press, Berkeley, CA.

Pitts-Singer, T. L., and B. T. Forschler. 2000. Influence of guidelines and passageways on tunneling behavior of *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks) (Isoptera: Rhinotermitidae). J. Insect Behavior 13: 273-290.

Potter, M. 1997. Termites. *In* S. A. Hedges [ed.], Handbook of Pest Control, 8th ed. Mallis. pp. 232-332. G.I.E., Cleveland, OH.

Potter, M. F., and A. E. Hillery. 2002. Exterior-targeted liquid termiticides: an alternative approach to managing subterranean termites (Isoptera: Rhinotermitidae) in buildings. Sociobiol. 39: 373-405.

Potter, M. F., E. A. Eliason, K. Davis, and R. T. Bessin. 2001. Managing subterranean termites (Isoptera: Rhinotermitidae) in the Midwest with a hexaflumuron bait and placement considerations around structures. Sociobiol. 38: 565-584.

Prabakaran, S. K. 2001. Eastern subterranean termite management using baits containing hexaflumuron in affected University of Iowa structures (Isoptera: Rhinotermitidae). Sociobiol. 37: 221-233.

Puche, H., and N.-Y. Su. 2001. Population density of subterranean termites (Isoptera: Rhinotermitidae) on tunnel formation in laboratory arenas. Sociobiol. 38: 523-530.

Ramakrishnan, R., D. R. Suiter, C. H. Nakatsu, and G. W. Bennett. 2000. Feeding inhibition and mortality in *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) after exposure to imidacloprid-treated soils. J. Econ. Entomol. 93: 422-428.

Ramakrishnan, R., D. R. Suiter, C. H. Nakatsu, R. A. Humber, and G. W. Bennett. 1999. Imidacloprid-enhanced *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) susceptibility to the entomopathogen *Metarhizium anisopliae*. J. Econ. Entomol. 92: 1125-1132.

Rath, A. C. 2000. The use of entomopathogenic fungi for control of termites. Biocontrol Sci. and Tech. 10: 563-581.

Reinhard, J., M. J. Lacey, and M. Lenz. 2002. Application of the natural phagostimulant hydroquinone in bait systems for termite management (Isoptera). Sociobiol. 39: 213-229.

Ripa, R., L. Castro, N.-Y. Su, and P. Palma. 2002. Laboratory estimate of wood consumption rates by *Reticulitermes* sp. (Isoptera: Rhinotermitidae) in Chile. Sociobiol. 39: 285-290.

Rojas, M. G., and J. A. Morales-Ramos. 2001. Bait matrix for delivery of chitin synthesis inhibitors to the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 94: 506-510.

Scharf, M. E., E. A. Buss, C. R. Ratliff, D. J. Brad, and G. W. Bennett. 2002. Invertebrate taxa associated with subterranean termite monitoring devices in the Eastern Midwest. Sociobiol. 39: 441-451.

Scheffrahn, R. H., and N.-Y. Su. 1994. Keys to soldier and winged adult termites (Isoptera) of Florida. Florida Entomol. 77: 460-475.

Sheets, J. J., L. L. Karr, and J. E. Dripps. 2000. Kinetics of uptake, transfer, and metabolism of hexaflumuron by eastern subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 93: 871-877.

Smythe, R. V., and F. L. Carter. 1970. Feeding responses to sound wood by *Coptotermes formosanus*, *Reticulitermes flavipes*, and *R. virginicus* (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Amer. 63: 841-847.

Snyder, T. E. 1935. Our Enemy the Termite. Comstock Publishing Company, Ithaca, New York. 196 Pp.

Snyder, T. E. 1954. Order Isoptera: The Termites of the United States and Canada. National Pest Control Assoc., New York. 64 Pp.

Stansly, P. A., N.-Y. Su, and J. M. Conner. 2001. Management of subterranean termites, *Reticulitermes* spp. (Isoptera: Rhinotermitidae) in a citrus orchard with hexaflumuron bait. Crop Protection 20: 199-206.

Su, N.-Y. 1994. Field evaluation of a hexaflumuron bait for population suppression of subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 87: 389-397.

Su, N.-Y. 2001a. Studies on the foraging of subterranean termites (Isoptera). Sociobiol. 37: 253-260.

Su, N.-Y. 2001b. A computerized system for remote monitoring of subterranean termites near structures. J. Econ. Entomol. 94: 1518-1525.

Su, N.-Y. 2002. Novel technologies for subterranean termite control. Sociobiol. 40: 95-101.

Su, N.-Y., and R. H. Scheffrahn. 1991. Laboratory evaluation of two slow-acting toxicants against Formosan and eastern subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 84: 170-175.

Su, N.-Y., and R. H. Scheffrahn. 1992. Penetration of sized-particle barriers by field populations of subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 85: 2275-2278.

Su, N.-Y., M. Tamashiro, and M. I. Haverty. 1987. Characterization of slow-acting insecticides for the remedial control of the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 80: 1-4.

Su, N.-Y., P. M. Ban, and R. H. Scheffrahn. 1991. Suppression of foraging populations of the Formosan subterranean termite (Isoptera: Rhinotermitidae) by field applications of a slow-acting toxicant bait. J. Econ. Entomol. 84: 1525-1531.

Su, N.-Y., P. M. Ban, and R. H. Scheffrahn. 1993. Foraging populations and territories of the eastern subterranean termite (Isoptera: Rhinotermitidae) in Southeastern Florida. Environ. Entomol. 22: 1113-1117.

Su, N.-Y., M. Tamashiro, J. R. Yates, and M. I. Haverty. 1982. Effects of behavior on the evaluation of insecticides for prevention of or remedial control of the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 75: 188-193.

Suiter, D. R., S. C. Jones, and B. T. Forschler. 2002. Biology of subterranean termites in the Eastern United States. USDA Coop. Ext. Serv. Bull. 1209. 8 Pp.

Thorne, B. L. 1998. Biology of Subterranean Termites of the Genus *Reticulitermes*. pp. 1-30. National Pest Control Assoc., Dunn Loring, VA.

Thorne, B. L., and B. T. Forschler. 2000. Criteria for assessing efficacy of stand-alone termite bait treatments at structures. Sociobiol. 36: 245-255.

Thorne, B. L., J. F. A. Traniello, E. S. Adams, and M. Bulmer. 1999. Reproductive dynamics and colony structure of subterranean termites of the genus *Reticulitermes* (Isoptera Rhinotermitidae): a review of the evidence from behavioral, ecological, and genetic studies. Ethology, Ecology and Evolution 11: 149-169.

van den Meiracker, K. G., P. A. Zungoli, E. P. Benson, and J. W. C. Bridges. 2001. Hexaflumuron-induced mortality in *Reticulitermes flavipes* and *Coptotermes formosanus* at constant and fluctuating temperatures (Isoptera: Rhinotermitidae). Sociobiol. 37: 1-13.

Watson, J. A. L., W. V. Brown, L. R. Miller, F. L. Carter, and M. J. Lacey. 1989. Taxonomy of *Heterotermes* (Isoptera: Rhinotermitidae) in south-eastern Australia: cuticular hydrocarbons of workers, and soldier and alate morphology. System. Entomol. 14: 299-325.

Weesner, F. M. 1970. Termites of the Nearctic Region. *In* K. Krishna and F. M. Weesner [eds.], Biology of Termites. pp. 477-525. Academic Press, New York.

Woodrow, R. J., J. K. Grace, L. J. Nelson, and M. I. Haverty. 2000. Modification of cuticular hydrocarbons of *Cryptotermes brevis* (Isoptera: Kalotermitidae) in response to temperature and relative humidity. Physiol. Chem. Ecol. 29: 1100-1107.

Yates, J. R., III, J. K. Grace, and J. N. Reinhardt. 2000. Installation guidelines for the basaltic termite barrier: a particle barrier to Formosan subterranean termites (Isoptera: Rhinotermitidae). Sociobiol. 35: 1-16.

Ye, W., C.-Y. Lee, R. H. Scheffrahn, J. M. Aleong, N.-Y. Su, G. W. Bennett, and M. E. Scharf. 2004. Phylogenetic relationships of nearctic *Reticulitermes* species (Isoptera: Rhinotermitidae) with particular reference to *Reticulitermes arenicola* Goellner. Mol. Phylogenet. Evol. 30: 815-822.

CHAPTER III. COLONY CHARACTERIZATION OF *RETICULITERMES FLAVIPES* (ISOPTERA: RHINOTERMITIDAE) ON A NATIVE TALLGRASS PRAIRIE

(Environmental Entomology)

ABSTRACT

In recent years, a growing need exists for a better understanding of basic termite ecology. Studies estimating foraging populations and colony sizes of subterranean termites have been conducted in many areas of the United States. However, characterizations of colonies of *Reticulitermes* occurring in the Central Great Plains have not been conducted. Results of studies conducted on three colonies of *Reticulitermes flavipes* (Kollar) in a native tallgrass prairie habitat are provided herein. Colonies were found to forage over areas ranging from 9.0 to 92.3m^2 and contained 10,357 to 183,495 foragers. Soldiers comprised 2.69 to 4.46% of the total foraging population. We conclude thatwidespread termite pressure in this native habitat is most likely the result of many small colonies foraging in close proximity to each other.

KEY WORDS subterranean termites, Rhinotermitidae, *Reticulitermes flavipes,* foraging populations, foraging territories, caste ratios, prairie, native grasslands, Lincoln Index, Weighted Mean Model

DEVELOPMENT AND IMPLEMENTATION OF BAITS and baiting systems for termite management during the past thirty yearshas revealed the need for a complete understanding of basic termite ecology. Historically, the primary method of controlling termites was to create an insecticidal barrier in the soil between wooden structures and termite colonies. The intention of baiting systems; however, is to suppress colony numbers, disrupt foraging, and potentially eliminate entire colonies. Evaluating the effects of such control methods requires the ability to delineate colonies (Getty et al. 2000) and estimate foraging population numbers (Su 1994). This has necessitated the development of persistent, non-toxic dyes such as Neutral Red (Esenther 1980), Nile Blue A (Su et al. 1991), and more recently a purple blend (Oi 2000; Atkinson et al. 2004).

Utilization of these dyes allows the foraging territory of a given colony to be visualized and delineated. When used in a mark-release-recapture protocol, foraging population numbers can be estimated. Studies employing these techniques have been conducted in many regions of North America including Toronto, Canada (Grace et al. 1989), Ft. Lauderdale, FL (Su et al. 1993), Tucson, AZ (Jones 1990), and Riverside, CA (Haagsma and Rust 1995). Estimates of *Reticulitermes flavipes* (Kollar) colonies, a commonly occurring termite in Oklahoma (Brown et al. 2004), range from small colonies containing less than ~ 1,000 foragers (Forschler and Townsend 1996) and covering less than $20m^2$ (Su et al. 1993) to huge colonies containing millions of foragers and covering thousands of square meters (Grace et al. 1989; Su et al. 1993).

Currently, no information is available on colony characteristics of *Reticulitermes* occurring in the Central Plains of the United States (U.S.). Further, although colony

characterizations in native/wildland sites have been conducted in other areas of the U.S. (Haagsma and Rust 1995; Haverty et al. 2000), nothing is known about the ecology of *Reticulitermes* in native tallgrass prairies. We report here foraging population estimates, foraging territory sizes, and caste ratios of three colonies of *Reticulitermes flavipes* from a native tallgrass prairie habitat in North-Central Oklahoma. This information provides insight into the role termites play in this vital ecosystem.

Materials and Methods

Study Site. The study site was located on the Nature Conservancy's Tallgrass Prairie Preserve in Osage county, ten miles north of Pawhuska, OK. In North America, only 10% of the historical 142 million acres of tallgrass prairie remain. The 38,700 acres of the current preserve that is home to ~ 3,200 bison, represent an important portion of this vanishing ecosystem. The tallgrasses, big bluestem *Andropogon gerardii* Vitman, Indiangrass *Sorghastrum nutans* (L.), and switchgrass *Panicum virgatum* L., for which the prairies were originally named dominate the landscape. While these grasses are probably their primary diet, *Reticulitermes flavipes* were noticed feeding on woody roots of live goat's rue *Tephrosia virginiana* (L.), Pers., a small flowering perennial containing rotenone in its roots. The study site was located within a 350 acre non-grazed prairie area near the preserve headquarters.

Monitoring Devices and Grid. Monitoring devices consisted ofcylindrical (10.2cm diameter × 20.3cm deep) below-ground monitoring stations and soil-surface ground-boards (Brown et al. 2004). In the summer of 2003, a grid of 292 stations spaced at 3m intervals was installed. Two hundred fifty-seven ground-boards were also installed within the grid so that each monitoring device subtended an area of 4.5m^2. Five larger

below-ground stations measuring 20.3cm in diameter by 25cm deepfilled with tightly rolled corrugated cardboard, were also installed to increase the number of termites collected for population estimations. This resulted in a grid of 554 monitoring devices covering an area of 2,313m^2 (Fig. 3.1).

Colony Characterizations. During the summers of 2004 and 2005, characterizations were made of three colonies of *Reticulitermes flavipes*. Voucher specimens were collected in 70% ethyl alcohol and placed in the K.C. Emerson Insect Museum, 135 Noble Research Center, OSU, Stillwater, OK. Estimates of foraging populations were made using the triple mark-release-recapture technique (Grace et al. 1989; Su et al. 1993; Haverty et al. 2000). Termites from a monitoring device with high foraging activity were collected, counted by caste, and fed filter paper impregnated with one of three dyes for 14 days. A monitoring device outside the territory of the previous colony/colonies was selected for initiation of characterization of the subsequent colony. Each colony received a different dye to avoid confusion that could be caused by potentially overlapping foraging territories. The dyes used were 0.1% (wt/wt) Nile Blue A (Aldrich, Milwaukee, WI) (Su et al. 1991), 0.5% (wt/wt) Neutral Red (Sigma Chemical Co., St. Louis, MO) (Esenther 1980), and a purple blend containing 0.1% Nile Blue and 0.25% Neutral Red in a 50:50 ratio (Oi 2000; Atkinson et al. 2004). Dyed termites were counted and returned to the monitor where they were originally collected. Surrounding monitors were evaluated 14d after release for the presence of dyed termites. All termites from surrounding monitors containing dyed termites were subsequently collected, counted by caste, again fed dyed filter paper and released back into their respective monitoring devices. Estimates of foraging populations of each colony were made based on data

obtained from a total of three cycles of this mark-release-recapture process. Both the Lincoln Index and the weighted mean model (Bailey 1951; Begon 1979; Grace et al. 1989; Su et al. 1993; Haverty et al. 2000) were used to estimate foraging populations and associated standard errors of each of the three colonies so that these two methods could be compared (Haverty et al. 2000). Numbers from the initial mark-release-recapture cycle were used in the Lincoln Index calculations. Caste ratio averages for each monitoring device and subsequently each colony were obtained based on data collected during the triple mark-release-recapture process. A chi-square test was used to compare soldier ratios from monitors centralized within the foraging territory of a single colony with ratios from those on the periphery (PROC FREQ; SAS Institute 2001).

To ensure a more complete delineation of foraging territories, the mark-release-recapture procedure was continued beyond the third cycle until no new monitoring devices were found to contain dyed termites. A total of six cycles were completed for each of the three colonies. Areas of each colonies foraging territory were calculated by summing the number of monitoring devices that contained dyed termites at some point during the monitoring period and multiplying by 4.5m^2 (Jones 1990). Maximum linear foraging distances were obtained by calculating the distance between the two monitoring devices within the foraging territory that were the farthest apart (Haverty et al. 2000).

Results and Discussion

Foraging Territory Delineations. During the study, wood associated with 223 of the 554 monitors was located and fed upon by foraging termites. This large percentage (40.3%) indicated a high level of termite pressure within the study site. Su et al. (1993) reported a colony of *Reticulitermes flavipes* with a foraging territory covering 2,361m^2,

indicating the possibility that foraging activity at our site could be attributed to a single colony. However, results of the mark-release-recapture procedure indicated that the termite pressure at our site could be attributed to the foraging activities of several small colonies, similar to the findings of Haverty et al. (2000). Our estimates of foraging territories ranged from 9.0 to 92.3m^2. Calculations of foraging territories as well as maximum foraging distances and are given in Table 3.1.

Foraging Population Estimations. Estimates of foraging populations are given in Table 3.2. For each cycle (1-3), M is the number of marked termites released, n is the number of marked plus unmarked termites recaptured, and m is the number of marked individuals among recaptured termites. Estimates ranged from ~10,000 to ~180,000 foragers. When compared with previous studies estimating *Reticulitermes* spp. foraging populations our estimates are substantially lower than the millions of foragers reported by Su et al. (1993) and Grace et al. (1989), but comparable to those reported by Forschler and Townsend (1996) and Haverty et al. (2000). One consideration when making such comparisons is that urban environments may provide more optimal habitats for termites, allowing their colonies to expand in number and size compared with native habitats (Haagsma and Rust 1995).

It is interesting to note that the estimates of foraging populations, the red colony having the largest estimate, did not necessarily correspond to size of the foraging territory. This phenomenon, while of note, is not unique to the current study (Su et al. 1993) and could be explained, at least in part, by the fact that estimates of foraging territories are most likely overestimates of the area the colony is actually using and defending (Thorne 1998; Haverty et al. 2000). However, such estimates are an important

indicator of the dispersion of termite colonies and provide a basis for comparisons between studies. Another possible explanation is that estimates of foraging populations of the red colony had higher standard deviations relative to the mean than those of the other two colonies, indicating that the red colony estimates are not as precise as those of the other two colonies.

When comparing estimations using the Lincoln Index to those using the Weighted Mean Model, only the purple colony's estimates agreed. The Weighted Mean Model estimates for the red and blue colonies were 2.3 and 7.4 times that of the Lincoln Index estimates, respectively. Similar marked differences between estimates, using these two methods, were noted by Haverty et al. (2000) with the weighted mean commonly yielding the larger estimate. The advantage of using the Lincoln Index is that it requires much less time and effort. Estimations based on either method do not account for termites that may not be actively foraging and therefore only estimates the foraging population of the colony. To date, no method for determining the validity of estimations made using either of these models has been developed. However, we agree with Haverty et al. (2000) that utilizing a 14-day dying period and recapture interval increases the probability of meeting the assumptions of the models.

Caste Ratio Determinations. Termite colonies exhibit social polymorphism with a well defined caste system. For all practical purposes these castes can be divided into three distinct groups; workers (including larvae, true workers, and pseudergates), soldiers, and reproductives (including primary and neotenic reproductives). Ratios of each of these castes are regulated within colonies. Within the colony, the primary role of the soldier caste is defense. Banks and Snyder (1920) as cited by Haverty (1977) reported that

soldier proportions in mature colonies of *R. flavipes* range from 8.4 to 10.8%. Soldier ratios of the three *R. flavipes* colonies evaluated in the current study ranged from 2.69 to 4.46% (Table 3.2). Soldier ratios from individual monitoring devices rarely exceeded 5% of the foraging population. Soldier ratios calculated from monitors that were centralized within the foraging range of the blue colony (monitors ER, 175, and FF) were compared with those from monitors on the periphery of the foraging territory (Figure 3.2; remaining monitors). The weighted soldier ratio from the three centralized monitors was 7.48% compared to 2.52% for those on the periphery. Results of the chi-square test indicated a significantly higher proportion of soldiers among the three centralized stations ($p < 0.0001$). These results should be interpreted cautiously as the exact locations of the main nest and potential satellite nests are unknown. However, these results indicate that this colony allocates a higher proportion of soldiers to areas that are more centralized within its foraging range.

In summary, colonies of *Reticulitermes flavipes* characterized on the Tallgrass Prairie Preserve are relatively small in both foraging population and territory compared with studies by Su et al. (1993) and Grace et al. (1989), but similar to those reported by Forschler and Townsend (1996) and Haverty et al. (2000) from both wildland and residential locations in Georgia and Northern California. It is likely that within our study site numerous colonies are foraging in close proximity to each other. However, soldier proportions are relatively low and seem to increase with proximity to the geographical center of the colony. Further colony characterizations at this site are warranted as numerous monitors with termite activity lie outside the territories of the three colonies described herein.

Acknowledgments

I thank Mike Doss, Matt Rawlings, Andrine Morrison, and Doug Keuhl for their assistance in field evaluations.

Literature Cited

Atkinson, T. H., A. J. Griffin, and J. E. King. 2004. Laboratory and field studies of a purple dye marker for *Reticulitermes* spp. (Isoptera: Rhinotermitidae). Sociobiol. 43: 259-267.

Bailey, N.T.J. 1951. On estimating the size of mobile populations from capture-recapture data. Biometrika 38: 293-306.

Banks, N., and T. E. Snyder 1920. A revision of the Nearctic termites with notes on biology and geographical distribution. U.S. Natl. Mus., Bull. 108. 228 Pp.

Begon, M. 1979. Investigating animal abundance: capture-recapture for biologists. University Park Press, Baltimore, MD.

Brown, K. S., B. M. Kard, and M. P. Doss. 2004. 2002 Oklahoma termite survey (Isoptera). J. Kansas Entomol. 77: 1-9.

Esenther, G. R. 1980. Estimating the size of subterranean termite colonies by a release-recapture technique. International Research Group on Wood Preservation 1-5.

Forschler, B. T., and M. L. Townsend. 1996. Mark-release-recapture estimates of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) colony foraging populations from Georgia, U.S.A. Environ. Entomol. 25: 952-962.

Getty, G. M., M. I. Haverty, K. A. Copren, and V. R. Lewis. 2000. Response of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) in Northern California to baiting with hexaflumuron with Sentricon Termite Colony Elimination System. J. Econ. Entomol. 93: 1498-1507.

Grace, J. K., A. Abdallay, and K. R. Farr. 1989. Eastern subterranean termite (Isoptera: Rhinotermitidae) foraging territories and populations in Toronto. Can. Entomol. 121: 551-556.

Haagsma, K. A., and M. K. Rust. 1995. Colony size estimates, foraging trends, and physiological characteristics of the western subterranean termite (Isoptera: Rhinotermitidae). Environ. Entomol. 24: 1520-1528.

Haverty, M. I. 1977. The proportion of soldiers in termite colonies: a list and a bibliography (Isoptera). Sociobiol. 2: 199-216.

Haverty, M. I., G. M. Getty, K. A. Copren, and V. R. Lewis. 2000. Size and dispersion of colonies of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) in a wildland and a residential location in northern California. Environ. Entomol. 29: 241-249.

Jones, S. C. 1990. Delineation of *Heterotermes aureus* (Isoptera: Rhinotermitidae) foraging territories in a Sonoran Desert grassland. Environ. Entomol. 19: 1047-1054.

Oi, F. M. 2000. Purple dye marker for *Reticulitermes* spp. (Isoptera: Rhinotermitidae). Florida Entomol. 83: 112-113.

SAS Institute. 2001. PROC user's manual, version 8.2. ed. SAS Institute, Cary, NC.

Su, N.-Y. 1994. Field evaluation of a hexaflumuron bait for population suppression of subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 87: 389-397.

Su, N.-Y., P. M. Ban, and R. H. Scheffrahn. 1991. Evaluation of twelve dye markers for population studies of the Eastern and Formosan subterranean termite (Isoptera: Rhinotermitidae). Sociobiol. 19: 349-362.

Su, N.-Y., P. M. Ban, and R. H. Scheffrahn. 1993. Foraging populations and territories of the eastern subterranean termite (Isoptera: Rhinotermitidae) in Southeastern Florida. Environ. Entomol. 22: 1113-1117.

Thorne, B. L. 1998. Biology of subterranean termites in the genus *Reticulitermes*. Part I, pp. 1-30. *In* NPCA research report on subterranean termites. National Pest Control Association, Dunn Loring, VA.

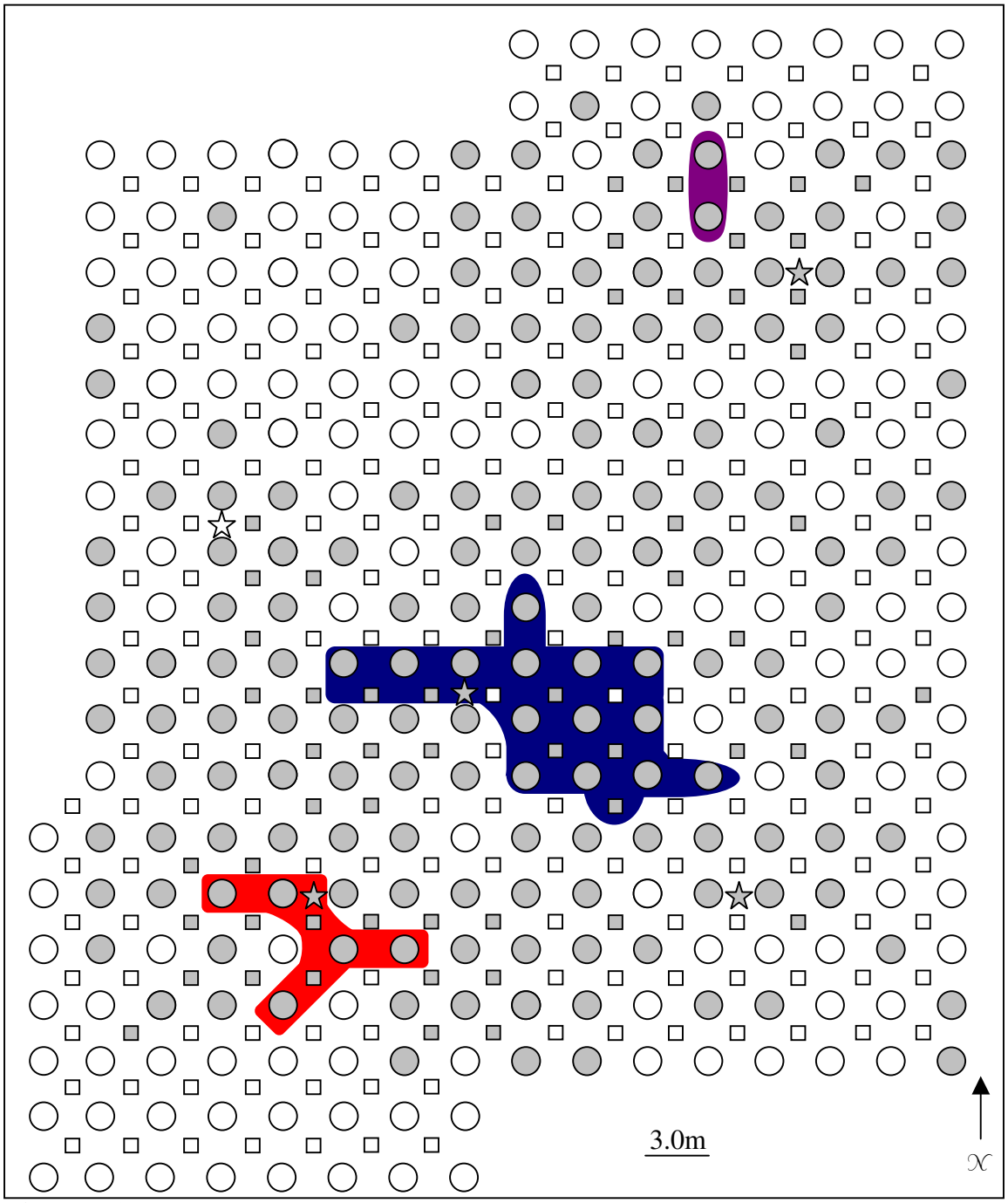

Figure 3.1. Foraging territories (colored areas) of three colonies of *Reticulitermes flavipes* located on the Nature Conservancy's Tallgrass Prairie Preserve north of Pawhuska, OK. Each circle denotes a 10.2cm diameter by 20.3cm deep in-ground monitoring station, squares denote surface ground boards, and stars denote 20.3cm diameter by 25cm deep in-ground monitoring stations. Solid and open figures represent monitoring devices with and without termite activity, respectively.

Table 3.1. Foraging territories and maximum linear foraging distances of three colonies of *Reticulitermes flavipes* on the Nature Conservancy's Tallgrass Prairie Preserve.

Colony	Number of active monitors	Foraging territory m^2	Maximum linear distance m
Purple	2	9.0	3.0
Blue	21	92.3 *	19.0
Red	6	24.8 †	9.5

*Twenty monitors subtended 4.5m^2 each, and one monitor (L-3) subtended 2.25m^2.

† Five monitors subtended 4.5m^2 each, and one monitor (L-4) subtended 2.25m^2.

Table 3.2. Foraging population estimates (Lincoln Index and Weighted Mean Model) and average caste ratios (expressed in percent soldiers) of three colonies of *Reticulitermes flavipes* on the Nature Conservancy's Tallgrass Prairie Preserve.

Colony	Monitor	Mark-release-recapture cycle*									Lincoln Index (SE)	Weighted Mean Model (SE)	Caste Ratio
		M1	n1	m1	M2	n2	m2	M3	n3	m3			
Purple	42	2489	484	45	409	22	1	22	638	67			4.19
	27	0	252	7	192	538	41	394	510	47			4.64
	Total	2489	736	52	601	560	42	416	1148	114	35,229 (4,710)	36,302 (2,523)	4.46
Blue	L-3	237	3208	72	2970	1326	185	1287	183	32			2.33
	EP	0	157	5	86	0	0	0	0	0			1.91
	160	0	0	0	0	421	14	405	856	37			3.37
	ER	0	0	0	0	49	2	45	0	0			4.08
	174	0	0	0	0	214	6	209	0	0			2.34
	175	0	0	0	0	59	3	57	295	20			8.19
	176	0	0	0	0	322	7	313	776	57			3.46
	FF	0	0	0	0	25	1	23	0	0			4.00
	189	0	0	0	0	91	4	86	277	22			5.71
	190	0	0	0	0	1696	39	1658	0	0			0.88
	FV	0	0	0	0	333	10	284	0	0			0.90
	144	0	0	0	0	0	0	0	245	7			2.86
	158	0	0	0	0	0	0	0	627	35			3.51
	161	0	0	0	0	0	0	0	75	4			12.00
	EO	0	0	0	0	0	0	0	222	5			0.90
	191	0	0	0	0	0	0	0	226	14			2.65
	Total	237	3365	77	3056	4536	271	4367	3782	233	10,357 (1,167)	76,812 (3,189)	2.69
Red	216	1387	114	2	96	22	1	17	164	2			2.16
	L-4	0	0	0	0	1567	4	1282	1215	27			3.67
	249	0	0	0	0	228	2	199	112	4			12.35
	235	0	0	0	0	0	0	0	322	2			3.11
	Total	1387	114	2	96	1817	7	1498	1813	35	79,059 (55,411)	183,495 (27,995)	3.65

*Numbers (1-3) indicate mark-release-recapture cycle. For each cycle, M indicates the number of marked termites released, n indicates the number of termites (marked and unmarked) recaptured, and m indicates the number of marked individuals among recaptured termites.

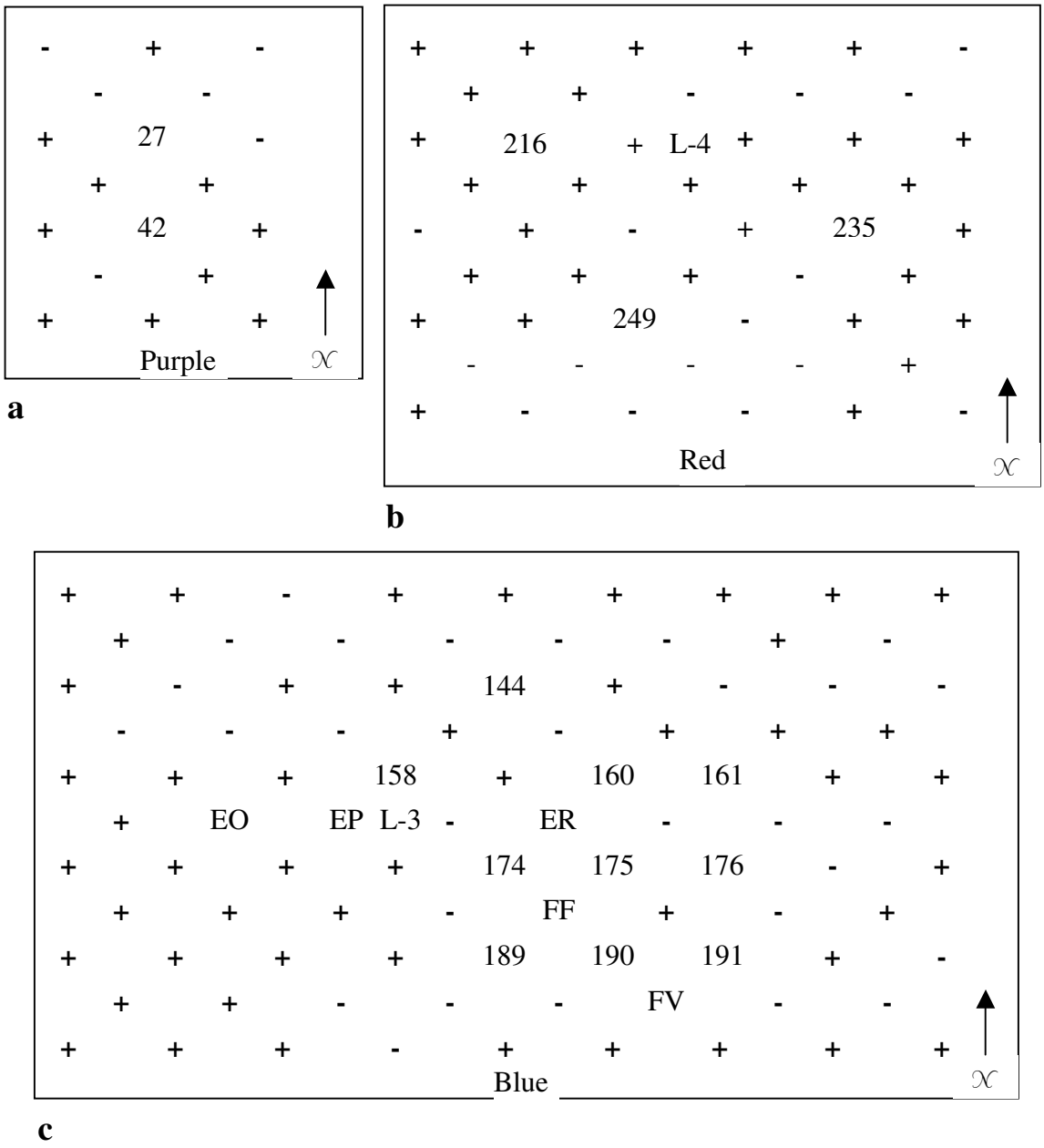

Figure 3.2. Monitoring device identifications (see Table 3.2) for (a)purple colony ; (b) red colony; (c) blue colony. Numbers and letters denote 10.2cm diameter below-ground stations and surface ground-boards, respectively. L-4 and L-3 denote 20.3cm diameter below-ground stations. For monitoring devices without dyed termites during initial three mark-release-recapture cycles, each "**+**" denotes devices with termite activity and each "**-**" denotes devices without termite activity.

CHAPTER IV. SOIL MOVEMENT BY *RETICULITERMES FLAVIPES* (ISOPTERA: RHINOTERMITIDAE)

(Environmental Entomology)

ABSTRACT

Movement of soil by foraging subterranean termites is important from both an ecological and control standpoint. Previous studies indicated a positive relationship between proportions of sand in a substrate and termite tunneling rates. The purpose of this study was to evaluate tunneling and soil movement capabilities of *Reticulitermes flavipes* (Kollar) in a no-choice test using field collected soils comprised of different proportions of sand, silt, and clay. Results indicate that soil texture significantly influences both the rate of tunneling and amount of soil removed from foraging tubes. Termites tunneled at a significantly faster rate and excavated significantly more soil from foraging tubes packed with higher proportions of sand. Soil texture had little effect on mortality or wood consumption. Soil movement capabilities of subterranean termites in a native tallgrass prairie habitat and the resulting impact on soil turnover in this unique ecosystem are discussed.

KEY WORDS subterranean termites, Rhinotermitidae, soil movement, soil particle size, soil texture, caste ratios

DURING THEIR NORMAL FORAGING ACTIVITY, subterranean termites construct shelter tubes as well as earthen nests from the surrounding soil. In addition, termites are capable of moving large quantities of soil throughout soil horizons. Movement of soil by foraging termites is important from both an ecological and control standpoint. Nutting et al. (1987) showed that apart from their important roles in litter decomposition and nutrient cycling, termites are the primary source of soil turnover in the Sonoran Desert. Because some termite species are incapable of either moving or penetrating specifically sized coarse sands, using sands as a termite barrier has been evaluated (Ebeling and Pence 1957; Smith and Rust 1991; Tamashiro et al. 1991, Su and Scheffrahn 1992, Kard 1996, Lewis et al. 1996). Termites also utilize soil from surrounding areas to build tunnels through termiticide-treated soil, resulting in treatment failures (McDaniel and Kard 1994). Houseman and Gold (2003) evaluated the tunneling capabilities of *Reticulitermes flavipes* (Kollar) using varying proportions of sand artificially mixed with soil in laboratory assays. Their results indicated that the proportion of sand in a substrate has a positive relationship to tunneling rates. The current study evaluates the tunneling capabilities of *R. flavipes,* the most commonly encountered termite in Oklahoma (Brown et al. 2004), in a no-choice test using soils of varying proportions of sand, silt, and clay collected intact from a native tallgrass prairie habitat. Results provide information on the effects of soil texture on termite foraging and on the soil movement capabilities of subterranean termites as it relates to soil turnover in this native habitat.

Materials and Methods

Termites. Termites used in these experiments were taken from two laboratory colonies of *R. flavipes*. Colony 1 was collected from a wildland site on the Nature Conservancy's Tallgrass Prairie Preserve located in Osage county, ten miles north of Pawhuska, OK. Colony 2 was collected from a monitoring device adjacent to a building (Noble Research Center) on the campus of Oklahoma State University, Stillwater, OK. Termites were maintained in 10gal galvanized steel cans containing moist sand/vermiculite substrate, and fed southern yellow pine (*Pinus* sp.) sapwood prior to use in these experiments (Kard et al. 2003).

Forty groups of 475 late-instar workers from each of the two colonies were used for these experiments. Soldiers were added to each group to provide stability as well as to create a more accurate representation of an actual foraging population. Groups of 500 termites were evaluated to determine soldier proportions in each colony, numbers of soldiers added to each group were based on observed proportions. Therefore, 25 and 10 soldiers were added to each group of termites from Colonies 1 and 2, respectively. Thus, 20,000 termites from Colony 1 and 19,400 termites from Colony 2, for a total of 39,400 termites were required.

Soils. Four distinct soils of different texture were used for this experiment. Three soils were collected on the tallgrass prairie preserve. The 38,700 acres that make up the current preserve are a remnant of the original tallgrass prairies that once covered over 142 million acres in North America. The preserve is grazed by ~3,200 bison and is dominated by the tallgrasses, big bluestem (*Andropogon gerardii* Vitman), Indiangrass (*Sorghastrum nutans* (L.)), and switchgrass (*Panicum virgatum* L.), with extensive areas

of black jack (*Quercus marilandica* Muenchh)/post oak (*Quercus stellata* Wangenh) crosstimbers. Soils from this site were selected based on their different textures (% sand:silt:clay). The first soil is classified in the Bigheart series thatconsists of shallow, well-drained to somewhat excessively-drained, moderately permeable soils formed from weathered sandstone (Soil Survey Staff 2005). The Bigheart sample used in this study was collected from an upper hill slope in a crosstimbers habitat and was classified as a Sandy Loam (75:17.5:7.5 sand:silt:clay). The Bates series, the second soil used, consists of moderately deep, well-drained, moderately permeable soils found on broad smooth ridge crests and sideslopes of hills (Soil Survey Staff 2005). This sample was classified as a Loam (37.5:47.5:15 sand:silt:clay). The third sample collected was from soil classified in the Summit series thatconsists of very deep, moderately wel l-drained, slowly permeable soil that formed in material weathered from residual shales or colluvial calcareous clays (Soil Survey Staff 2005). This sample was classified as a Clay Loam (22.5:45:32.5 sand:silt:clay). The fourth soil was collected from a shipment of sand excavated at Perkins, OK (Continental Sand), that is typically used as afoundation fill for residential and commercial construction. This soil is classified in the Goodnight series that consists ofvery deep, excessively -drained, rapidly permeable soil formed in sandy eolian sediments (Soil Survey Staff 2005). This sample was classified as a Loamy Sand (77.5:15:7.5 sand:silt:clay). All texture classifications were determined by the Soil, Water and Forage Analytical Laboratory on the campus of Oklahoma State University, Stillwater, OK.

To determine field moisture content, 100g portions of each soil type were weighed, dried at 105^{o}C for 24h, and reweighed. So that accurate comparisons of initial and final

dry weights of soil used in the experiment could be obtained, each of the four soil samples was dried in its entirety at 105°C for 24h and dry weights of portions of each soil type needed to fill the foraging tubes were determined. Samples were then rehydrated to their observed field moisture contents by adding relative amounts of sterile, distilled, deionized water.

Experimental Design. A test unit consisted of two foraging arenas (initial release arena and satellite arena) connected by a 30cm section of Tygon® tubing (12.7mm inside diameter, 17.5mm outside diameter) packed with a known weight of one of the four soils (Figure 4.1). Cylindrical plastic containers (10cm diameter, 6.6cm height) with a removable lid (Pioneer Plastics, Inc., Dixon, KY) were used for both the initial release and satellite arenas. A circular hole (17.5mm diameter) was drilled in the sidewall 0.5cm up from the bottom of each arena to receive the foraging tube, and a rubber stopper was used to plug each hole. Each arena was partially filled with a mixture of 162g sand, 18g vermiculite, and 45ml sterile, distilled, deionized water, resulting in a sand/vermiculite mixture in a 9:1 ratio (wt:wt) and 20% moisture content by weight. The substrate covered the top of the drill holes when tamped down evenly. A block of Radiata pine (*Pinus radiata* D. Don) measuring 2.0 × 2.0 × 1.2cm was placed centrally in each arena. Each block was dried at 105°C for 24h, weighed, and remoistened by soaking in sterile, distilled, deionized water prior to placement in an arena.

Ten replicates for each of two colonies and four soil types were used for this experiment. Termites were placed in the initial release arena and allowed to acclimatize for 24h. After 24h, the rubber stoppers were removed and opposite ends of the packed foraging tubes were inserted into the initial release and satellite arenas. Termites were

allowed to forage freely for 4wk. Test units were assessed daily for presence of termites in satellite arenas. The elapsed time, in days, taken for termites to tunnel through the foraging tube and reach the satellite arena was recorded for each test unit. After 4wk, each unit was dismantled, surviving termites counted, and soil remaining in the foraging tube was dried at 105^{o}C and weighed.

Data Collected and Statistical Analyses. The four response variables analyzed were elapsed time taken (days) for termites to reach the satellite arena (tunneling rate), weight of soil removed from foraging tubes, wood consumption, and mortality. A PROC MIXED (SAS Institute 2001) procedure was used to compare results between colonies and soil types. A PROC CORR (SAS Institute 2001) procedure was used to identify correlations between pairs of response variables. Significance was determined at the $P \leq 0.05$ level.

Results and Discussion

Tunneling Rate and Removal of Soil From Foraging Tubes. Tunneling rates (Table 4.1) indicated that termites tend to tunnel faster through soils with higher proportions of sand and lower proportions of clay. This trend was more apparent in Colony 2, where the time to reach the satellite arena significantly increased as the percent of sand in each soil type decreased. It is interesting to note that termites from Colony 1 (collected from the prairie preserve), while tunneling at a significantly quicker rate through loamy sand, tunneled at similar rates through the other soils collected from the prairie preserve, regardless of texture.

Weights of soil removed from foraging tubes (Table 4.2) generally followed the same trends as the tunneling rates, with termites excavating more soil from tubes containing

higher proportions of sand, although this trend was less obvious for Colony 1. Termites from both colonies excavated a significantly higher amount of loamy sand than the three other soil types. Although both colonies excavated clay loams the least, the amounts were only significantly different with regard to Colony 2.

Wood Consumption. Generally, wood consumption was not affected by soil type, as few differences in wood weight loss were significant (Table 4.3). Interestingly, there were few significant differences in consumption data between initial release and satellite arenas.

Mortality. Mortality was mixed and did not follow any recognizable trend relative to soil types, with the exception that mortality was lowest for both colonies in clay loam (Table 4.4). Colony 1 sustained higher mortality than Colony 2 when data were averaged over allsoil type s. It is interesting to note that while termites tunneled the slowest and excavated the least soil from foraging tubes with the lowest proportion of sand, this trend had no obvious negative affects on either mortality or wood consumption.

In general, soil texture affected both rate of tunneling and amount of soil excavated although no soil tested in this study was impervious to termites. These results, using naturally occurring soils from north-central Oklahoma, were consistent with those of Houseman and Gold (2003), who used soils artificially mixed with varying proportions of sand. Termites in their study excavated more soil and tunneled the quickest through foraging tubes with the highest proportion of sand. Because clayey soils have a smaller average particle size than sandy soils, our results add to the growing body of evidence that the effort required by foraging termites to tunnel through soils has an inverse relationship to the average particle size of the soil (Houseman and Gold 2003; Cornelius

2005). This conclusion is supported by the correlation analyses (Table 4.5). Results of correlation analyses showed a highly significant negative correlation between wood consumption and the amount of soil excavated from foraging tubes ($P < 0.0001$), and a highly significant positive correlation between wood consumption and time taken to reach the satellite arena ($P = 0.0015$). These correlations indicate that more energy was required (exhibited by higher consumption levels) by termites tunneling through clayey soils (smaller average particle size, smaller amounts of soil excavated, and increased time required to reach the satellite arena) than those tunneling through sandy soils (larger average particle size, larger amounts of soil excavated, and decreased time required to reach satellite arena). Correlation analyses also indicated a significant negative relationship between the amount of soil excavated and the time taken to reach the satellite arena ($P < 0.0001$), indicating that termites removed more soil from foraging tubes that were easily tunneled (sandy soils). There were no significant correlations between mortality and any of the other three response variables.

Active termite foraging populations on the tallgrass prairie preserve have been estimated to range from 10,357 to 183,495 individuals (Brown et al. unpublished). In the current study termites excavated 3.232×10^{-4} to 1.149×10^{-3}g of soil/termite/day. It follows then, that the average termite colony on the tallgrass prairie is capable of moving 3.35 to 210.89g of soil/day, possibly vertically and horizontally throughout soil horizons depending on the number of foragers and texture of soil. Normal foraging activities of subterranean termites undoubtedly have an important impact on nutrient cycling and soil turnover in this native tallgrass prairie habitat (Nutting et al. 1987). Further studies of termite impact on soil factors would be enlightening.

Acknowledgments

Thanks to Greg Broussard, Jake Boyett, and Jeremy Dennis for assistance in field collections and identification of soils, as well as to Brian Carter for guidance in experimental design.

Literature Cited

Brown, K. S., B. M. Kard, and M. P. Doss. 2004. 2002 Oklahoma Termite Survey (Isoptera). J. Kansas Ento. Soc. 77: 61-69.

Cornelius, M. L. 2005. Effect of particle size of different sands on the tunneling behavior of the Formosan subterranean termite (Isoptera: Rhinotermitidae). Sociobiol. 45:173-184.

Ebeling, W., and R. J. Pence. 1957. Relation of particle size to the penetration of subterranean termites through barriers of sand or cinders. J. Econ. Entomol. 50: 690-692.

Houseman, R. M. and R. E. Gold. 2003. Factors that influence tunneling in the Eastern subterranean termite, *Reticulitermes flavipes* (Kollar). J. Agric. and Urban Entomol. 20: 69-81.

Kard, B. M. 1996. High-grade stainless-steel mesh and sand-barrier field tests for control of subterranean termites. Proc. National Conf. Urban Entomol. Ferbruary 18-20, 1996. Arlington TX. 1 Pp. (Abstract).

Kard, B. M., J. L. Etheridge, E. J. Mallete, and N. M. Rich. 2003. Procedures for preparing subterranean termites for laboratory studies (Isoptera: Rhinotermitidae). Sociobiol. 41: 495-511.

Lewis, V. R., M. I. Haverty, D. S. Carver, and C. Fouche. 1996. Field comparison of sand or insecticide barriers for control of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) infestations in homes in Northern California. Sociobiol. 28: 327-336.

McDaniel, C. A., and B. M. Kard. 1994. The latest in termiticide degradation. Pest Control Tech. 22: 80, 84, 86, 90-91.

Nutting, W. L., M. I. Haverty, and J. P. Lafage. 1987. Physical and chemical alteration of soil by two subterranean termite species in Sonoran Desert grassland. J. Arid Environs. 12: 233-239.

SAS Institute. 2001. PROC user's manual, version 8.2. ed. SAS Institute, Cary, NC.

Smith, J. L. and M. K. Rust. 1991. Factors affecting the tunneling behavior of the western subterranean termite, *Reticulitermes hesperus* Banks. pp. 28-33. *In* Haverty, M. I. and W. W. Wilcox [technical coordinators], Proc. symposium on current research on wood-destroying organisms and future prospects for protecting wood in use. USDA For. Serv. Gen. Tech. Rpt. PSW-128. 65 Pp.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. 2005. Official Soil Series Descriptions [Online WWW]. Available URL: "http://soils.usda.gov/technical/classification/osd/index.html" [Accessed 9 June 2004].

Su, N-Y., and R. H. Scheffrahn. 1992. Penetration of sized particle barriers by field populations of subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 85: 1453-1457.

Tamashiro, M., J. R. Yates, R. T. Yamamoto, and R. H. Ebesu. 1991. Tunneling behavior of the Formosan subterranean termite and basalt barriers. Sociobiol. 19: 163-170.

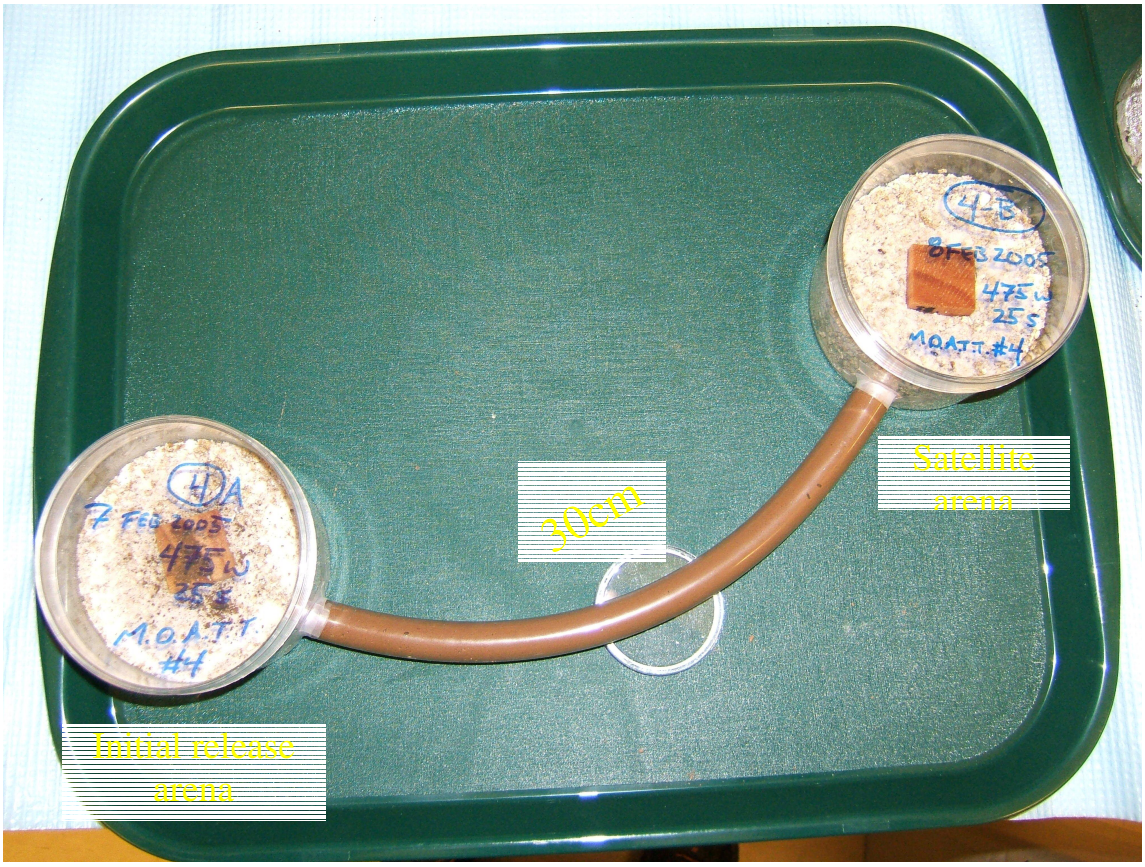

Figure 4.1. No-choice test unit with initial release and satellite arenas containing sand/vermiculite substrate and a pine block, connected by a soil-packed 30cm foraging tube.

Table 4.1. Elapsed time (days) to tunnel to a satellite arena by *R. flavipes* during a 4 wk foraging test.

	Elapsed Time, Mean ± SEM*		
Soil Texture†	Colony 1	Colony 2	Colony 1+2
Loamy Sand	1.60 ± 0.16a,x	1.10 ± 0.10a,x	1.35 ± 0.11a
Sandy Loam	2.80 ± 0.29b,x	1.90 ± 0.10b,y	2.35 ± 0.18b
Loam	2.60 ± 0.16b,x	2.60 ± 0.16c,x	2.60 ± 0.11b
Clay Loam	2.70 ± 0.15b,x	3.30 ± 0.21d,y	3.00 ± 0.15c

*Means followed by the same letter are not significantly different, $P > 0.05$ (PROC MIXED; SAS Institute 2001); a, b, c, and d, down columns; x and y across rows.

† Soils are listed in order of decreasing proportions of sand and increasing proportions of clay.

Table 4.2. Weight (g) of soil removed from foraging tubes by*R. flav ipes* during a 4 wk foraging test.

	Soil removed, Mean ± SEM*		
Soil Texture†	Colony 1	Colony 2	Colony 1+2
Loamy Sand	16.09 ± 1.01b,x	14.96 ± 1.04d,x	15.53 ± 0.72c
Sandy Loam	7.56 ± 0.36a,x	6.67 ± 0.47b,x	7.11 ± 0.31b
Loam	6.35 ± 0.74a,x	10.79 ± 0.49c,y	8.57 ± 0.67b
Clay Loam	5.74 ± 0.47a,x	4.39 ± 0.33a,x	5.07 ± 0.32a

*Means followed by the same letter are not significantly different, $P > 0.05$ (PROC MIXED; SAS Institute 2001); a, b, c, and d, down columns; x and y across rows.

† Soils are listed in order ofdecreasing proportions of sand and increasing proportions of clay.

Table 4.3. Weight loss(g) of pine blocks due to *R. flavipes* feeding during a 4 wk foraging test.

Soil Texture[†]	Wood weight loss, Mean ± SEM[*]		Sum of Initial Release and Satellite Arenas ± SEM
	Initial Release Arena	Satellite Arena	
		Colony 1	
Loamy Sand	0.259 ± 0.066a,x	0.332 ± 0.056a,x	0.591 ± 0.016a
Sandy Loam	0.295 ± 0.062ab,x	0.450 ± 0.082a,x	0.745 ± 0.040b
Loam	0.363 ± 0.039ab,x	0.456 ± 0.044a,y	0.820 ± 0.037b
Clay Loam	0.444 ± 0.044b,x	0.383 ± 0.063a,x	0.827 ± 0.061b
		Colony 2	
Loamy Sand	0.342 ± 0.057a,x	0.348 ± 0.079a,x	0.690 ± 0.049a
Sandy Loam	0.429 ± 0.030ab,x	0.289 ± 0.043a,x	0.718 ± 0.024a
Loam	0.335 ± 0.050a,x	0.392 ± 0.055a,x	0.727 ± 0.083a
Clay Loam	0.545 ± 0.034b,x	0.332 ± 0.039a,y	0.877 ± 0.015b

[*]Within colonies, means followed by the same letter are not significantly different, $P > 0.05$ (PROC MIXED; SAS Institute 2001); a, b, c, and d, down columns; x and y across rows.

[†] Soils are listed in order of decreasing proportions of sand and increasing proportions of clay.

Table 4.4. Mortality of *R. flavipes* during a 4 wk foraging test.

	Mortality, Mean ± SEM* (%)		
Soil Texture†	Colony 1	Colony 2	Colony 1+2
Loamy Sand	56.10 ± 6.73 (11.2)b,x	33.80 ± 5.35 (7.0)ab,y	44.95 ± 4.90 (9.1)b
Sandy Loam	71.70 ± 3.49 (14.3)c,x	49.80 ± 6.36 (10.3)c,y	60.75 ± 4.33 (12.3)c
Loam	54.20 ± 6.30 (10.8)ab,x	43.70 ± 2.68 (9.0)bc,x	48.95 ± 3.54 (9.9)bc
Clay Loam	40.20 ± 4.91 (8.0)a,x	22.70 ± 5.53 (4.7)a,y	31.45 ± 4.12 (6.4)a

*Means followed by the same letter are not significantly different, $P > 0.05$ (PROC MIXED; SAS Institute 2001); a, b, c, and d, down columns; x and y across rows.

† Soils are listed in order of decreasing proportions of sand and increasing proportions of clay.

Table 4.5. Correlation analyses among the four response variables.

	Pearson Correlation Coefficients (*P* value)			
	Days*	Move†	Cons‡	Mort§
Days	1.0000	-0.5803 (< 0.0001)	0.3483 (0.0015)	-0.1182 (0.2964)
Move	-0.5803 (< 0.0001)	1.0000	-0.4617 (< 0.0001)	-0.0197 (0.8627)
Cons	0.3483 (0.0015)	-0.4617 (< 0.0001)	1.0000	-0.2051 (0.0680)
Mort	-0.1182 (0.2964)	-0.0197 (0.8627)	-0.2051 (0.0680)	1.0000

*Elapsed time taken (days) for termites to reach the satellite arena (tunneling rate).

† Weight of soil removed from foraging tubes.

‡ Wood consumption.

§ Mortality.

CHAPTER V. VARIABLES AFFECTING CUTICULAR HYDROCARBON PROFILES IN TERMITES (ISOPTERA: RHINOTERMITIDAE)

(Journal of Chemical Ecology)

ABSTRACT

Preparation protocols for extracting cuticular hydrocarbons from subterranean termites for chemotaxonomic studies are evaluated. Hydrocarbon profiles from fresh (frozen) specimens are compared with those of dried specimens. Hexane extracts of groups of 200 subterranean termite workers dried at 70°C for 6-hrs contained components that were predominantly internal and showed similarity to hydrocarbons isolated from total lipid extracts. Intact, brush-collected termites were held at -20°C prior to lipid extraction in hexane. These extracts contained no internal components when visualized by thin-layer chromatography. Thus,the isolated hydrocarbons more accurately reflected cuticular hydrocarbons compared with dried termite extracts. The possibility of internal lipid contamination due to cuticular damage during desiccation and aspiration is discussed and preparation methodologies that ensure extraction of only cuticular lipid components are proposed.

KEY WORDS subterranean termites, Rhinotermitidae, *Reticulitermes flavipes*, cuticular hydrocarbons, gas chromatography, thin-layer chromatography

THE FUNDAMENTAL TAXONOMIC TREATMENT of the termites of North America was completed over eighty-five years ago by Banks and Snyder (1920). Subsequently, many termite researchers have indicated the need for taxonomic revisions (Weesner, 1970; Haverty and Nelson, 1997; Thorne, 1998; Haverty et al., 1999; Jones, 2000). Termite species identification is problematic because workers are the most abundant caste in termite colonies but are not morphologically useful in species determination. Identifications are made based on soldiers that comprise only 1-5% of the caste ratio at any given time, and on winged reproductives that are only seasonally found in colonies.

Correct identification of termites in the genus *Reticulitermes* based solely on morphological characteristics can be ambiguous due to overlap in measurements among species. In the past twenty years, this has prompted research efforts focusing on developing additional methods which may be used to accurately identify species. One such method is cuticular hydrocarbon analysis. Hydrocarbons, organic compounds containing only carbon and hydrogen, frequently comprise the major fraction of lipids on the surface of insect cuticle and are vitally important in the prevention of dessication (Blomquist et al., 1987). Insect cuticular hydrocarbons are also known to have many semiochemical functions including territory marking, sex attractants, defense secretions, and species and caste recognition cues (Howard and Blomquist, 1982). These functions compel us to investigate cuticular hydrocarbons as a tool for termite identification.

Many factors are known to affect profiles of termite species established by cuticular hydrocarbon analysis (Haverty et al., 1996a). Among these are the number of specimens used, handling of specimens prior to extraction, choice of solvent, and duration of extraction. The optimal extraction protocol is one that isolates the complete cuticular

hydrocarbon profile without extracting internal lipids (Blomquist et al., 1987). It is also preferable to have a standardized protocol so that comparisons can be made between collaborators. The current standard protocol for analyzing termite hydrocarbons involves a 10min. extraction in hexane of 100 – 200 workers, depending on size of the termite species being studied (Haverty et al., 1996a). The methodology for handling specimens prior to extraction has evolved from first freezing specimens at -20°C (Howard et al., 1980,1982; Haverty et al., 1988), to using live specimens (Watson et al., 1989; Haverty et al., 1991), to heat-drying specimens (Haverty et al., 1996a,b; Haverty and Nelson, 1997; Haverty et al., 1999; Jenkins et al., 2000; Nelson et al., 2001). Resulting profiles of hydrocarbons extracted from live and dried specimens were compared by Haverty et al. (1996a). Placing live specimens in hexane caused them to evacuate their gut contents. It was concluded that drying specimens prior to extraction was preferable (Haverty et al., 1996a).

During our initial attempts to utilize cuticular hydrocarbons for termite species identification, we noted marked differences between hydrocarbon profiles resulting from fresh (frozen at -20°C) compared with dried specimens. We know of no study to date that compares termite hydrocarbon profiles extracted from intact frozen specimens with those of heat-dried specimens. Additionally, aspirating termites during collection caused them to evacuate their gut contents and also caused mechanical cuticular damage which could result in contamination of cuticular hydrocarbons by internal components during the extraction process.

Therefore our objectives were, first, to identify the location of termite hydrocarbon components, determining those that are predominantly internal or external (cuticular).

Second, we examined and compared hydrocarbon profiles resulting from specimens prepared by drying, drying and agitating (to simulate shipment), aspirating and freezing, or brush-collecting and freezing, for possible contamination by internal components.

Materials and Methods

Termites. *Reticulitermes flavipes* were collected from an active colony on the campus of Oklahoma State University, Stillwater, and maintained in the laboratory (Kard et al., 2003). Workers beyond the third instar were selected for analyses.

Hydrocarbon Isolation. For each hydrocarbon extraction replicate, 200 termites were collected from the laboratory colony into 7ml scintillation vials. All glassware used in the extraction process was pre-rinsed with glass distilled *n*-hexane to remove any residual hydrocarbon contamination. Cuticular lipids were extracted by immersing termites in 5ml redistilled *n*-hexane for 10min. Solvent from each extraction was transferred via glass pipette to a clean vial. Fifty microliters of 0.2µg/µl *n*-triacontane (*n*C30)in hexane was added to each vial as an internal standard. The mixture was then evaporated to dryness under nitrogen at 60^{o}C. Hydrocarbons were isolated according to Blailock et al., (1976) by dissolving samples in 200µl *n*-hexane and transferring the solution to a 100-200 mesh silica gel (Sigma-Aldrich Co., St. Louis, MO) minicolumn (14.5mm × 6mm ID). Each sample vialwas rinsed twice with an additional 200µl *n*-hexane and collective washes were also added to the minicolumn. Hydrocarbons were eluted with 6mls *n*-hexane and evaporated to dryness under nitrogen at 60^{o}C. All samples were stored at -20^{o}C until analysis.

Gas Chromatography. GC analysis was preformed with a Hewlett-Packard (HP) 5890 Gas Chromatograph interfaced with HP Chemstation software. Hydrocarbon samples

were dissolved in 50µl *n*-hexane, and 1µl aliquots were automatically injected onto a DB-1 capillary column with a 0.1µm film thickness (30m × 0.25mm ID; J&W Scientific, Folsum, CA) using an HP 7673A Automatic Sampler (Hewlett Packard, Sunnyvale, CA). Samples were introduced using a splitless injector set at 250°C with a purge time of 1.85min to 59.50min. Ultrapure helium was the carrier gas at 1ml/min with the GC oven parameters as follows: 180°C for 1.75min, then + 3°C/min to 320°C, and hold final temperature for 18.00min. Peaks were detected with a flame ionization detector operating at 350°C. Hydrocarbon equivalent chainlengths were determined by comparing retention times with reference hydrocarbon standards (Sigma Aldrich Co., St. Louis, MO) of which 1µl of a 1mg/ml solution was automatically injected.

Hydrocarbon Location. To establish whether isolated hydrocarbons originated internally or externally, three replicates of two groups of termites were examined (200/replicate). Termites for each group were initially collected via aspiration and frozen at -20°C for 24hrs prior to extraction. Both external (cuticular) and internal hydrocarbons were examined in the first experimental group (split group) as follows: cuticular lipids were extracted first by immersing termites in 5ml *n*-hexane for 10min, then internal lipids were isolated from the same insects by extracting the total remaining lipids according to Bligh and Dyer (1959). Hydrocarbons were isolated from each extract via silica gel minicolumns. In the second experimental group, total hydrocarbon profiles (cuticular + internal) were examined by extracting total lipids and then isolating hydrocarbons (Bligh and Dyer, 1959) from total lipid extracts as described above. All isolated hydrocarbons were held in a dry state at -20°C until GC analysis.

Effects of Specimen Preparation Protocols. To determine quantitative and qualitative effects of different sample preparation protocols on hydrocarbon profiles, termites were collected and stored under the following four treatments (200/replicate × 4 replicates): GROUP 1 – collected via aspiration and dried at 70°C for 6hrs; GROUP 2 - collected via aspiration, dried at 70°C for 6hrs, then agitated for a 10sec burston an o rbit shaker (Labline Instruments Inc., Melrose Park, IL) at 3rps to simulate shipping; GROUP 3 - collected via aspiration and frozen at -20°C; GROUP 4 - brush-collected and frozen at -20°C. Cuticular lipids were extracted from each treatment group (see *Hydrocarbon Isolation*). Quantitative GC analysis was conducted on purified hydrocarbon extracts from replicates 1 – 3. Total amounts of extracted hydrocarbons were calculated from resulting profiles and comparisons were made using PROC MIXED; TUKEY (SAS Institute 2001) procedure. Qualitative analysis of internal lipid contamination was determined via high performance thin-layer chromatography (HPTLC) of replicate 4. HPTLC was conducted by dissolving replicate 4 with 100µl of chloroform and spotting 1µl onto a clean HPTLC-GHLF plate (10 × 20-cm, 150µ-thick uniplates, Analtech, Newark, DE). Plates were developed in an 80:20:1 (v:v:v) hexane:diethyl ether:acetic acid solvent system. Developed plates were treated with 3% cupric acetate in a 15% phosphoric acid solution and heated until lipids were visualized. Lipid components were identified by visual comparison with two external standards (1µl of 10mg/ml Nu-Check TLC standard 18-5, Nu-Check Prep. Inc., Elysian, MN, and 1µl of 10mg/ml 1-eicosene, Sigma, St. Louis, MO).

Results and Discussion

Hydrocarbon Location. Examples of profiles of cuticular hydrocarbons (split group), internal hydrocarbons (split group), and total hydrocarbons are given in Figure 5.1. Profiles indicate two different sets of hydrocarbons. One set is predominantly internal and one set corresponds to cuticular hydrocarbons. All three replicates of external hydrocarbons consisted of nine peaks eluting between 18.62 and 25.89min that correspond to hydrocarbons of chain length 23 to 27 with no peaks beyond the internal standard, n-C30 (Figure 5.1a). Internal hydrocarbon traces consisted of 42 to 46 peaks eluting between 16.59 and 57.92min which correspond to hydrocarbons of chain length 22 to 42, with several peaks beyond the internal standard (Figure 5.1b). Comparisons between the external, internal, and total hydrocarbon traces (Figure 5.1a,b,c) indicate a majority of hydrocarbons in the insect are internal.

Effects of Specimen Preparation Protocols. Hydrocarbon profiles in Figure 5.2 indicate termite preparation methods affect resulting traces. Termites dried prior to extraction produced hydrocarbon traces consisting of 45 to 46 peaks eluting between 16.58 and 57.91min which correspond to hydrocarbons of chain length 22 to 42 (Figure 5.2a). These hydrocarbon profiles are similar to those visualized in the internal and total hydrocarbon profiles from Experiment 1 (Figure 5.1b,c). Agitating dried samples (Group 2; Figure 5.2b) produced little change in profiles compared with Group 1 (Figure 5.2a). Profiles from a subsequent group of termites that were dried and shipped via first class mail were quantitatively and qualitatively similar to profiles of Groups 1 and 2 (data not shown).

Hydrocarbon profiles from specimens aspirated and frozen prior to extraction (Group 3) resulted in nine peaks eluting between 18.62 and 25.89min corresponding to hydrocarbons of chain length 23 to 26 with no peaks beyond the internal standard n-C30 (Figure 5.2c). Peak number and location of hydrocarbon profiles from termites collected via aspiration/freezing or brush collection/freezing were similar (Figure 5.2d). Quantitative analysis of hydrocarbons extracted using each of the four collection/preparation protocols indicate a significant difference in amounts extracted from dried specimens compared with amounts from frozen specimens (Table 5.1). Species differentiation based on cuticular hydrocarbon profiles relies on differences in composition of the hydrocarbon mixture, as well as the relative amounts of each component. Therefore, it is important to identify differences in peak number and location in profiles resulting from different preparation protocols, and identify any quantitative differences that may result. Table 5.1 illustrates the differences in the average total amount of hydrocarbon extracted from termites using each of the four preparation protocols, as well as the total lipid extraction method. The largest total amount of hydrocarbon was extracted from specimens that were dried and agitated prior to extraction. Total amounts of hydrocarbon extracted from specimens that were dried and those that were dried and then agitated were not significantly different from the amounts extracted using the total lipid extraction method, indicating the presence of internal contaminants.

HPTLC results further confirmed the contamination of cuticular extracts with internal lipid components due to sample preparation technique (Figure 5.3). Both dried and dried/agitated extracts contained high levels of triglycerides and phospholipids. These

components are primarily internal lipids and have not been reported from the cuticle of an insect. Aspirated/frozen samples contained much lower amounts of internal lipids and extracts of brush collected specimens contained only hydrocarbon, free fatty acid, and cholesterol which are likely cuticular lipid components. Because workers are soft bodied, aspirating may damage the cuticle and result in contamination of the extract with internal components.

These results have important implications for termite chemotaxonomic and kin recognition studies. Termite taxonomic hierarchies determined from samples containing both internal and external hydrocarbons, although reported as cuticular only, have been shown to be reliable discriminators of species (Haverty et al., 1996b; Haverty and Nelson, 1997; Haverty et al., 1997; Haverty et al., 1999; Haverty et al., 2000; Jenkins et al., 2000; Nelson et al. 2001; Copren et al., 2005). It must be emphasized that there is currently little information on the role of termite internal hydrocarbons or their variability due to environmental factors or diet, therefore, it must be recognized that due to sampling and sample handling there is a definable proportion of internal hydrocarbons in past studies. Dietary effects on both internal and cuticular hydrocarbons are important areas for future studies. One such study by Rojas et al. (2005) indicated that diet may affect relative ratios of termite cuticular hydrocarbons. However, the study evaluated changes in the free fatty acid fraction and did not address cuticular hydrocarbons. Further investigation into such dietary affects on cuticular components is warranted. In addition, solid phase microextraction (SPME) has recently been evaluated as a tool for elucidating termite cuticular hydrocarbons (Bland et al. 2003; Florane et al. 2004). In light of our findings, we agree with Bland et al. (2004) that direct contact SPME would be preferable

to headspace SPME (HS-SPME) for extracting only surface components as dessication involved in HS-SPME would likely result in the extraction of internal components.

This paper indicates that care must be used during specimen collection and preparation to ensure that only those hydrocarbons from the insect cuticle are extracted. Damage to the termite cuticle from drying or rough handling and/or termite defecation or regurgitation must be minimized, as we have demonstrated, to ensure the integrity of a hydrocarbon sample claiming to contain only surface components. Therefore, the authors suggest that brush-collected/frozen termites provide the most accurate cuticular hydrocarbon profiles.

Acknowledgments

Thanks to Robin Madden and Jack Dillwith for guidance with experimental design, laboratory procedures, and manuscript preparation.

Literature Cited

Banks, N. and T. E. Snyder. 1920. Revision of the Nearctic termites. U.S. Natl. Mus. Bull. 108: 228pp.

Blailock, T. T., G. J. Blomquist, and L. L. Jackson. **1976.** Biosynthesis of z-methylalkanes in the crickets *Nemobius fasciatus* and *Gryllus pennsylvanicus*. Biochem. Biophys. Res. Commun. 68: 841-849.

Bland, J. M., W. L. A. Osbrink, M. L. Cornelius, A. R. Lax, and C. B. Vigo. 2003. Detection of termite cuticular hydrocarbons by solid-phase microextraction (SPME). Sociobiol. 41: 91-104.

Bligh, E. G. and W. J. A. Dyer. 1959. Rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911-917.

Blomquist, G. J., D. R. Nelson, and M. de Renobales. 1987. Chemistry, biochemistry, and physiology of insect cuticular lipids. Arch. Insect Biochem. Physiol. 6: 227-265.

Copren, K. A., L. J. Nelson, E. L. Vargo, and M. I. Haverty. 2005. Phylogenetic analyses of mtDNA sequences corroborate taxonomic designations based on cuticular hydrocarbons in subterranean termites. Mol. Phylogenet. Evol. 35: 689-700.

Florane, C. B., J. M. Bland, C. Husseneder, and A. K. Raina. 2004. Diet-mediated inter-colonial aggression in the Formosan subterranean termite *Coptotermes formosanus*. J. Chem. Ecol. 30: 2559-2573.

Haverty, M. I., M. Page, L. J. Nelson, and G. J. Blomquist. 1988. Cuticular hydrocarbons of dampwood termites, *Zootermopsis*: Intra- and intercolony variation and potential as taxonomic characters. J. Chem. Ecol. 14: 1035-1058.

Haverty, M. I., L. J. Nelson, and M. Page. 1991. Preliminary investigations of the cuticular hydrocarbons from North American *Reticulitermes* and tropical and subtropical *Coptotermes* (Isoptera: Rhinotermitidae) for chemotaxonomic studies. Sociobiol. 19: 51-76.

Haverty, M. I., B. L. Thorne, and L. J. Nelson. 1996a. Hydrocarbons of *Nasutitermes acajutlae* and comparison of methodologies for sampling cuticular hydrocarbons of Caribbean termites for taxonomic and ecological studies. J. Chem. Ecol. 22: 2081-2109.

Haverty, M. I., B. T. Forschler, and L. J. Nelson. 1996b. An assessment of the taxonomy of *Reticulitermes* (Isoptera: Rhinotermitidae) from the Southeastern United States based on cuticular hydrocarbons. Sociobiol. 28: 287-318.

Haverty, M. I. and L. J. Nelson. 1997. Cuticular hydrocarbons of *Reticulitermes* (Isoptera: Rhinotermitidae) from Northern California indicate undescribed species. Comp. Biochem. Physiol. 118B: 869-880.

Haverty, M. I., M. S. Collins, L. J. Nelson, and B. L. Thorne. 1997. Cuticular hydrocarbons of termites of the British Virgin Islands. J. Chem. Ecol. 23: 927-964.

Haverty, M. I., L. J. Nelson, and B. T. Forschler. 1999. New cuticular hydrocarbon phenotypes of *Reticulitermes* (Isoptera: Rhinotermitidae) from the United States. Sociobiol. 34: 1-21.

Haverty, M. I., R. J. Woodrow, and J. K. Grace. 2000. Cuticular hydrocarbons of termites of the Hawaiian Islands. J. Chem. Ecol. 26: 1167-1191.

Howard, R. W. and G. J. Blomquist. 1982. Chemical ecology and biochemistry of insect hydrocarbons. Ann. Rev. Entomol. 27: 149-172.

Howard, R. W., C. A. McDaniel, and G. J. Blomquist. 1980. Chemical mimicry as an integrating mechanism: Cuticular hydrocarbons of a termitophile and its host. Science 210: 431-433.

Howard, R. W., C. A. McDaniel, and G. J. Blomquist. 1982. Chemical mimicry as an integrating mechanism for three termitophiles associated with *Reticulitermes virginicus* (Banks). Psyche 89: 157-167.

Jenkins, T. M., M. I. Haverty, C. J. Basten, L. J. Nelson, M. Page, and B. T. Forschler. 2000. Correlation of mitochondrial haplotypes with cuticular hydrocarbon phenotypes of sympatric *Reticulitermes* species from the Southeastern United States. J. Chem. Ecol. 26: 1525-1542.

Jones, S. C. 2000. Subterranean termites. *In* R. E. Gold and S. C. Jones [eds.], Handbook of Household and Structural Insect Pests. pp. 119-124. Entomol. Soc. Amer., Lanham, MD.

Kard, B. M., J. L. Etheridge, E. J. Mallete, and N. M. Rich. 2003. Procedures for preparing subterranean termites for laboratory studies (Isoptera: Rhinotermitidae). Sociobiol. 41: 495-511.

Nelson, L. J., L. G. Cool, B. T. Forschler, and M. I. Haverty. 2001. Correspondence of soldier defense secretion mixtures with cuticular hydrocarbon phenotypes for chemotaxonomy of the genus *Reticulitermes* in North America. J. Chem. Ecol. 27: 1449-1479.

Rojas, M. G., C. Werle, N. Cottrell, and J. A. Morales-Ramos. 2005. Changes in ratios of four cuticular hydrocarbons in Formosan subterranean termite workers (*Coptotermes formosanus*) (Isoptera: Rhinotermitidae) due to diet. Sociobiol. 46: 131-140.

SAS Institute. 2001. PROC user's manual, version 8.2. ed. SAS Institute, Cary, NC.

Thorne, B. L. 1998. Biology of Subterranean Termites of the Genus *Reticulitermes*. pp. 1-30. National Pest Control Assoc., Dunn Loring, VA.

Watson, J. A. L., W. V. Brown, L. R. Miller, F. L. Carter, and M. J. Lacey. 1989. Taxonomy of *Heterotermes* (Isoptera: Rhinotermitidae) in south-eastern Australia: Cuticular hydrocarbons of workers, and soldier and alate morphology. System. Entomol. 14: 299-325.

Weesner, F. M. 1970. Termites of the Nearctic Region. *In* K. Krishna and F. M. Weesner [eds.], Biology of Termites. pp. 477-525. Academic Press, New York.

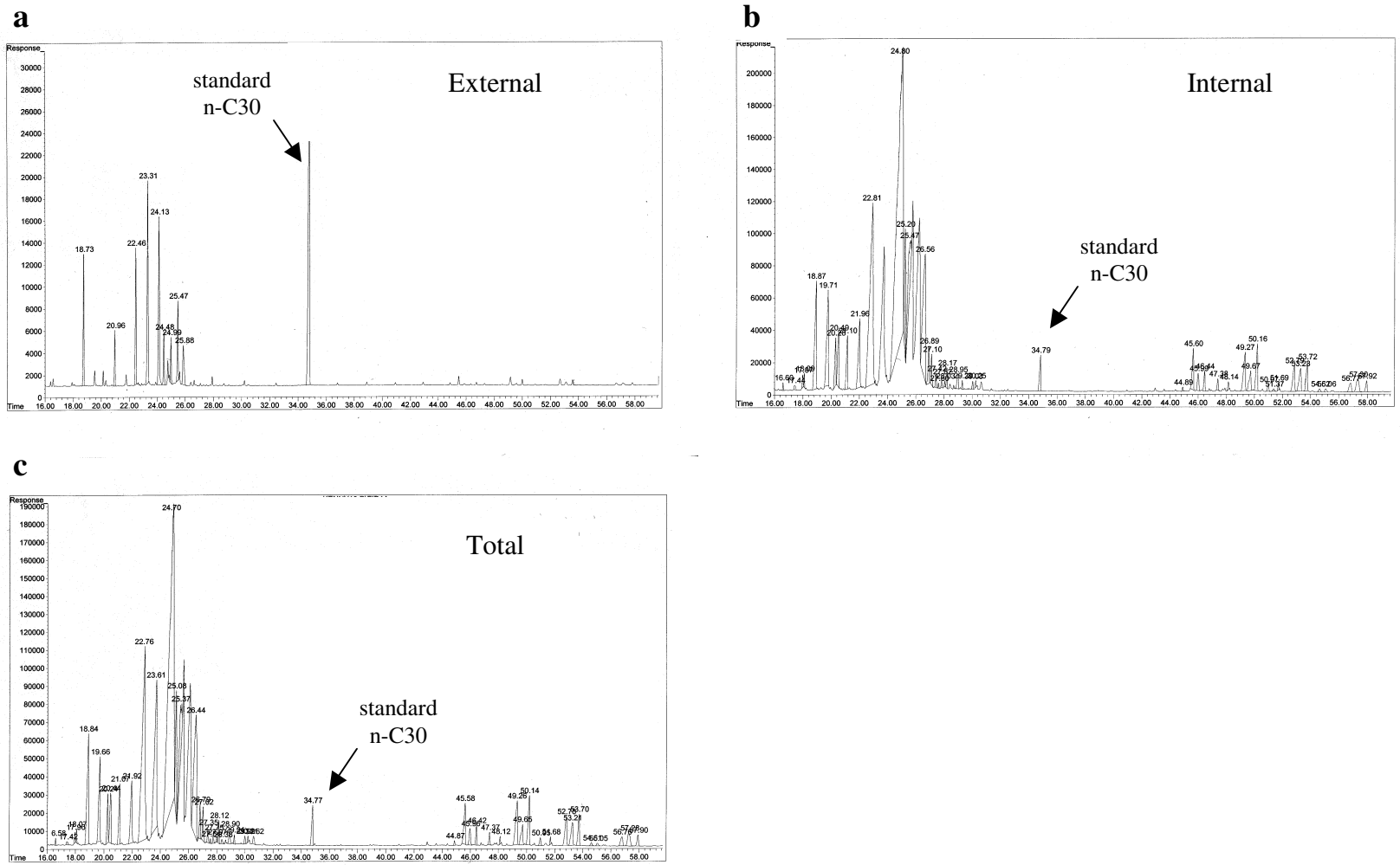

Figure 5.1. Traces of (a) external hydrocarbons from split group; (b) internal hydrocarbons from split group; and (c) total hydrocarbon extract (Bligh and Dyer 1959).

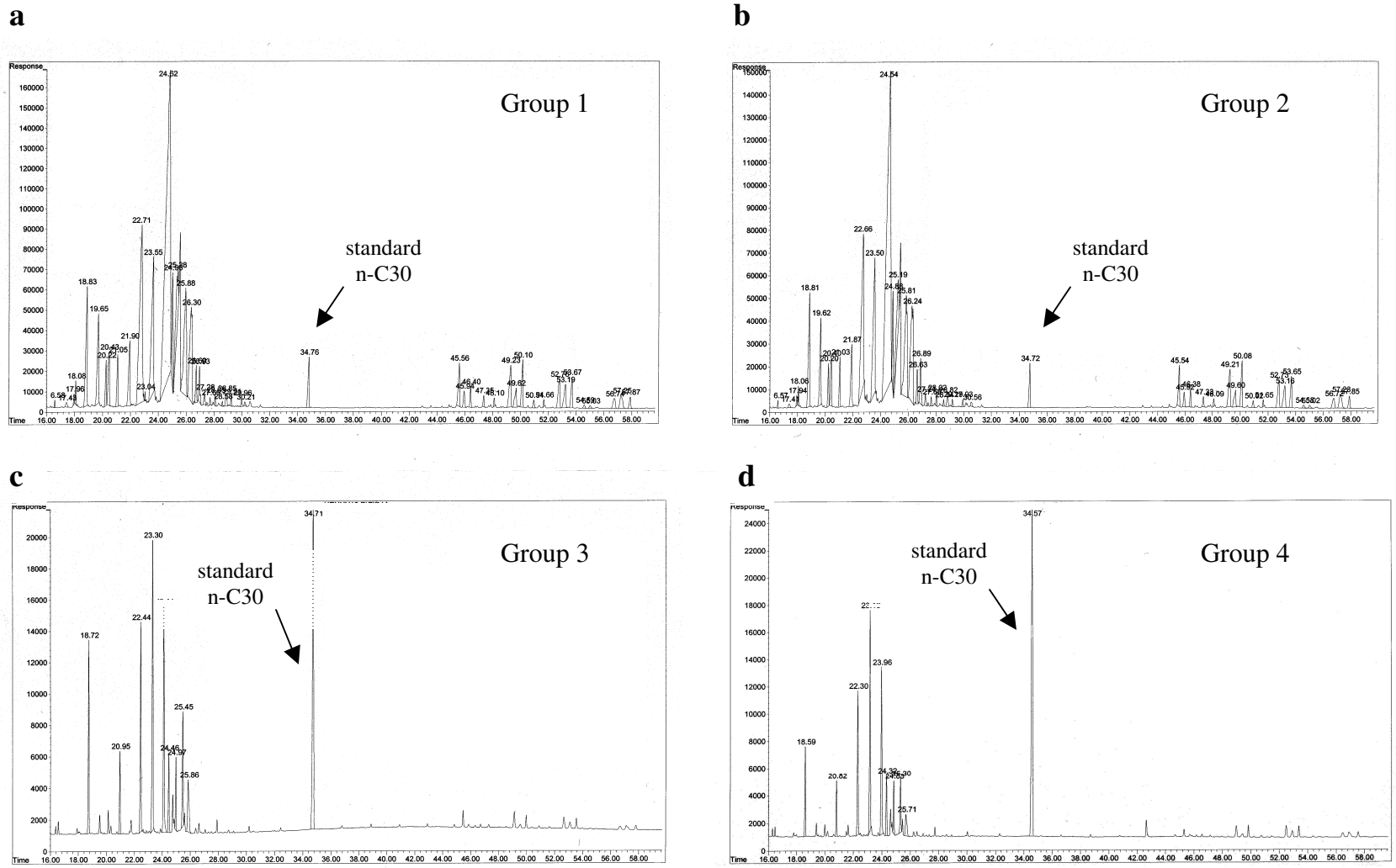

Figure 5.2. Traces of specimens prepared by (a) drying (70°C); (b) drying/agitating; (c) aspirating/freezing (-20°C); (d) brush-collecting/freezing (-20°C).

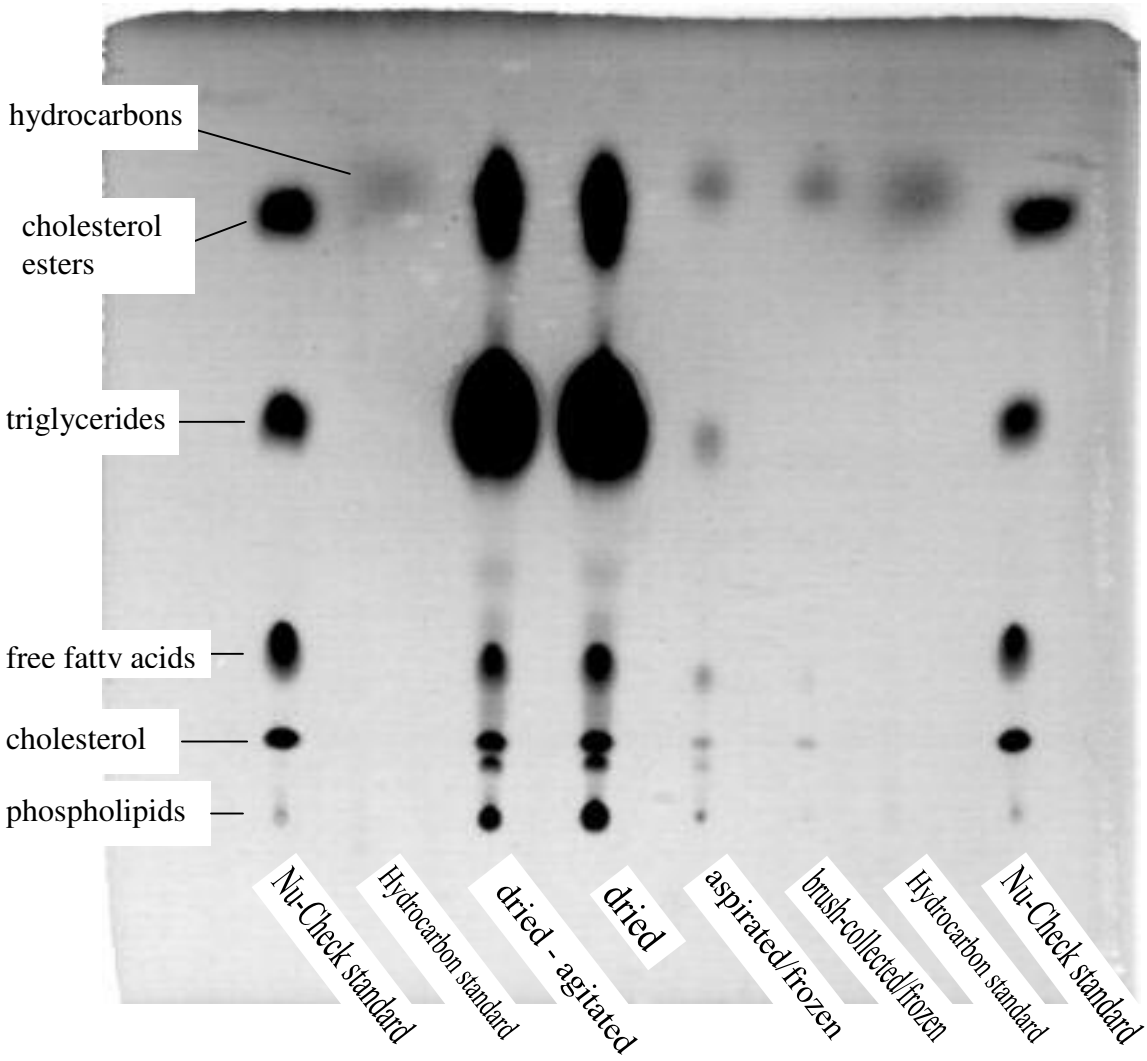

Figure 5.3. High performance thin layer chromatography (HPTLC) of lipid extracts showing differences resulting from different sample preparation protocols.

Table 5.1. Average total amounts (μg) of hydrocarbons extracted using different termite preparation protocols.[*]

Preparation method	Total HC ± SEM[†]
Total (Bligh and Dyer)	678.44 ± 122.3a
Dried (70°C)	711.67 ± 27.60a
Dried (70°C)– Agitated	727.42 ± 45.49a
Aspirated/Frozen (-20°C)	32.23 ± 2.51b
Brush-Collected/Frozen (-20°C)	99.15 ± 15.96b

[*] n = 3.

[†] Means followed by the same letter are not significantly different, $P \leq 0.05$ (PROC MIXED; TUKEY; SAS Institute 2001).

CHAPTER VI. SUMMARY AND CONCLUSIONS

The research described herein provides valuable information on identification and ecology of the termites of Oklahoma. Results of the colony characterization study indicate that the relatively high termite pressure on the tallgrass prairie is most likely a result of many small colonies of *R. flavipes* foraging in close proximity to each other. When compared with estimates of foraging territories of *R. flavipes* in other areas of the United States, colony territories in this Oklahoma native habitat are somewhat small. One possible explanation for this phenomenon is that the colonies evaluated in this study could be insipient colonies that had not reached full maturity. This conclusion is, in part, supported by the low proportion of soldiers within the colonies examined. Estimates of foraging numbers; however, indicate that these colonies, while having relatively small foraging ranges, are comprised of tens to hundreds of thousands of foragers. This would lead one to conclude that there are enough cellulose resources in the tallgrass habitat to sustain large populations of termites within relatively small areas.

Results of the second study indicate that termites tunnel faster through soils containing greater proportions of sand. Colonies of *R. flavipes* evaluated in this study also excavated more soil from foraging tubes that were packed with higher percentages of sandy soils compared with more loamy or clayey soils. Correlation analyses indicated that termites expend more energy, thus requiring more food, when tunneling through clayey soils than when tunneling through sandy soils. This conclusion is supported by a study on the Formosan subterranean termite *Coptotermes formosanus* Shiraki conducted by Cornelius (2005). When coupled with foraging population estimates, results from this study show that termite colonies on the tallgrass prairie preserve are capable of moving 3.35 to 210.89-g of soil/day depending on the size of the colony and texture of the soil.

This is strong evidence that termites are vital to soil turnover in this native tallgrass prairie ecosystem.

Finally, results of the third study indicate that current procedures for preparing termite specimens for cuticular hydrocarbon analyses likely result in contamination with internal hydrocarbons. Currently, little is known about the role of internal hydrocarbons or their variability due to environmental factors or diet. It follows then, that this contamination factor may potentially explain some of the variability in species determinations based on cuticular hydrocarbon analysis seen in the literature (Haverty et al. 1996; Haverty and Nelson 1997; Haverty et al. 1999). Results of this study prove that brush-collecting specimens and then freezing them at -20^{o}C results in no internal contamination and must be a primary method used for preparing termites for cuticular hydrocarbon evaluations.

Literature Cited

Cornelius, M. L. 2005. Effect of particle size of different sands on the tunneling behavior of the Formosan subterranean termite (Isoptera: Rhinotermitidae). Sociobiol. 45:173-184.

Haverty, M. I., B. T. Forschler, and L. J. Nelson. 1996. An assessment of the taxonomy of *Reticulitermes* (Isoptera: Rhinotermitidae) from the Southeastern United States based on cuticular hydrocarbons. Sociobiol. 28: 287-318.

Haverty, M. I., and L. J. Nelson. 1997. Cuticular hydrocarbons of *Reticulitermes* (Isoptera: Rhinotermitidae) from Northern California indicate undescribed species. Comp. Biochem. Physiol. 118B: 869-880.

Haverty, M. I., L. J. Nelson, and B. T. Forschler. 1999. New cuticular hydrocarbon phenotypes of *Reticulitermes* (Isoptera: Rhinotermitidae) from the United States. Sociobiol. 34: 1-21

VITA

Kenneth Scott Brown

Candidate for the Degree of

Master of Science

Dissertation: BIOLOGY AND BEHAVIOR OF OKLAHOMA SUBTERRANEAN TERMITES (ISOPTERA: RHINOTERMITIDAE)

Major Field: Entomology

Biographical:

Personal: Born in Cheyenne, Wyoming on November 15, 1977 to Steve and Diana Brown. Married Kathryn Michelle Mannin on June 16, 2001. Son Reece Anthony Brown was born on August 7, 2003.

Education: Graduated from Monett High School, Monett, Missouri in May 1996. Received Bachelor of Science degree in Biology (cum laude) from Missouri Southern State College, Joplin, Missouri in July 2000. Received Master of Science degree in Entomology from Oklahoma State University in December 2002. Completed the requirements for the Doctor of Philosophy degree with a major in Entomology at Okalahoma State University in December 2005.

Experience: Conducted research within the Dept. of Plant Pathology, University of Arkansas, Fayetteville, Arkansas, May to August 1999. Teaching/Research Assistant, Dept. of Entomology and Plant Pathology, Oklahoma State University, Stillwater, Oklahoma, August 2000 to present.

Professional and Honor Memberships: Entomological Society of America, 2000 to present; Southwestern Entomological Society, 2003 to present; Kansas Entomological Society, 2003 to present; Pi Chi Omega National Professional Pest Control Fraternity, 2004 to present; Oklahoma Microscopy Society, 2003 to present; Sanborn Entomology Club, 2000 to present; Alpha Chi National College Honor Scholarship Society, February 2000 to present; Phi Kappa Phi National College Honor Scholarship Society, April 2002 to present; Sigma Pi Social Fraternity, December 1997 to present.

Name: Kenneth Scott Brown Date of Degree: December 2005

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: BIOLOGY AND BEHAVIOR OF OKLAHOMA SUBTERANEAN TERMITES (ISOPTERA: RHINOTERMITIDAE)

Pages in Study: 96 Candidate for the Degree of Doctor of Philosophy

Major Field: Entomology

Scope and Method of Study: The objectives of these studies were to investigate the biology and behavior of Oklahoma subterranean termites including colony characteristics in a native tallgrass prairie habitat, soil movement capabilities, and variables affecting cuticular hydrocarbon profiles. A greater understanding of the basic biology and ecology of termites has become increasingly necessary in recent years as control methods have become more targeted. In the first of the three studies, characterizations of three colonies of subterranean termites including foraging population estimates, foraging territory delineations, and caste ratio determinations were conducted on the Nature Conservancy's Tallgrass Prairie Preserve north of Pawhuska, OK. The second study evaluated the soil movement capabilities of two colonies of *Reticulitermes flavipes* (Kollar) in artificial arenas containing four distinct soil types based on texture. During the final study, preparation techniques for extracting cuticular hydrocarbons from subterranean termites for chemotaxonomic studies were evaluated. Statistical analyses were conducted using PC SAS Version 8.1.

Findings and Conclusions: *R. flavipes* on the Tallgrass Prairie Preserve were found to forage in areas ranging from 9.0 to 92.3-m^2 and contain 10,357 to 183,495 active foragers. Soldiers comprised 2.69 to 4.46% of the total foraging populations. It is concluded that the high termite pressure in this native habitat is likely the result of many small colonies foraging in close proximity to each other. Soil texture significantly influenced both the rate of tunneling and amount of soil removed from foraging tubes over a 4-wk test. Termites tunneled at a significantly faster rate and excavated significantly more soil from foraging tubes packed with higher proportions of sand. Termite hydrocarbon location experiments indicated two distinct sets of hydrocarbons, one that is predominantly internal and one that corresponds to cuticular hydrocarbons. Lipid extracts from termites that were dessicated and/or aspirated prior to extraction contained internal components. Extracts of termites prepared by brush-collection and freezing at -20^{o}C prior to extraction contained no such internal component contamination. It was determined that brush-collecting/freezing specimens prior to extraction yields the most accurate cuticular hydrocarbon profiles and should be the methodology used for termite chemotaxonomic studies.

ADVISOR'S APPROVAL: Bradford M. Kard