LSU Doctoral Dissertations																																												Graduate School

2011

Role of substrate moisture, relative humidity and temperature on survival and foraging behavior of Formosan subterranean termites

Bal Krishna Gautam
Louisiana State University and Agricultural and Mechanical College, bgauta3@tigers.lsu.edu

Recommended Citation

ROLE OF SUBSTRATE MOISTURE, RELATIVE HUMIDITY AND TEMPERATURE ON SURVIVAL AND FORAGING BEHAVIOR OF FORMOSAN SUBTERRANEAN TERMITES

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Entomology

by
Bal Krishna Gautam
M. S., G. B. Pant University of Agriculture and Technology, 2001
December 2011

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to my major professor Dr. Gregg Henderson. He has been a very important person in my life whose unwavering support in every step during my Ph. D. program enabled me to accomplish this degree successfully. I feel myself lucky to have such an excellent and generous mentor and will be guided in the future with his principles of academic integrity. Besides academics, I and my family enjoyed his and his wife Sue's invitations to every year's 'burn' party, thanks from our heart to them both. My committee members Drs. Seth Johnson, Michael Stout and Richard Story deserve my sincere appreciation. Their correct guidance and suggestions, whether research related or course related, always inspired me to think the problems critically and find the solutions. I humbly thank for their support during my Ph. D. program. Thanks are due to Dr. Lixin Mao, Poornima Jayasimha, Chaoling Qi, Ahmad Evans, Luo Pan, Nikhil Nagendra, Cai Wang, Yanxi Liu, Gerald Soderstrum and Amelia Burns for their assistance in numerous occasions in the laboratory and field work. They helped me in big laboratory experiments and gave me good company while collecting termites in scorching summer days. I wish to thank Dr. Timothy Schowalter, head of the Department, who always helped me in my time of need. I am grateful to all other professors in the Department, office staff and all graduate students who made my years in the department very pleasant and comfortable.

At this moment I cannot forget the contribution made by my parents, Meghnath Gautam and Bhima Gautam, whose selfless love and wish to see me successful probably gave me the greatest strength to succeed. It is definitely not easy for them to have me away from home but their sole desire to see me succeed and be happy made it all possible. I have gotten an invaluable help from my brothers and sisters right from my childhood together to this stage thousands of

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The Formosan subterranean termite is an important structural pest in the southern United States, especially Louisiana. Baiting systems and spot treatment with non-repellent delayed action termiticides are among the most eco-friendly control approaches. However, to achieve a desired success from these approaches requires a sophisticated knowledge of foraging behavior and food transfer system of these social insects. Moisture and temperature play a vital role in influencing the foraging behavior of these desiccation prone insects. A series of laboratory experiments were conducted to understand the subtleties of foraging behavior of *Coptotermes formosanus* Shiraki in relation to moisture, relative humidity and temperature. Foraging behavior, food consumption and distribution pattern were studied in a suitably designed sand moisture gradient arena. Results showed that *C. formosanus* can adapt to a wide range of sand moisture levels but not in dry and saturated sand. Next, termites were studied to determine how combinations of various food moisture and temperature levels impact their foraging behavior. *C. formosanus* was unable to maintain sustained feeding when the wood moisture was lower than the fiber saturation point (~ 25% wt/wt). In the absence of moist soil, *C. formosanus* always preferred the highest moisture wood with the optimum feeding and survival obtained at $28\,^{\circ}$C.

Relative humidity and temperature mainly determine the water loss from an organism. Soldiers and workers of *C. formosanus,* which are extremely prone to desiccation, were exposed to various temperature and relative humidity conditions to determine their survival. Even the near saturated relative humidity (~99%) level was not sufficient to keep them from desiccating, indicating that a continuous supply of moisture either from moist substrate or food is required for their normal survival. Likewise, impact of food source disturbance on termites' escape behavior was studied with wet versus dry sand conditions in the periphery of the food source, and its

implications for termite baiting is discussed. Finally, exploratory tunneling behavior was examined in different substrates in different moisture and temperature situations. With the same moisture level, sand was found to be the preferred substrate for aggregation and tunnel construction to sandy loam and loam.

CHAPTER 1

INTRODUCTION

Termites

Termites are eusocial insects with a complex social system (Wilson 1971). They belong to the order Isoptera, which in Greek means equal wings, referring to the similar size, shape and venation of the four wings in adults. They are polymorphic and live in large communities of several hundred to several million individuals (Henderson and Fei 2002). As eusocial insects, they are characterized by 1) an overlapping of generations, 2) cooperative care of young, and 3) presence of a reproductive division of labor, or a caste system comprising of workers, soldiers and reproductive forms (Wilson 1975, Henderson and Fei 2002). Termites are among the non-hymenopteran eusocial arthropods evolved separately from the social Hymenoptera (Donovan et al. 2000).

Origin and Phylogeny

Termites are reported to have evolved approximately 200 million years ago (Krishna 1992) from cockroach-like insects (Prestwich 1983). Systematists generally agree that mantids, cockroaches and termites, which form a super order Dictyoptera, are phylogenetically closely related (Boudreaux 1979, Henning 1981). However, the exact relationship of termites with the other two dictyopterans is not yet fully resolved (Eggleton 2001). Cleveland et al. (1934) suggested that termites might be closely related to the social wood-feeding cockroach *Cryptocercus* on the basis of morphological and behavioral similarities as well as shared flagellate gut symbionts between *Cryptocercus* nymphs and lower termite pseudergates, while Boudreaux (1979) suggested that termites are the sister group of a mantid + cockroach clade and *Cryptocercus* is placed within a cockroach clade, a view also supported by Thorne and Carpenter (1992). Recent molecular studies by Maekawa et al. (1999) and Lo et al. (2000) have suggested that termites are nested within the cockroaches.

Classification

Termites are classified into seven families where Mastotermitidae is placed as the most basal termite group in the family tree (Donovan et al. 2000, Kambhapati and Eggleton 2000, Thompson et al. 2000). Other termite families ordered from most basal to advanced are: Hodotermitidae, Termopsidae, Kalotermitidae, Serritermitidae, Rhinotermitidae and Termitidae (Donovan et al. 2000). Donovan et al. (2000) described all termite families except Termitidae as lower termites. Although all lower termite families do not form a clade they have shared features in having: a simple gut structure and symbiotic protozoans in their gut (Noirot 1995). Higher termites, Termitidae, which are often described as the most derived family, represent about two thirds of all described species (Donovan et al. 2000). Unlike many species of lower termites in which workers are immature ("false workers" or "pseudergates"), all of the species of higher termites possess a sterile worker caste (Noirot 1985).

Distribution

Termites are the major invertebrates in a tropical ecosystem (Wood et al. 1982, Collins 1983, Eggleton et al. 1996), with the highest species richness found in tropical forests (Eggleton 2000). They are sometimes referred to as "ecosystem engineers" due to their vital role in plant decomposition (Wood and Johnson 1986) and other ecosystem services, such as altering soil composition and structure (Lee and Wood 1971), improving drainage and aeration and increasing exchangeable cations (Eggleton 2000, Holt and Lepage 2000, Donovan et al. 2001). Termites' niches include a wide range of terrestrial environments.

Food Habit and Economic Importance

Almost all species of termites feed on cellulosic plant materials at different stages of decay (Bignell and Eggleton 2000) and specialized or occasional food may include fungi, algae,

lichens and members of their own colony (Wood 1978, Hinze et al. 2002). Cellulosic plant materials include living, dead but fresh, dead decomposing and decomposed plant materials (humus). In the quest of their staple food, cellulose, termites inflict indiscriminate damage to even non-cellulosic materials causing significant loss to human interests. In the United States, a conservative estimate of economic loss associated with termites is up to $11 billion annually (Su 2002). Due to this very destructive nature of termites, their important role in nature is often overshadowed and people see them as harmful foes rather than ecological friends.

Termites have complex symbiotic relationships with gut microbes which help to break the complex cellulose to simple digestible sugars (Breznak 2000). Lower termites have dominant populations of flagellate protozoa in their guts (Inoue et al. 2000). In higher termites, however, the flagellates have been lost and replaced by fungi and prokaryotes (in Macrotermitinae of Termitidae) or prokaryotes alone (in other Termitidae) (Eggleton and Tayasu 2001).

According to their feeding habit and moisture requirement, lower termites are categorized as drywood termites, dampwood termites and subterranean termites. Drywood termites (Kalotermitidae) feed on sound dry wood (≈ 13% moisture) and do not need an extra moisture source. Dampwood termites (Termipsidae) infest damp and decaying wood. Subterranean termites attack moist wood and need a continuous supply of moisture for sustained feeding (Potter 2004). For this, subterranean termites require some kind of contact with the ground soil, or in the case of Formosan subterranean termites, ground contact can be cut off if the moisture source is available above ground (Henderson and Fei 2002). Foraging in subterranean termites is accomplished through the extensive excavation of soil to construct underground tunnels or aboveground galleries around the nest, sometimes consisting of a number of satellite nests connected by a tunnel network up to 100 m from the parent nest (King and Spink 1969).

Although there are more than 2600 described species of termites in 281 genera (Kambhapati and Eggleton 2000), only 183 species are known to attack buildings worldwide and 83 of them are considered responsible for significant damage (Edwards and Mill 1986, Su and Scheffrahn 2000). Subterranean termites constitute 80% of these economically important species. In the United States, a total of 45 termite species have been reported (Su and Scheffrahn 1990a) but only 9 of them are considered economically important. They include one higher termite, *Nasutitermes costalis* (Holmgren); two drywood termites, *Cryptotermes brevis* (Walker) and *Incisitermes minor* (Hagen); and six subterranean termites, *Coptotermes formosanus* Shiraki, *C. gestroi* (Wasmann), *Reticulitermes flavipes* (Kollar), *R. virginicus* (Banks), *R. hesperus* Banks and *Heterotermes aureus* (Snyder) (Su and Scheffrahn 1990a, Scheffrahn and Su 1995, Su et al. 1997, Scheffrahn et al. 2002, Baker and Marchosky 2005, Su et al. 2005).

The Formosan Subterranean Termite

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, was first described from Formosa (Taiwan) in 1903 by Tokuichi Shiraki (Abe 1937). This termite is widely believed to have originated in mainland China and transported to Japan and Taiwan in the 16th Century or even before (Su and Tamashiro 1987). Introduction of this species to other tropical and subtropical parts of the world is primarily by commerce (Su and Tamashiro 1987). This termite was first reported from Hawaii at the advent of the 20th Century and along the coast of the continental United States in the mid-20th Century (Su and Tamashiro 1987).

The introduction of the Formosan subterranean termite in the mainland US is believed to be through infested military cargo ships returned from Japan during World War II (Spink 1967, Gay 1969). Although originally misidentified, *C. formosanus* was first reported from Charlseton, South Carolina in 1957 (Chambers 1988) and eight years later, it was found in a Shipyard in

Texas (Spink 1967). In Louisiana, established colonies of *C. formosanus* were first discovered in New Orleans and Lake Charles in 1966 (Spink 1967). Surveys done that same year indicated that *C. formosanus* were introduced in the areas many years earlier (Spink 1967). Woodson et al. (2001) reported that *C. formosanus* was found established in 11 states (including Louisiana) and USAPHC (2010) reported the detection of isolated colonies of this termite in additional 4 states namely Arizona, Arkansas, New Mexico and Virginia. *Coptotermes formosanus* disperse to new habitats either by flying, budding or by passive transport (Forschler et al. 2001). Adults (winged forms) are weak flyers, flying a maximum of 1 km from the parent nest (Messenger and Mullins 2005). Budding, a process where a portion of a parent colony loses contact or migrates away from that parent colony, establishes a separate colony (satellite colony) by formation of secondary reproductives (Thorne et al. 1999). Budding in *Reticulitermes flavipes* has been reported via primary reproductives in Wisconsin where they 'walk away' from mother nest and start a new colony (personal communication, G. Henderson). If not aided by man's activities, budding is also responsible for short distance dispersal. Dispersal through passive transport of infested materials such as soil, wood or plant materials is the main mode of long distance dispersal of this species (Spink 1967, Gay 1969, Weesner 1970, Oi 1998).

Coptotermes formosanus is the most destructive subterranean termite in Louisiana (McMichael 1998, Henderson 2001, Henderson and Fei 2002). Unlike native subterranean termites, *Reticulitermes* spp., *C. formosanus* can attack water-bound trees, can establish above-ground nests, can build colony populations to an extremely high number (up to several million individuals) and is very aggressive in nature (Spink 1967, Tamashiro et al. 1980, Chambers 1988, Henderson 1996, Henderson 2001, Henderson and Fei 2002). Armed with all these features, this invasive species is posing a huge challenge in Louisiana (and other southern states)

by attacking economically important structures such as buildings, homes, boats, utility poles, railroad tiles and underground telephone poles, including live trees and mulches (Henderson and Dunaway 1999, Henderson 2001). Recently, Henderson (2008) suggested that the piping on sloped soil levees due to Formosan subterranean termites' tunneling activities as well as eating of bagasse in the floodwall seams may have possibly compromised the soundness of levee system in New Orleans. New Orleans is believed to have the most severe infestation by *C. formosanus* in North America, if not in the world (Lax and Osbrink 2003). The economic loss due to this invasive pest was estimated to be over $1 billion nationwide with more than $300 million in New Orleans alone (Suszkiw 1998, Lax and Osbrink 2003).

Control Strategies and Understanding Foraging Behavior

From time immemorial, humans have sought to protect their possessions from termites with various measures. The history of termite control practices includes the use of wood preservatives, physical barriers, application of liquid termiticides and baiting systems. Early in the 1900s, arsenical compounds were popular wood preservatives (Randall and Doody 1934, Preston 2000). Beginning from the late 1940s, the United States termite control industries began to rely heavily on the use of cyclodiene (organochlorine) soil termiticides such as chlordane, aldrin and dieldrin to prevent and control subterranean termites (Lewis 1980, Su and Scheffrahn 1990b). The cyclodienes became extremely popular termiticides as they were economical, very effective and stable in soil, sometimes protecting homes from subterranean termites for decades (Su and Scheffrahn 1990b, Grace et al. 1993). Unfortunately, the 'panacea' for subterranean termites did not last long as people started to see the negative effects of these chemicals to the environment and human health. Their long residual toxicity raised huge concerns about their impact in the environment and their fat soluble nature resulted in biomagnification (accretion of

chemical in the fatty tissues) in humans and other animals (Carson 1962, Lewis 1980, Su and Scheffrahn 1990b, Wood and Pierce 1991, Stevens et al. 1993, EPA 1998, Jitunari et al. 1995, Nasir et al. 1998, Walker and Newton 1998). In 1988, EPA banned the use of these chemicals in all forms (EPA 1998).

The next termiticides that came to the market were new formulations of organophosphates (chlorpyrifos, pryfon) and synthetic pyrethroids. However, chlorpyrifos quickly became unpopular due to its short residual activity and unwanted interaction with environmental factors such as binding with clay and cellulose particles thus making it less detectable and less toxic to subterranean termites (Lenz et al. 1990, Smith and Rust 1993). In addition, chlorpyrifos was highly toxic to birds and aquatic animals and posed health risks to human beings (Odenkirchen and Eisler 1988). These factors ultimately led the cancellation of its use as a termiticide in 2000 (EPA 2000). Pyrethroids, on the other hand, effectively kill contacted termites but their repellent property allows foragers to detect the chemical and avoid the treated zones (Su and Scheffrahn 1990b, Rust and Smith 1993, Su et al. 1993, Forschler 1994). Therefore, instead of being killed, termites were more likely to be funneled to the structures through the treatment gaps (Forschler 1994). Non-repellent and delayed action termiticides, such as fipronil, imidacloprid and chlorfenapyr that appeared in the market from late 1990s and early 2000s addressed some of the issues of earlier chemicals such as not repelling the foragers and exhibiting delayed mortality thus allowing the termites to transfer toxicants to their nestmates (Aventis 2001a, b, BASF Corp. 2001, Potter and Hillery 2001, Wagner et al. 2003). However, all of these non-repellent termiticides are more expensive compared to the cyclodiene termiticides that people were used to (Wagner et al. 2003). Moreover, creation of a toxic soil barrier needs applications of large volumes of chemical in soil

which naturally raised concerns of the negative impacts to the environment (Su and Scheffrahn 1998, Potter and Hillery 2001, Verkerk and Bravery 2001).

With the continued issues of health risks and environmental hazards posed by the use of liquid termiticides, renewed interest in termite baiting systems began to emerge in the United States (Jones 1989, Tamashiro et al. 1991, Robertson and Su 1995). From the mid 1990s, commercial termite baits, such as Sentricon™ system (active ingredient: hexaflumuron; Dow AgroSciences), FirstLine® (active ingredient: sulfuramid; FMC Corp.), hydramethylnon bait (Metabolic inhibitor; American Cyanamid Co.), Exterra® (active ingredient: diflubenzuron; Ensystex), and other baits with different designs and active ingredients became available in the market (Su et al. 1995, Pawson and Gold 1996, Henderson and Forschler 1997, Su and Scheffrahn 1998, Potter et al. 2001). Subterranean termite baiting systems exploit the termites' foraging behavior and their food transfer system (trophallaxis) to reduce the population or to eliminate the entire colony from an area (Esenther and Beal 1979, French 1991, Su et al. 1995). For a baiting system to succeed, foragers must find the bait stations, recruit more foragers, consume sufficient amount of bait matrix, carry the toxicant back to the colony and transfer a sufficient dose to the rest of the colony members by trophallaxis (Su 1995, Grace et al. 1996, Su and Scheffrahn 1996, Grace and Su 2001). However, in practice, the subterranean termite foraging behaviors are variable and not adequately understood (Thorne et al. 1996) thus the success of a baiting system is also unpredictable (Forschler 1996).

Knowledge of termite foraging behavior is actually less important when the control approach involves the use of wood preservatives or a large volume of soil barrier termiticides. Success of baiting systems, on the other hand, relies heavily on the detailed understanding of the complex foraging behavior of these cryptic social insects. Not only the baiting system but also

the spot treatment by non-repellent delayed action termiticides cannot be accomplished successfully without understanding the subtleties of their foraging and food distribution systems. However, subterranean termite foraging behavior is very unpredictable as it is influenced by an array of biotic and abiotic factors. Of the abiotic factors, moisture and temperature probably play the most important role in determining which areas are the most suitable for these desiccation-prone insects (Emerson 1955, Rudolph et al. 1990). Previous studies (Delaplane and La Fage 1989, Rudolph et al. 1990, Sponsler and Appel 1990, Delaplane et al. 1991, Forschler and Henderson 1995, Fei and Henderson 1999, 2002, Su and Puche 2003, Fei and Henderson 2004, Nakayama et al. 2004, 2005, Hu and Song 2007, Cornelius and Osbrink 2010) have shed light on some of the aspects of water and temperature relations of *C. formosanus*, however, there still remain many questions unanswered. This dissertation attempts to answer some of those unanswered questions by conducting a series of studies in the laboratory and discussing their real world implications. More specifically, the studies examine the role of substrate moisture, food moisture, relative humidity and ambient temperature on survival and foraging behavior of Formosan subterranean termites.

The dissertation is divided into seven chapters where the first chapter is a general introduction on termites and their economic importance with particular emphasis on Formosan subterranean termites. The study in the second chapter investigates how sand (substrate) moisture level impacts the feeding and habitat selection by these cryptic insects. Since substrate moisture and food moisture are different things, the study in the third chapter examines how various wood moisture levels and ambient temperatures influence their foraging behavior and survival. After understanding the role of substrate moisture and food moisture in their foraging behavior, the study in the fourth chapter demonstrates the influence of various relative humidity

and temperature levels on the survival of these desiccation prone termites in starved condition. The fifth chapter describes a study that attempts to find an answer whether a mechanical disturbance cause *C. formosanus* to abandon the already attacked food, and also to assess the difference in escape behavior when the substrate in the periphery of the food source varies in moisture content. This study will have implications in termite baiting systems where an inevitable disturbance occurs during inspection of monitors and/or replacement with baits. Finally, in the sixth chapter, an examination of the exploratory tunneling behavior of these cryptic insects in various substrate types, substrate moisture content and ambient temperature conditions is conducted. The seventh and the last chapter provides a summary of all the studies. The findings from these studies are important in contributing to the overall understanding of the foraging behavior of *C. formosanus* and hopefully will aid in the design of more robust, more economical and more environmental friendly termite management practices.

References

(EPA) Environmental Protection Agency. 1998. Iris Substance File: Chlordane (Technical) CAS 12789-03-6 (http://www.askthenerd.com/ocol/IRIS/SUBST/0142.HTM Accessed on August 1, 2011).

(EPA) Environmental Protection Agency. 2000. Chlorpyrifos revised risk assessment and agreement with registrants (http://www.ibiblio.org/ecolandtech/NAFEX/message-archives/old/pdf00000.pdf Accessed on August 1, 2011).

Abe, Y. 1937. On the distribution of the oriental termite, *Coptotermes formosanus* Shiraki in Japan. Sci. Rep. Tohoku Univ. 11: 463-472.

Aventis Environmental Science. 2001a. Termidor®SC termiticide label. Montvale, NJ.

Aventis Environmental Science. 2001b. Termidor® Reference Manual. Montvale, NJ.

Baker, P. B., and R. J. Marchosky. 2005. Arizona termites of economic importance. Cooperative Extension, College of Agriculture and Life Sciences, The University of Arizona, Tucson (http://cals.arizona.edu/pubs/insects/az1369.pdf Accessed on August 28, 2011).

BASF Corporation. 2001. Phantom® termiticide label. Triangle Park, NC.

Bignell, D. E., and P. Eggleton. 2000. Termites in ecosystems, pp. 363– 408. *In* T. Abe, D. E. Bignell and M. Higashi (eds.), Termites: Evolution, Sociality, Symbiosis, Ecology. Kluwer Academic Publ., Dordrecht, NL.

Boudreaux, H. C. 1979. Arthropod Phylogeny with Special Reference to Insects. John Wiley and Sons Inc., NY.

Breznak, J. A. 2000. Ecology of prokaryotic microbes in the guts of wood- and litter- feeding termites, pp. 209–231. *In* T. Abe, D. E. Bignell and M. Higashi (eds.), Termites: Evolution, Sociality, Symbiosis, Ecology. Kluwer Academic Publ., Dordrecht, NL.

Carson, R. 1962. Silent Spring. Houghton Mifflin, Boston, MA.

Chambers, D. M. 1988. The distribution, ecology and control of the Formosan subterranean termite,*Coptotermes formosanus* Shiraki, in South Carolina. Ph.D. Dissertation, Dept. Entomol., Clemson Univ., Clemson, South Carolina.

Cleveland, L. R., S. R. Hall, E. P. Sanders, and J. Colier. 1934. The wood-feeding roach *Cryptocercus*, its protozoa, and the symbiosis between protozoa, and the roach. Mem. Am. Acad. Arts Sci. 17: 185-342.

Collins, N. M. 1983. Termite populations and their role in litter removal in Malalaysian rain forests, pp. 311-325. *In* S. L. Sutton, T. C. Whitmore and A. C. Chadwick (eds.), Tropical rain forest: Ecology and management. Blackwell Scientific Publications, Oxford.

Cornelius, M. L., and W. L. A. Osbrink. 2010. Effect of soil type and moisture availability on the foraging behavior of the Formosan Subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 103: 799-807.

Delaplane, K. S., A. M. Saxton, and J. P. La Fage. 1991. Foraging phenology of Formosan subterranean termite (Isoptera: Rhinotermitidae) in Louisiana. Am. Midi. Nat. 125: 222-230.

Delaplane, K. S., and J. P. La Fage. 1989. Preference for moist wood by the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 82: 95-100.

Donovan, S. E, P. Eggleton, W. E. Dubbin, M. Batchelder, and L. Dibog. 2001. The effect of a soil-feeding termite, *Cubitermes fungifaber* (Isoptera: Termitidae) on soil properties: termites may be an important source of soil microhabitat heterogeneity in tropical forests. Pedobiologia 45: 1–11.

Donovan, S. E., D. T. Jones, W. A. Sands, and P. Eggleton. 2000. The morphological phylogenetics of termites (Isoptera). Biol. J. Linn. Soc. 70: 467-513.

Edwards, R., and A. E. Mills. 1986. Termites in buildings: their biology and control. Rentokil Ltd., East Grinstead, U.K.

Eggleton P., D. E. Bignell, W. A. Sands, N. A. Mawdsley, J. H. Lawton, T. G. Wood, and N. C. Bignell. 1996. The diversity, abundance, and biomass of termites under differing levels of disturbance in the Mbalmayo Forest Reserve, southern Cameroon. Phil. Trans. R. Soc. Lond**.** B 351**:** 51-68.

Eggleton, P. 2000. Global patterns of termite diversity, pp. 25-52. *In* T. Abe, M. Higashi and D. E. Bignell (eds.), Termites: Evolution, Sociality, Symbiosis, Ecology. Kluwer Academic Publ., Dordrecht, NL.

Eggleton, P., 2001. Termites and trees: a review of recent advances in termite phylogenetics. Insectes Soc. 48: 187–193.

Eggleton, P., and I. Tayasu. 2001. Feeding groups, life types and the global ecology of termites. Ecol. Res. 16: 941–960.

Emerson, A. E. 1955. Geographic origins and dispersion of termite genera. Fieldiana Zool. 37: 465‑521.

Esenther, G. R., and R. H. Beal. 1979. Termite control: decayed wood bait. Sociobiology 4: 215-320.

Fei, H., and G. Henderson. 1999. Effect of moisture and two nitrogen sources on nest site choice by alates and dealates of *Coptotermes formosanus in* the laboratory (Isoptera: Rhinotermitidae). Sociobiology 34: 581-589.

Fei, H., and G. Henderson. 2002. Formosan subterranean termite (Isoptera: Rhinotermitidae) wood consumption and worker survival as affected by temperature and soldier proportion. Environ. Entomol. 31: 509-514.

Fei, H., and G. Henderson. 2004. Effects of temperature, directional aspects, light conditions and termite species on subterranean termite activity (Isoptera: Rhinotermitidae). Environ. Entomol. 33: 242-246.

Forschler, B. T. 1994. Survivorship and tunneling activity of *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) in response to termiticide soil barriers with and without gaps of untreated soil. J. Entomol. Sci. 29: 43-54.

Forschler, B. T., and G. Henderson. 1995. Subterranean termite behavioral reaction to water and survival of inundation: Implications for field populations. Environ. Entomol. 24: 1592-1597.

Forschler, B. T., J. Harron, and T. M. Jenkins. 2001. Case histories involving the Formosan subterranean termite in Atlanta, Georgia, USA (Isoptera: Rhinotermitidae). Sociobiology 37: 311-322.

Forschler, B.T. 1996. Incidence of feeding by the eastern subterranean termite (Isoptera: Rhinotermitidae) in laboratory bioassays. Sociobiology 28: 265-273.

French, J. R. J. 1991. Baits and foraging behavior of Australian species of *Coptotermes*. Sociobiology 19: 171-186.

Gay, F. J. 1969. Species introduced by man, pp. 459-494. *In* K. Krishna and F. M. Weesner (eds.), Biology of termites, Vol. I. Academic Press, New York.

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

Grace, J. K., C. H. M. Tome, T. G. Shelton, R. J. Ohsiro, and J. R. Yates. 1996. Baiting studies and considerations with *Coptotermes formosanus* (Isoptera: Rhinotermitidae) in Hawaii. Sociobiology 28: 511-520.

Grace, J. K., J. R. Yates, M. Tamishiro, and R.T. Yamamoto. 1993. Persistence of organochlorine insecticides for Formosan subterranean termite (Isoptera: Rhinotermitidae) control in Hawaii. J. Econ. Entomol. 86: 761-766.

Henderson, G. 1996. Alate production, flight phenology, and sex-ratio in *Coptotermes formosanus* Shiraki, an introduced subterranean termite in New Orleans, Louisiana. Sociobiology 28: 319-326.

Henderson, G. 2001. Practical considerations of the Formosan subterranean termite in Louisiana: a 50-year-old problem. Sociobiology 37: 281-292

Henderson, G. 2008. The termite menace in New Orleans: Did they cause floodwalls to tumble? American Entomologist 54: 156-162.

Henderson, G., and C. Dunaway. 1999. Keeping Formosan termites away from underground telephone lines. La. Agr. 42: 5–7.

Henderson, G., and H. Fei. 2002. Comparison of native subterranean termite and Formosan subterranean termite: biology, ecology, and methods of control. Forest Products Society Conference, 11-13 February 2002. Radisson Resort Parkway, Kissimmee, Florida.

Henderson, G., B.T. Forschler. 1997. Termite bait tests. Louisiana Agriculture 40: 9-11.

Henning, W. 1981. Insect phylogeny. Wiley and Sons. New York.

Hinze, B., K. Crailsheim, and R. H. Leuthold. 2002. Polyethism in food processing and social organisation in the nest of *Macrotermes bellicosus* (Isoptera, Termitidae). Insectes Soc. 49: 31–37.

Holt, J. A., and M. Lepage, 2000. Termites and soil properties, pp. 389–407. *In* T. Abe, D. E. Bignell and M. Higashi (eds.), Termites: Evolution, Sociality, Symbiosis, Ecology. Kluwer Academic Publ., Dordrecht, NL.

Hu, X. P., and D. Song. 2007. Behavioral reactions of two subterranean termites (Isoptera: Rhinotemitidae) to instant freezing or chilling temperatures – cold avoidance and survival strategy. Environ. Entomol. 36: 1450-1456.

Inoue, T., O. Kitade, T. Yoshimura, and I. Yamaoka. 2000. Symbiotic associations with protests, pp. 275–288. *In* T. Abe, D. E. Bignell and M. Higashi (eds.), Termites: Evolution, Sociality, Symbiosis, Ecology. Kluwer Academic Publ., Dordrecht, NL.

Jitunari, F., F. Asakawa, N. Takeda, S. Suna, and Y. Manabe. 1995. Chlordane compounds and metabolite residues in termite workers blood. Bull. Environ. Contam. Toxicol. 54: 855-862.

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

Kambhampati, S., and P. Eggleton, 2000. Phylogenetics and Taxonomy, pp. 1–23. *In* T. Abe, D. E. Bignell and M. Higashi (eds.), Termites: Evolution, Sociality, Symbiosis, Ecology. Kluwer Academic Publ., Dordrecht, NL.

King, E. G. J., and W. S. Spink. 1969. Foraging galleries of the Formosan subterranean termite, *Coptotermes formosanus,* in Louisiana. Ann. Entomol. Soc. Am. 62: 536-542.

Krishna, K. 1992. Order Isoptera, pp. 234-241. In D. J. Borrer, C. A. Triplehorn and N. F. Johnson (eds.), An Introduction to the Study of Insects, 6th Edition. Harcourt Brace College Publishers. Fort Worth, TX.

Lax, A. R., and W. L. A. Osbrink. 2003. United States Department of Agriculture-Agriculture Research Service research on targeted management of the Formosan subterranean termite *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). Pest Management Science 59: 788-800.

Lee, K. E., and T. G. Wood. 1971. Termites and Soils. Academic Press, London.

Lenz, M, J. A. L. Watson, R. A. Barrett, and S. Runko. 1990. The effectiveness of insecticidal soil barriers against subterranean termites in Australia. Sociobiology.17: 9-35.

Lewis, D. L. 1980. Environmental and health aspects of termite control chemicals. Sociobiology 5: 197-203.

Lo, N., G. Tokuda, H. Watanabe, H. Rose, M. Slaytor, K. Maekwa, C. Bandi, and H. Noda. 2000. Evidence from multiple gene sequences indicate that termites evolved from wood-feeding cockroaches. Curr. Biol. 10: 801–804.

Maekawa, K., O. Kitade, and T. Matsumoto. 1999. Molecular phylogeny of orthopteroid insects based on the mitochondrial cytochrome oxidase II gene. Zool. Sci. 16: 175–184.

McMichael, J. A. 1998. Survey of subterranean termites (Isoptera: Rhinotermitidae) in trees in Sam Houston Jones State Park, West Lake, Louisiana. M.S. thesis. Department of Entomology, Louisiana State University, Baton Rouge, LA.

Messenger M. T., and A. J. Mullins. 2005. New flight distance recorded for *Coptotermes formosanus* (Isoptera: Rhinotermitidae). Fla. Entomol. 88: 99-100.

Nakayama, T., T. Yoshimura, and Y. Imamura. 2004. The optimum temperature-humidity combination for the feeding activities of Japanese subterranean termites. J. Wood Sci. 50: 530-34.

Nakayama, T., T. Yoshimura, and Y. Imamura. 2005. Feeding activities of *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* (Kolbe) as affected by moisture content of wood. J. Wood Sci. 51: 60-65.

Nasir, K., K. K. Bilto, and Y. Al-Shuraiki. 1998. Residues of chlorinated hydrocarbon insecticides in human milk of Jordanian women. Environ. Poll. 99: 141-148.

Noirot, C. 1985. The caste system in higher termites, pp. 75-86. *In* J. A. L. Watson , B. M. Okot-Kotber and C. Noirot (eds.), Caste Differentiation in Social Insects, Pergamon Press, New York.

Noirot, C. 1995. The gut of termites (Isoptera). Comparative anatomy, systematics, phylogeny 1. Lower termites. Ann. Soc. Entomol. Fr. 31: 197–226.

Odenkirchen, E. W., and R. Eisler. 1988. Chlorpyrifos hazards to fish, wildlife, and invertebrates: a synoptic review. US Fish Wildlife Serv. Biol. Rep. 85, 34 pp.

Oi, F. M. 1998. Formosan subterranean termites. Alabama Cooperative Extension System Circular ANR-1035.

Pawson, B. M., and R. E. Gold. 1996. Evaluation of baits for termites (Isoptera: Rhinotermitidae) in Texas. Sociobiology 28: 485-491.

Potter, M. F. 2004. Termites, pp. 217-361. *In* A. Mallis, S. A. Hedges and D. Moreland (eds.), Handbook of Pest Control, Ninth Edition. GIE Media Inc., Cleveland, OH.

Potter, M. F., and A. E. Hillery. 2001. Exterior-targeted liquid termiticides: an alternative approach to managing subterranean termites (Isoptera: Rhinotermitidae) in buildings. Sociobiology 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. Sociobiology 38: 565-584.

Preston, A. F. 2000. Wood preservation: trends of today that will influence the industry tomorrow. Forest Products Journal 50: 13–19.

Prestwich, G. D. 1983. The chemical defenses of termites. Sci. Am. 29: 76-87.

Randall, M., and T. C. Doody. 1934. Wood preservatives and protective treatments, pp. 463-476. In C.A. Kofoid (ed.), Termites and Termite Control. University of California Press, Berkeley, CA.

Robertson, A. S., and N.-Y. Su. 1995. Discovery of an effective slow-acting insect growth regulator for controlling subterranean termites. Down to Earth. 50: 1-7.

Rudolph, D., B. Glocke, and S. Rathenow. 1990. On the role of different humidity parameters for the survival, distribution and ecology of various termite species. Sociobiology 17: 129-140.

Rust, M. K., and J. L Smith. 1993. Toxicity and repellency of components in formulated termiticides against western subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 86: 1131-1135.

Scheffrahn, R. H., and N. Y. Su. 1995. A new subterranean termite introduced to Florida: *Heterotermes* Froggatt (Rhinotermitidae: Heterotermitinae) established in Miami. Florida Entomologist 78: 623-627.

Scheffrahn, R. H., B. J. Cabrera, W. H. Kern, and N.-Y. Su. 2002. *Nasutitermes costalis* (Isoptera: Termitidae) in Florida: first record of a non-endemic establishment by a higher termite. Fla. Entomol. 85: 273-275.

Smith, J. L., and M. K. Rust. 1993. Cellulose and clay in sand affect termiticide treatments. J. Econ. Entomol. 86: 53-60.

Spink, W. T. 1967. The Formosan subterranean termite in Louisiana. Louisiana State University Circ. 89.

Sponsler, R. C., and A. G. Appel. 1990. Aspects of the water relations of the Formosan and eastern subterranean termites (Isoptera: Rhinotermitidae). Environ. Entomol. 19:15-20.

Stevens, M. F., G. F. Ebell, and P. Psailasavona. 1993. Organochlorine pesticides in Western Australian nursing mothers. Med. J. Australia 158: 238-241.

Su, N.-Y. 1995. Population control of subterranean termites using bait matrix containing hexaflumuron. Down to Earth 1: 8-19.

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

Su, N.-Y., and H. Puche. 2003. Tunneling activity of subterranean termites (Isoptera: Rhinotermitidae) in sand with moisture gradients. J. Econ. Entomol. 96: 88-93.

Su, N.-Y., and M. Tamashiro. 1987. An overview of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the world, pp. 3-15. *In* M. Tamashiro and N.-Y. Su (eds.), The biology and control of the Formosan subterranean termite. College Trop. Ag. Human Resources, Univ. of Hawaii, Honolulu.

Su, N.-Y., and R. H. Scheffrahn. 1990a. Economically important termites in the United States and their control. Sociobiology 17: 77-94.

Su, N.-Y., and R. H. Scheffrahn. 1990b. Comparison of eleven soil termiticides against the Formosan subterranean termite and the eastern subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 83: 1918-1924.

Su, N.-Y., and R. H. Scheffrahn. 1996. A review of the evaluation criteria for bait-toxicant efficacy against field colonies of subterranean termites (Isoptera). Sociobiology 28: 521-530.

Su, N.-Y., and R. H. Scheffrahn. 1998. A review of subterranean termite control practices and prospects for integrated pest management programs. IPM Rev 3: 1-13.

Su, N.-Y., and R. H. Scheffrahn. 2000. Termites as pest of buildings, pp. 437-453. *In* T. Abe, D. E. Bignell and M. Higashi (eds.), Termites: Evolution, Sociality, Symbiosis, Ecology. Kluwer Academic Publ., Dordrecht, NL.

Su, N.-Y., E. M. Thomas, P. M. Ban, and R. H. Scheffrahn. 1995. Monitoring baiting stations to detect and eliminate foraging populations of subterranean termites (Isoptera, Rhinotermitidae) near structures. J. Econ. Entomol. 88: 932-936.

Su, N.-Y., R. H. Scheffrahn, and B. J. Cabrera. 2005. Double trouble: UF/IFAS researchers find another termite in South Florida as destructive as Formosan ʼsuper termiteʼ (http://news.ufl.edu/2005/04/08/super-termite Accessed on August 1, 2011).

Su, N.-Y., R. H. Scheffrahn, and P. M. Ban. 1993. Barrier efficacy of pyrethroid and organophosphate formulations against subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 86: 772-776.

Su, N.-Y., R. H. Scheffrahn, and T. Weissling. 1997. A new introduction of a subterranean termite, *Coptotermes havilandi* Holmgren (Isoptera: Rhinotermitidae) in Miami, Florida. Florida Entomologist 80: 408-411.

Suszkiw, J. 1998. The Formosan termite: a formidable foe. Agric. Res. 46: 4–9.

Tamashiro, M., J. R. Yates, P. Y. Lai, J. K. Jujii, and N-Y. Su. 1980. Size and structure of *Coptotermes formosanus* Shiraki colonies in Hawaii. Proc. 16th Int. Cong. Entomology, 311 pp.

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

Thompson, G. J., O. Kitade, N. Lo, and R.H. Crozier, 2000. Phylogenetic evidence for a single ancestral origin of a "true" worker caste in termites. J. Evol. Biol. 13: 869–881.

Thorne, B. L., and, J. M. Carpenter. 1992. Phylogeny of the Dictyoptera. Syst. Entomol. 17: 253-268.

Thorne, B. L., E. Russek-Cohen, B. T. Forschler, N. L. Breisch, and J. F. A Traniello. 1996. Evaluation of mark release recapture methods for estimating forager population size of subterranean termite (Isoptera: Rhinotermitidae) colonies. Environ. Entomol. 25: 938-951.

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. Ethol. Ecol. Evol. 11: 149–169.

USAPHC (U. S. Army Public Health Command). 2010. Just the facts... Formosan suterranean termites (http://phc.amedd.army.mil/PHC%20Resource%20Library/Formosansubterranean termitesJan2010.pdf Accessed on August 28, 2011)

Verkerk, R. H. J. and A. F. Bravery. 2001. The UK termite eradication programme: justification and implementation. Sociobiology 37: 351-360.

Wagner T., J. Mulrooney, T. Shelton, and C. Petersen. 2003. Reduced risk products steal spotlight. Termiticide Rep. 71: 16–23.

Walker, C. H., and I. Newton. 1998. Effects of cyclodiene insecticides on the sparrowhawk (*Accipiter nisus*) in Britain – a reappraisal of the evidence. Ecotoxicology 7: 185-189.

Weesner, F. M. 1970. Termites of the nearctic region, pp. 477-524. *In* K. Krishna and F. M. Weesner (eds.), Biology of Termites. Academic Press, NY.

Wilson, E. O. 1971. The insect Societies. Harvard Univ. Press, Cambridge, MA.

Wilson, E. O. 1975. Sociobiology. Harvard University Press, Cambridge, MA and London UK.

Wood, T. G., and M. J. Pierce. 1991. Termites in Africa: The environmental impact of control measures and damage to crops, trees, rangeland and rural buildings. Sociobiology 19: 221-234.

Wood, T. G., and R. A. Johnson. **1986.** The biology, physiology, and ecology of termites, pp. 1-68. *In* S. B. Vinson (ed.), The econonic impact and control of social insects. Praeger, NY.

Wood, T. G., R. A. Johnson, S. Bacchus, M. O. Shittu, and J. M. Anderson. 1982. Aundance and distribution of termites (Isoptera) in a riparian forest in the southern Guinea savanna zone of Nigeria. Biotropica 14: 25-39.

Wood, T.G. 1978. Food and feeding habits of termites, pp. 55-80. *In* M.V. Brian (ed.), Production Ecology of Ants and Termites. Cambridge Univ. Press Cambridge, MA.

Woodson, W. D., B. A. Wiltz, and A. R. Lax. 2001. Current distribution of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the United States. Sociobiology 37: 661-671.

CHAPTER 2

**EFFECTS OF SAND MOISTURE LEVEL ON FOOD CONSUMPTION AND
DISTRIBUTION OF FORMOSAN SUBTERRANEAN TERMITES (ISOPTERA:
RHINOTERMITIDAE) WITH DIFFERENT SOLDIER PROPORTIONS[1]**

---

[1] Reprinted by permission of "Journal of Entomological Science"

Introduction

Field studies have indicated that subterranean termites display seasonal variation in activity (Haverty et al. 1974, Haagsma and Rust 1995, Forschler 1996), which is perhaps influenced by extrinsic factors such as moisture and temperature (Haverty et al. 1974). Moisture is one of the most important environmental requirements for the survival of subterranean termites. Because of their soft cuticle with poor water-retaining properties (Moore 1969), subterranean termites lose moisture through the integument more readily than from respiration (Sponsler and Appel 1990) rendering them very susceptible to desiccation. Subterranean termites obtain moisture either from the abiotic environment or from the food source. The water content of the food source is, in turn, directly dependent on the water content of the substrate or soil where the food source is located (Williams 1934). The moisture content of the nest material provides a glimpse of their habitat moisture requirements. Sponsler and Appel (1990) examined the nest material of several species of subterranean termites and reported a moisture content ranging from 23 - 60% by weight. The variation in the moisture content of the nest material may be due to the different types of substrates used by the different species of the termites and their ecological requirements.

Green et al. (2005) studied the substrate moisture preference for 3 species of *Reticulitermes* and reported that *Reticulitermes flavipes* (Kollar) and *Reticulitermes tibialis* Banks had a slightly narrower substrate moisture preference range than *Reticulitermes virginicus* (Banks). Unlike *Reticulitermes* spp., *Coptotermes formosanus* Shiraki is not native to the US and is an extremely aggressive species (Tamashiro et al. 1980) that poses a greater threat to wooden structures. Henderson (2001) reported a dramatic increase in the number of parishes in Louisiana infested by *C. formosanus* by the end of 1990s, reaching 29 parishes from only 5 or 6 infested

parishes reported by La Fage (1987). One of the main factors for its successful establishment in a new area is its ability to forage and adapt to the new environment. Unlike its native counterparts, *C. formosanus* is known to build above-ground nests in trees and buildings (King and Spink 1969) thereby reducing their dependency on below-ground nests (Forschler and Henderson 1995, Henderson and Fei 2002). The adaptation of *C. formosanus* to the environment separated from ground soil leads us to hypothesize that they readily forage in substrates having a relatively wide moisture range. Although there have been some studies involving the moisture effects on *C. formosanus* (Forschler and Henderson 1995, Fei and Henderson 1999, Su and Puche 2003), no studies have been conducted to determine their substrate moisture level preference in multiple choice situations.

Soldier proportion in *C. formosanus* varies seasonally, which is regulated by both environmental as well as colony factors (Haverty 1979, Waller and La Fage 1988, Delaplane et al. 1991, Henderson 1998, Mao et al. 2005). Wells and Henderson (1993) suggested that the abnormally low number of soldiers in the foraging population of *C. formosanus* showed less movement into new areas as compared with high number of soldiers while foraging. We were interested to see if high or low soldier proportion has any role in the selection and aggregation among various soil moisture levels. This study, therefore, was aimed at determining the substrate moisture level preference for distribution and consumption by *C. formosanus* with different soldier proportions in a 3-dimensional discrete moisture gradient arena.

Materials and Methods

Termites. Workers and soldiers from 3 colonies of Formosan subterranean termites were collected from Brechtel Park, New Orleans, LA, on 29 May, 14 June and 26 July 2007 using milk crate traps. This trap consists of a plastic milk crate (external dimension: L = 33.2, W =

33.2 and H = 28.1 cm; Rehrig Pacific Company, Los Angeles, CA) that houses a wooden lattice structure composed of 44 pieces of softwood lumber of 2 different dimensions viz., 3.5 x 3.5 x 29.5 cm (for horizontal arrangement) and 3.5 x 3.5 x 27 cm (for vertical arrangement). A potential Formosan subterranean termite infested area is identified in the field by inspecting in or around live and dead trees, logs, tree stumps or any type of wooden structure. Once an area has been identified as infested with the termites, the crates are buried within a few meters of the infested spot and covered with 3 - 5 cm of soil. After 3 - 6 wks, the crates are examined for termite infestation by pulling 1 or 2 wood pieces out of the crates. If sufficiently infested, the crates are retrieved and replaced with new ones.

For the present experiments, termites were maintained in the laboratory for 1 – 3 months before they were used in the bioassays. Healthy and uniform-looking (by size) termites were collected using moist brown paper towels as described in Gautam and Henderson (2008).

Bioassay. The bioassay arena was constructed with 7 peripheral plastic containers (8.5 cm diam x 3.4 cm) and 1 central container (12.4 cm diam x 3.6 cm) (Pioneer Plastics©, North Dixon, KY).The central container (release chamber) was connected to all the peripheral containers (moisture chambers) with clear vinyl tubes (9.5 mm outside diam, 6.5 mm inside diam, Watts Clear Vinyl Tubing, North Andover, MA) and all the peripheral containers were connected side by side in a way that termites could have easy access from one chamber to another through the tubes (Fig. 2.1).

Forty grams of dry sterilized sand (fine beach sand) were put in each peripheral container. Distilled water was then added to create the required moisture content, viz., 4%, 8%, 12%, 16%, 20%, 24% and 28% wt/wt where 28% was the saturation level. The sand moisture level in the peripheral chambers was increased sequentially from one direction (left to right in
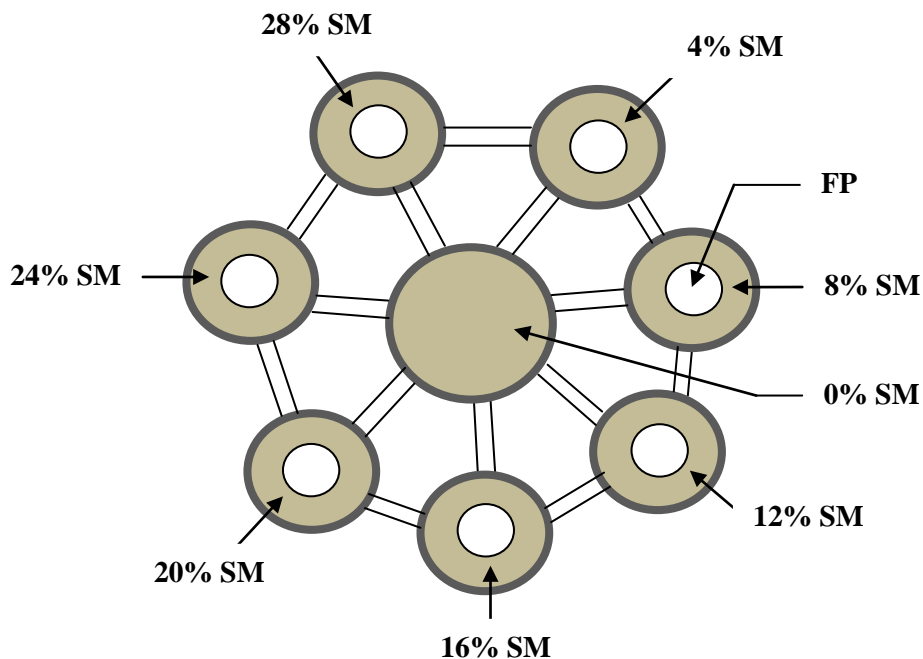
Figure 2.1. Moisture gradient bioassay arena with the central release chamber containing dry sand (0% moisture) with no filter paper and the peripheral chambers containing sand having moisture levels from 4 to 28% with a filter paper disc as a food source. Chambers are connected in a way that termites can go to all the chambers from the central chamber and they would have access to both higher and lower moisture chambers from any of the peripheral chambers (FP: filter paper, SM: sand moisture).

some replications and right to left in others) to maintain a moisture gradient in the arena. The

sand and water was evenly mixed with a steel rod and leveled. The central chamber contained 80

g of dry sand. Dry filter paper discs (42.5 mm diam, Whatman®) were weighed and placed in

each of the peripheral chambers as a food source. No filter paper was put in the central release

chamber. After 2 h, the moisture content of each filter paper was measured and found to be

>100% by weight, i.e., even at 4% sand moisture chamber the moisture content of the filter paper

was very high. The relative humidity (RH) was also near saturation (> 98%) in all the moisture

chambers regardless of whether the sand moisture level was 4 or 28%, possibly because the

airspace inside the closed container was very small and became saturated with just a little

amount of moisture from the substrate.

Three hundred termites with 3 different soldier proportions, viz., 4% (low), 16% (normal) and 32% (high) were released in the central release chamber. Three colonies of termites were used for the experiment with 3 replications for each soldier proportion from each colony. Altogether, 27 experimental arenas were prepared. The experimental arenas were then placed in a dark corner of the laboratory at 24 ± 1 °C. Preliminary tests were conducted to determine the water loss after 5 d in the plastic containers that were set up identical to bioassays but without termites. The weight of the containers taken before and after 5 d indicated that there was no substantial change in moisture level for any treatment chamber.

Daily inspections were made to record foraging patterns, particularly the aggregation pattern of the termites and movement from one moisture level to another. On day 6, the experiment was terminated. The chambers were carefully, but quickly, detached from each other and the openings of the chambers (or tubings) were sealed with Parafilm® immediately. The filter papers were removed, cleaned of any debris with soft forceps and a small brush and air-dried before taking weights. The loss of weight before and after the experiment was the estimation of the filter paper consumption. Location counts of the termites in each chamber were conducted by dumping the sand from each container into a large plastic tray and counting termites.

Statistical Analysis. Data were analyzed using SAS software (SAS 9.1, 2002 - 2003). To stabilize the variance, data were transformed using appropriate transformation methods. Location count data were square-root transformed, and the consumption data were log transformed before they were subjected to analysis of variance using proc mixed models. Means were compared using Tukey's honestly significant difference (HSD) at a = 0.05. Untransformed means were used

for reporting. A simple linear regression analysis was conducted to determine the relationship between distribution and consumption.

Results

Distribution (Aggregation). Colony had no significant effect on termite distribution in different moisture chambers (F= 0.07; df = 2, 206; *P*- 0.9337). Similarly, soldier proportion also had no significant effect on termite distribution (F= 0.11; df = 2, 206; *P* = 0.9001). Because there were no colony and soldier proportion effects, the data for all 3 colonies and 3 soldier proportion were pooled to determine the effects of sand moisture levels. Sand moisture level had a significant effect on termite distribution (F= 6.39; df = 7, 192; *P*< 0.0001). Tukey's HSD revealed that there were no significant differences in distribution among moisture levels from 4 - 24% but significant differences were present when these moisture levels were compared with either 0 or 28% moisture levels (Fig. 2.2). The distribution pattern as shown in Fig. 3 was obtained only after calculating and using the average of all 27 replications, which differed from each individual replication (or experimental arena).
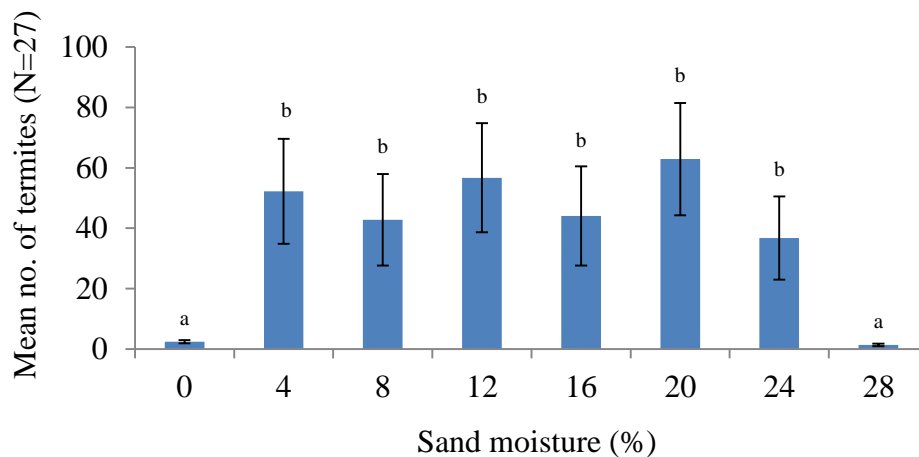
Figure 2.2. Mean ± SEM distribution of Formosan subterranean termites in various moisture chambers recovered at the end of termination 6-d bioassay. Means with the same letter are not significantly different (*P* > 0.05) using Tukey's HSD.
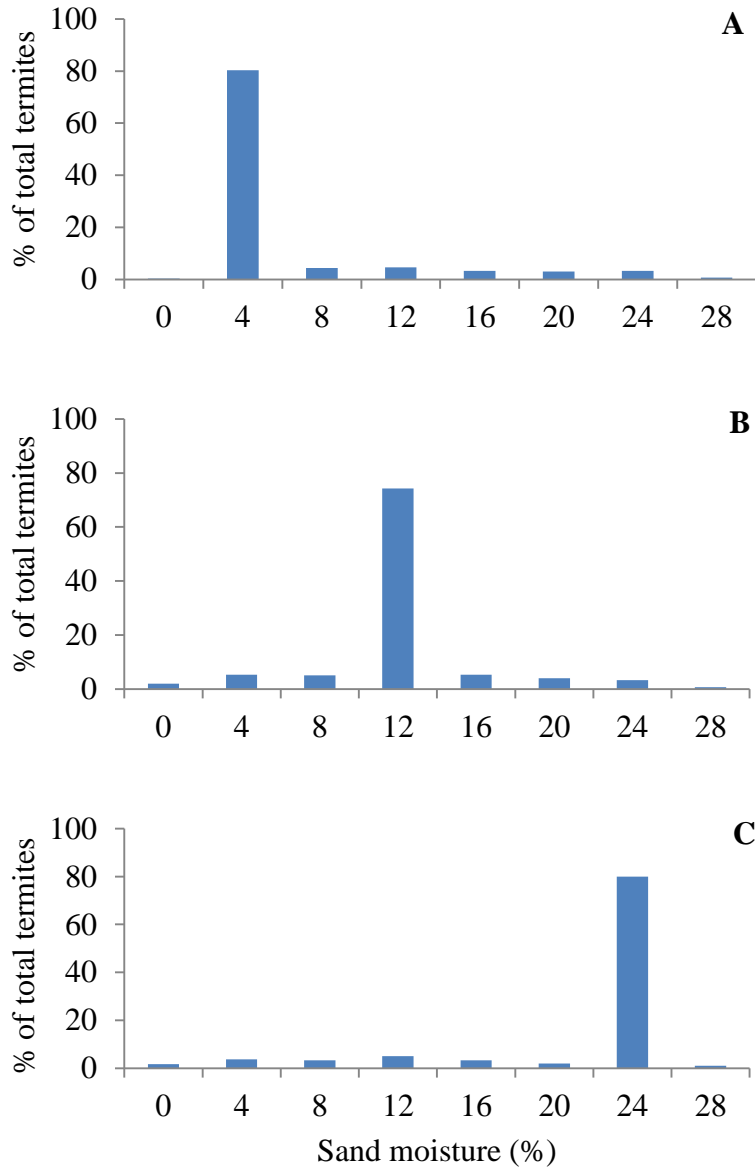
Figure 2.3. Distribution pattern of Formosan subterranean termites at various moisture chambers for 3 individual replications (A, B, C).

Immediately after release, termites began to explore the surroundings of the dry release chamber. They quickly found their way out through the peripheral tubes leading to the moisture chambers. After a few minutes, the release chambers were completely evacuated, and termites were gathered in one or more of the peripheral moisture chambers. In the initial few hours, termites were observed moving from one moisture level to another except for the one with

saturated sand moisture level (28%), where they would immediately retreat to the 24% or 4%

moisture level, depending on the direction from which they arrived. Similarly, termites would

not go back to the dry sand chamber. From day 2, there was clear evidence that termites

aggregated to 1 of the 6 moisture chambers ranging from 4 - 24% in each replicate. Examination

of each experimental arena revealed a distribution pattern where about 70 - 80% of the released

termites aggregated in 1 of the 6 moisture chambers and the remainder of the termites were

distributed in the remaining 5 of the 6 moisture chambers. A sample of individual experimental

arenas showing the distribution pattern of the termites is shown in Fig. 2.3(A, B, C). All 27

experimental arenas exhibited this distribution pattern. Table 2.1 shows the number of

experimental arenas (replications) that termites aggregated in a particular moisture chamber.

Once the termites were found to be aggregated in a chamber with a specific moisture level, the

majority of the termites remained in the same chamber for the entire experimental period, i.e., for

6 d (Table 2.2). Although termites wandered from one chamber to another, the movement did not

change the overall distribution pattern.

Table 2.1. Number of experimental arenas showing the aggregation of termites in different sand moisture levels out of total 27 experimental arenas

Sand moisture level (% by wt.)	# experimental arenas termites aggregated (Out of total 27)
0	0
4	5
8	4
12	5
16	4
20	6
24	3
28	0

Table 2.2. Termite aggregations from day 2 to day 6 in various sand moisture chambers

Treatments		Termite aggregation in different sand moisture %				
		2nd day	3rd day	4th day	5th day	6th day
4% soldier Colony1	R1	12%, 16%	12%, 16%	12%	12%	12%
	R2	4%	4%	4%	4%	4%
	R3	24%	24%	24%	24%	24%
Colony 2	R1	20%	20%	20%	20%	20%
	R2	8%	8%	8%	8%	8%
	R3	20%	20%	20%	20%	20%
Colony 3	R1	4%	4%	4%	4%	4%
	R2	20%	20%	20%	20%	20%
	R3	12%	12%	12%	12%	12%
16% soldier Colony 1	R1	8%	8%	8%	8%	8%
	R2	4%	4%	4%	4%	4%
	R3	8%	8%	8%	8%	8%
Colony 2	R1	16%	16%	16%	16%	16%
	R2	24%	24%	24%	24%	24%
	R3	20%	20%	20%	20%	20%
	R2	8%	8%	8%	8%	8%
	R3	8%	8%	8%	8%	8%
32% soldier Colony 1	R1	16%	16%	16%	16%	16%
	R2	20%	20%	20%	20%	20%
	R3	16%	16%	16%	16%	16%
Colony 2	R1	4%, 12%	4%, 12%	12%	12%	12%
	R2	4%	4%	4%	4%	4%, 8%
	R3	16%	16%	16%	16%	16%
Colony 3	C1	4%	4%	4%	4%	4%
	C2	20%	20%	20%	20%	20%
	C3	20%	4%	4%	4%	4%

Consumption. Colony had no significant effect on filter paper consumption $(F = 1.13$; df $= 2, 180$; $P = 0.3262)$. Similarly, soldier proportions also had no significant effect on filter paper

consumption *(F= 0.25; df = 2, 180; P = 0.7828)*. Owing to no significant effects of colony or soldier proportions on consumption, the data were pooled to determine the effects of sand moisture levels. Sand moisture had a significant effect on filter paper consumption (F= 18.24; df = 6, 166; *P* < 0.0001). Filter paper consumption by the termites in the respective chambers corresponded to their distribution (r = 0.829; Fig. 2.4). Tukey's means comparison showed that filter paper consumption at 28% moisture level was significantly lower (in fact, it was almost nonexistent) as compared with 4 - 24% moisture levels. However, no significant difference in filter paper consumption among 4 - 24% was observed (Fig. 2.5). Like the distribution pattern, the consumption pattern of individual replication also was very uneven; however, by using the average of all 27 replications we obtained the consumption graphs as shown in Fig. 2.5.
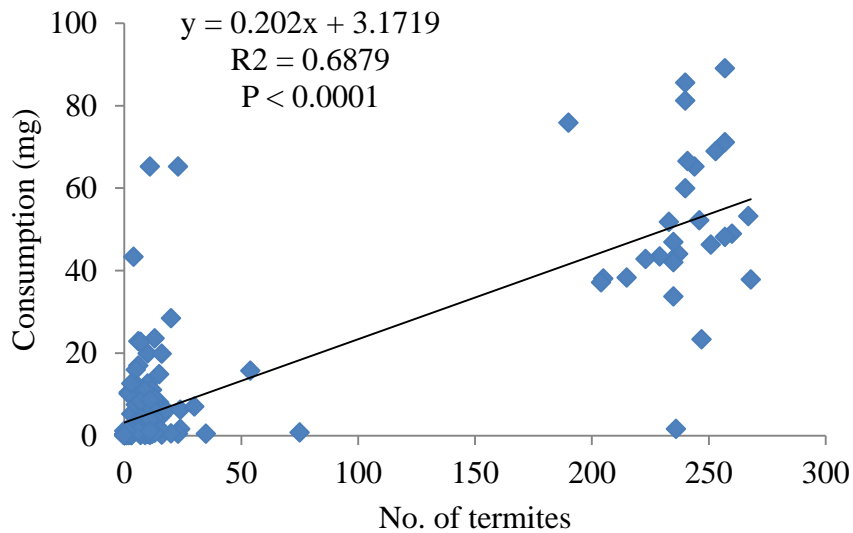
Figure 2.4. Relationship between numbers of termites retrieved in various moisture chambers and the filter paper consumption in corresponding chambers.
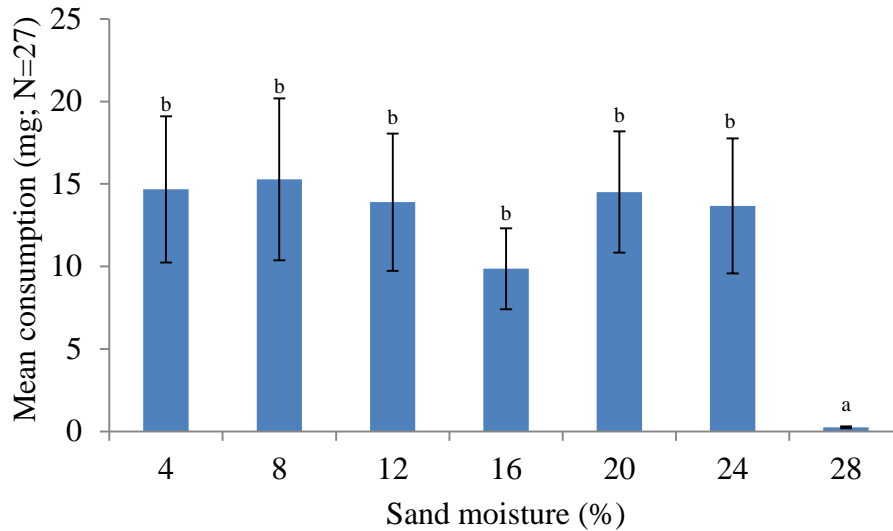
Figure 2.5. Mean ± SEM consumption of filter paper in various moisture chambers by Formosan subterranean termites over the 6-d bioassay. Means with the same letter are not significantly different ($P > 0.05$) using Tukey's HSD.

Discussion

Acceptable Range of Substrate Moisture for Foraging. The distribution pattern of *C. formosanus* to different moisture chambers in multiple choice arenas suggests that a range of substrate moisture levels are acceptable for *C. formosanus* provided other conditions are suitable. Populations of subterranean termites often encounter different levels of moisture in nature (Forschler and Henderson 1995). This may range from absolutely dry to full saturation or even inundation. The fact that Formosan subterranean termites are found to infest water-bound trees and are abundant in areas near levees (Forschler and Henderson 1995, Henderson 2008) suggests that they may be able to forage near areas having a high moisture level. It is also possible that their presence near waterways reflects their mode of dispersal via ships. On the other hand, unlike *Reticulitermes spp., C. formosanus* constructs above-ground nests (King and Spink 1969, Forschler and Henderson 1995), sometimes in complete isolation from the ground nest indicating their ability to forage in relatively dry substrates. The current study confirmed that *C.*

32

formosanus randomly foraged in the treatment chambers that had a range of sand moisture levels from 4 - 24%. Although *C. formosanus* exhibits a range of acceptable moisture levels preferences, they immediately evacuated the dry sand chamber, indicating that they quickly avoid the unfavorable conditions. Our preliminary test with a filter paper in the center dry sand chamber had shown that termites would not stay in the dry sand chamber irrespective of filter paper presence. In this chamber, the filter paper was also dry, and the relative humidity of the chamber was very low (~55%). A couple of termites that were present in the dry sand chamber were found dead by the next day. We believe that subterranean termite nest and gallery systems are designed to maintain a required moisture level and high RH.

Our results suggest that *C. formosanus* activities would be drastically reduced if substrates are saturated. This is consistent with the findings of field studies by Forschler and Henderson (1995) who reported a sharp decline in subterranean termite populations in the field due to heavy rainfall. Similarly, Snyder (1962) reported the elimination of *Reticulitermes* from an area coupled with frequent inundations. In the present experiment, we observed that *C. formosanus* avoided the saturated substrate. However, when suddenly inundated with water subterranean termites were found to enter a state of quiescence until more favorable conditions prevailed (Forschler and Henderson 1995).

The moisture-retaining capacity of any soil primarily depends on the soil type. The sand substrate that we used in the present study has a low water retention property, with a saturation point at 28% by wt. Addition of clay or vermiculite in the sand would increase the moisture absorption capacity making the saturation point higher. A mixture of equal volume of sand and vermiculite (saturation point: 55% by wt.) was used by Green et al. (2005) to test the substrate moisture preference for 3 species of *Reticulitermes*. They reported that most *R. flavipes* and *R.*

33

tibialis showed the substrate moisture preference range of 35 - 55% and *R. virginicus* 25 - 55% in multiple choice arenas having moisture gradient from 5 - 55%. Although it may not be appropriate to directly compare their study with our present study because of different substrates used, all indications are that Formosan subterranean termites can forage in a relatively drier substrate as compared with *Reticulitermes* spp.

Our present results showing no significant difference in filter paper consumption by *C. formosanus* among 6 moisture chambers (from 4 - 24%) is consistent with the field findings on *R. flavipes* by Potter et al. (2001) who showed that there was no significant difference in damage rate (bait consumption) between Sentricon® (Dow AgroSciences, INpolis, IN) stations placed in soil having different moisture levels. Henderson et al. (1998) documented that ground monitors placed near areas considered conducive for subterranean termite activity, i.e., near water and food sources were attacked in greater numbers. Interestingly, according to the present findings, any soil surface that is not completely dry or saturated might be an area conducive for *C. formosanus* from a moisture point of view. This, perhaps, makes it even more complex to predict the probable area of *C. formosanus* infestation but may help in avoiding the risks of overlooking possible infestation sites based on some preconceived notion.

Aggregation Pattern. Aggregation is defined as a higher temporal and spatial density of individuals than in the surrounding area (Southwood 1966, Camazine et al. 2001). It is one of the most basic social phenomena, especially in social insects like termites (Deneubourg et al. 2002). Mutual interactions mediated by information transfer among individuals result in the origin and stability of social aggregates. This can induce group behaviors that are not merely the sum of individual behaviors (Parrish et al. 1997). Group behaviors, like trophallaxis, allogrooming and contact stimulation furnish the organizational glue to keep termite colonies cohesive and

functional (Nalepa and Bandi 2000). In the present experiment, we reported that *C. formosanus* aggregated in various moisture chambers. Aggregation to a particular chamber may have been evoked by the trail pheromone produced by the pioneer foragers. However, it is surprising that sand moisture levels ranging from 4 - 24% seemed to be equally acceptable sites for aggregation. We noticed that termites started to aggregate in one chamber after a few hours of the release and by the second day aggregations were observed in all the experimental arenas. By visual estimation, about 70 - 80% of the total released termites were found to be aggregated in one chamber from day 2 to day 5. This held true when we counted the termites on day 6 after terminating the test. Moreover, the aggregation was consistently on the first chosen chamber throughout the experimental period (6 days) in all the experimental arenas indicating that *C. formosanus* feed heavily on the first attacked food before they move to the next one. Previous studies have also reported that *C. formosanus* remained longer and consumed heavily on the first attacked food whereas *Reticulitermes* spp. were not found so (Delaplane and La Fage 1987,1989, Polizzi and Forschler 1999). The results from the present experiment suggest that there is an equal probability of finding groups of *C. formosanus* in a soil irrespective of the soil moisture levels except dry or saturated conditions. This type of random aggregation pattern exhibited by *C. formosanus* in a wide range of substrate moisture levels should be taken into consideration when placing monitors and baits in termite baiting systems.

Role of Soldiers in Aggregation. The primary role of soldiers in a termite colony is defense (Deligne et al. 1981, Mill 1982, Noirot 1990). In some species, in addition to alarm and defense, soldiers also are found to play a leading role in food search. For example, *Nasutitermes costalis* (Holmgren) soldiers are reported to be responsible for discovering new food sources and communicating their find to workers (Traniello 1981). On the other hand, worker-initiated

35

foraging has been reported in species like *R. santonensis* Feytaud and *R. flavipes* where soldiers

arrive after the food source has been found (Robson et al. 1995, Reinhard et al. 1997). These

termite species have rather low numbers of soldiers (1 - 5%) in their population. The role of

soldiers in food search in foraging population of *C. formosanus* is not well established. Wells

and Henderson (1993) reported that *C. formosanus* with low numbers of soldiers (2.4%) showed

less tendency to move to new locations as compared with groups having a higher soldier

numbers (18.3%). They speculated that soldiers may be responsible for movement into new

areas. In the present study, although we did not determine if soldiers had a leading role in

initiation of the movements, we demonstrated that termite aggregation preferences for different

sand moisture levels were not affected by soldier proportion.

References

Camazine, S., J. L. Oeneubourg, N. Franks, J. Sneyd, G. Theraulaz, and E. Bonabeau. 2001. Self-organization in biological systems. Princeton Univ. Press, Princeton, NJ. 535 pp.

Delaplane, K. S., A. M. Saxton, and J. P. La Fage. 1991. Foraging phenology of Formosan subterranean termite (Isoptera: Rhinotermitidae) in Louisiana. Am. Midi. Nat. 125: 222-230.

Delaplane, K. S., and J. P. La Fage. 1987. Variance in feeding on equivalent wood blocks by the Formosan subterranean termite in laboratory choice tests. Sociobiology 13: 227-233.

Delaplane, K. S. and J. P. La Fage. 1989. Foraging tenacity of *Reticulitermes flavipes* and *Coptotermes formosanus* (Isoptera: Rhinotermitidae). Sociobiology 16: 183-189.

Deligne, J., A. Quennedy, and M. S. Blum. 1981. The enemies and defense mechanisms of termites, Pg. 176. In H, R. Hermann (ed.), Social Insects, Vol. 2. Academic, New York.

Deneubourg, J. L., A. Lioni, and C. Detrain. 2002. Dynamics of aggregation and emergence of cooperation. Biol. Bull. 202: 262-267.

Fei, H., and G. Henderson. 1999. Effect of moisture and two nitrogen sources on nest site choice by alates and dealates of *Coptotermes formosanus in* the laboratory (Isoptera: Rhinotermitidae). Sociobiology 34: 581-589.

Forschler, B. T. 1996. Baiting *Reticulitermes* (Isoptera: Rhinotermitidae) field colonies with abamectin and zinc borate-treated cellulose in Georgia. Sociobiology 28: 459-485.

Forschler, B. T., and G. Henderson. 1995. Subterranean termite behavioral reaction to water and survival of inundation: Implications for field populations. Environ. Entomol. 24: 1592-1597.

Gautam, B. K. and G. Henderson. 2008, Effects of m-tyrosine on feeding and survival of Formosan subterranean termites (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 11: 1088-1093.

Green, J. M., M. E. Scharf, and G. W. Bennett. 2005. Impacts of soil moisture level on consumption and movement of three sympatric subterranean termites (Isoptera: Rhinotermitidae) in a laboratory assay. J. Econ. Entomol. 98: 933-937.

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. 1979. Soldier production and maintenance of soldier proportions in laboratory experimental groups of *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). Insectes Soc. 26: 69-84.

Haverty, M. I., J. P. La Fage, and W. L. Nutting. 1974. Seasonal activity and environmental control of foraging of the subterranean termite, *Heterotermes aureus* (Snyder), in desert grassland. Life Sci. 15: 1091-1101.

Henderson, G. 1998. Primer pheromones and possible soldier caste influence on the evolution of sociality in lower termites, pp. 314-330. *In* R. K. Vander Meer, M. D. Breed, K. E. Espelie and M. L. Winston (eds.), Pheromone Communication in Social Insects - Ants, Wasps, Bees, and Termites. Westview Press, Boulder, Colorado.

Henderson, G. 2001. Practical considerations of the Formosan subterranean termite in Louisiana: A 50-year-old problem. Sociobiology 37: 281-292.

Henderson, G. 2008. The termite menace in New Orleans: Did they cause the floodwalls to tumble? Am. Entomol. 54: 156-162.

Henderson, G., and H. Fei. 2002. Comparison of native subterranean termite and Formosan subterranean termite: biology ecology and methods of control. In Enhancing the Durability of Lumber and Engineered Wood Products Conference, 11-13 Feb. 2002. Orlando, FL. Forest Products Society, Madison, Wisconsin. 320pp.

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. Fla. Entomol. 81: 461-464.

King, E. G., and W. T. Spink. 1969. Foraging galleries of the Formosan subterranean termite, *Coptotermes formosanus,* in Louisiana. Ann. Entomol. Soc. Am. 62: 537-542.

La Fage, J. P. 1987. Practical considerations of the Formosan subterranean termite in Louisiana: A 30-year-old problem, Pp. 37-42. *In* M. Tamahiro and N. Y. Su (eds.), Biology and control of the Formosan subterranean termite, Research and Extension Series 083. College of Tropical Agriculture and Human Resources, Univ. Hawaii, Honolulu.

Mao, L., G. Henderson, Y. Liu, and R. A. Laine. 2005. Formosan subterranean termite (Isoptera: Rhinotermitidae) soldiers regulate juvenile hormone levels and caste differentiation in workers. Ann. Entomol. Soc. Am. 98: 340-345.

Mill, A. E. 1982. Foraging and defensive behavior in neotropical termites. Ph. D. diss. Univ. Southampton. 264 pp.

Moore, B. P. 1969. Biochemical studies in termites, pp. 407-432. *In* K. Krishna and F. Weesner (eds.), Biology of the Termites, Vol. 1. Academic, New York.

Nalepa, C. A., and C. Bandi. 2000. Characterizing the ancestors: Paedomorphosis and termite evolution, pp. 53-76. *In* T. Abe, D. E. Bignell and M. Higashi (eds.), Termites: Evolution, Sociality, Symbiosis, Ecology. Kluwer Academic Publ., Dordrecht, NL.

Noirot, C. 1990. La caste des soldats chez les termites: originalite, evolution. Actes des Colloques. Insectes Soc. 6: 21-26.

Parrish, J. K., W. M. Hammer, and C.T. Prewitt. 1997. Introduction - from individuals to aggregations: unifying properties, global framework, and the holy grails of congregation, pp. 1-14. *In* J. K, Parrish and W. M. Hamner (eds.), Animal groups in three dimensions. Cambridge Univ. Press, Cambridge.

Polizzi, J, M., and B. T. Forschler. 1999. Lack of preference by *Reticulitermes* spp. (Isoptera: Rhinotermitidae) for termite feeding stations with previous termite exposure. J. Agric. Urban Entomol. 16: 197-205.

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 consideration around structures. Sociobiology 38: 565-584.

Reinhard, J., H. Hertel, and M. Kaib. 1997. Systematic search for food in the subterranean termite *Reticulitermes santonensis* De Feylaud (Isoptera: Rhinotermitidae). Insectes Soc. 44: 147-158.

Robson, S. K., M. G. Lesniak, R. V. Kothandapani, J. F. A. Traniello, B. L. Thorne, and V. Fourcassie. 1995. Nonrandom search geometry in subterranean termites. Naturwissenschaften 82: 526-528.

Snyder, T. E. 1962. Termite research around the world. Report on the 4th Congress of the International Union for the Study of Social Insects, Pavia, Italy. Pest Contr. 30: 48-50.

Southwood, T. R. E. 1966. Ecological Methods. Methuen and Co., London. 391 pp.

Sponsler, R. C., and A. G. Appel. 1990. Aspects of the water relations of the Formosan and eastern subterranean termites (Isoptera: Rhinotermitidae). Environ. Entomol. 19:15-20.

Su, N. Y. and H. Puche. 2003. Tunneling activity of subterranean termites (Isoptera: Rhinotermitidae) in sand with moisture gradients. J. Econ. Entomol. 96: 88-93.

Tamashiro, M., J. R. Yates, P. Y. Lai, J. K. Fuji, and N. Y. Su. 1980. Size and structure of *Coptotermes formosanus* Shiraki colonies in Hawaii. *In* Proc. 16th International Congr. Entomol., Kyoto, Japan. 311 pp.

Traniello, J. F. A. 1981. Enemy deterrence in the recruitment strategy of a termite: soldier-organized foraging in *Nasutitermes costalis.* Proc. Natl. Acad. Sci. USA 78: 1976-1979.

Waller, D. A., and J. P. La Fage. 1988. Environmental influence on soldier differentiation in *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitdae). Insectes Soc. 35: 144-152.

Wells, J. D. and G. Henderson. 1993. Predation by *Solenopsis invicta* (Hymenoptera: ormicidae) on introduced and native subterranean termites (Isoptera: Rhinotermitidae): effect of termite soldier proportion. Ecol. Entomol. 18: 270-274.

Williams, O. L. 1934. Some factors limiting the distribution of termites, pp. 42-44. *In* C. A. Kofoid (ed.), Termites and Termite Control. Univ. California Press, Berkeley.

CHAPTER 3

WOOD CONSUMPTION BY FORMOSAN SUBTERRANEAN TERMITES (ISOPTERA: RHINOTERMITIDAE) AS AFFECTED BY WOOD MOISTURE CONTENT AND TEMPERATURE[2]

Introduction

Moisture is one of the most important environmental requirements for the survival of subterranean termites (Collins 1969). Unlike many other insects, subterranean termites have a very thin, soft cuticle that readily desiccates (Moore 1969, Sponsler and Appel 1990). As a survival strategy, subterranean termites always associate with moist and humid environments. Moisture can be obtained from many sources, including metabolic breakdown of sugars (food source) and wet food materials (Pearce 1997). Wood being the principle food of subterranean termites, its moisture is one of the important factors that affect their mode of feeding and ultimately their survival. Delaplane and La Fage (1989) suggested that high moisture content makes wood Þber soft and easy to masticate. They also reported that *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) preferred wet wood blocks over dry blocks. Behr et al. (1972) showed a positive correlation between wood moisture level and the feeding by *Reticulitermes flavipes* (Kollar).

In addition to moisture on the food source, relative humidity of the surrounding atmosphere is of utmost importance to subterranean termites. Generally, high moisture on the food source contributes to increased atmospheric humidity. However, high relative humidity alone may not be sufficient for long-term survival if the food moisture content is not adequate. Although wood and nest material moisture level of >16% makes the relative humidity of termite galleries near saturation (Sponsler and Appel 1990), *R. flavipes* cannot survive on wood having ≤16% moisture (McManamy et al. 2008).

Temperature is another important factor for feeding and survival of subterranean termites. The highest feeding rate by *C. formosanus* was reported at 30$^\text{o}$C (Nakayama et al. 2004). By employing an acoustic emission (AE) technique, Imamura and Fuji (1995) reported the highest

wood-attacking activity by these termites at 36°C. However, despite feeding rate being the highest at these temperatures, termite survival is the critical factor for total consumption. The highest survival of *C. formosanus* as reported by Fei and Henderson (2002) was obtained at 30°C. Given the importance of these factors, a fluctuation in either moisture or temperature will impact the overall termite consumption and survival. Previous studies were undertaken either at one constant temperature with different moisture levels or one constant moisture level with different temperature conditions (Smythe and Williams 1972, Delaplane and La Fage 1989, Fei and Henderson 2002, Nakayama et al. 2005). For example, Fei and Henderson (2002) reported that wood consumption rates increased with increase in temperature, up to 30°C, but did not look at the impacts by change in wood moisture level. Similarly, Delaplane and La Fage (1989) considered only the moisture content change in wood but did not consider the impacts by fluctuations in temperature. Given that environments are often heterogeneous, these studies may not determine the optimum temperature and wood moisture combination for termites. Our present study examines the impact of several ambient temperature and initial wood moisture combinations on feeding and survival of *C. formosanus* at a constant high relative humidity condition in both choice and no choice laboratory tests.

Materials and Methods

Termites. Formosan subterranean termites were collected from Brechtel Park and along a floodway servitude bordering Tchoupitoulas Street in New Orleans, Louisiana in August 2008 using milk crate traps (Gautam and Henderson 2011). These termites were assumed to be from different colonies based on the >1,000 m distance between the collection sites. The termites were maintained in the laboratory for a maximum of one month before they were used for the

bioassays. Healthy and active workers (externally undifferentiated larvae developed to at least the third instar) and soldiers were selected for the experiment.

Modification of Moisture Content of Wood. Southern yellow pine (*Pinus* sp.) wood was cut to equal sized blocks (1.9 ×1.9 × 0.9 cm) with an electric band saw. The blocks were oven-dried at 105°C for 24 hours and weighed. The blocks initially had 9-11% moisture content at laboratory conditions and after oven-drying the dry blocks had moisture content from 0 to 3%. This is because the oven-dried wood blocks had to expose for several seconds during the weighing process and that probably increased the moisture content up to 3% in some blocks. The dry blocks were then soaked in deionized water to make the desired initial moisture content of the wood by weight. For high moisture content (125-150%), wood blocks were soaked in water for 5 days. For medium moisture content (70-90%), the blocks were soaked for 5 hours. Low moisture content (22-24%) was obtained by keeping the wood blocks in a container having saturated relative humidity (RH) for 3 days. Finally, oven-dried wood blocks (0-3% moisture content) were used as a control. The moistened wood blocks were weighed to determine the moisture content before placing on the treatment dish and any blocks that had higher or lower moisture content than a given range for a particular category were discarded from the tests.

No-choice Bioassay. The experiment consisted of 12 combinatorial treatments of four wood moisture levels and three temperature levels. The three temperature levels, *viz.*, 19°C, 28°C and 35°C were maintained in three incubators. Required number of wood blocks of four different moisture levels were prepared as described above. A single block having designated moisture content was placed in each Petri dish (100 by 15 mm, Fisherbrand, Thermo Fisher Scientific, Waltham, MA) and 115 termites (103 workers and 12 soldiers) were released (Fig. 3.1A). Prior to this, termites required for the tests were counted and put in separate Petri dishes

43

containing a moistened filter paper. A total of six replications were conducted with termites taken from two different colonies. The Petri dishes were then placed in humidity chambers (described below) to provide high relative humidity throughout the experiment and put in three incubators set to the three different temperatures. No water was added to the units during the bioassay period. On day 6, the units were taken down, termite mortality was recorded and the wood blocks were cleaned and oven dried to calculate the total wood consumption.

Choice Bioassay. Choice tests used larger Petri dishes (100 by 15 mm, Fisherbrand, Thermo Fisher Scientific) than the ones used for no-choice tests. Each dish consisted of four wood blocks having four different moisture levels and 250 termites (200 workers and 25 soldiers) were released on the central point of the dish (Fig. 3.1B). The choice tests also consisted of six replications with termites from two colonies. Total wood consumption and termite mortality was recorded at the end of the experiment as described in the no-choice tests.
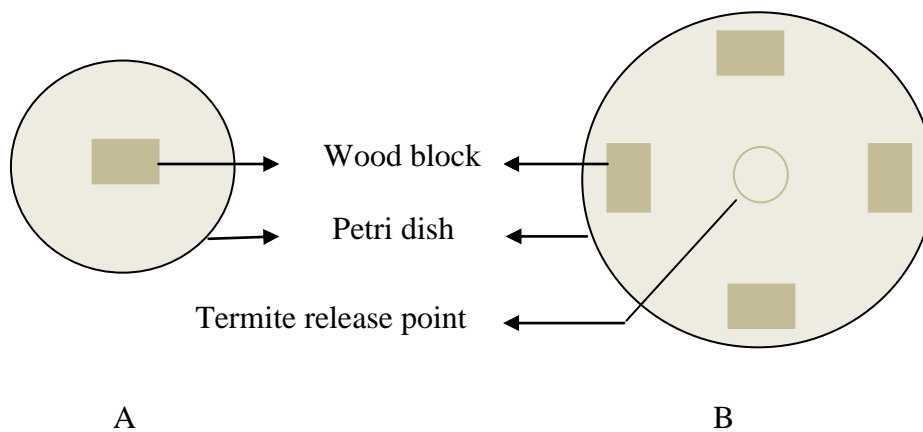

Wood block

Petri dish

Termite release point

A   B

Figure 3.1. (A) No-choice feeding test apparatus. (B) Choice feeding test apparatus.

Preparation of Humidity Chamber. A Rubbermaid® clear box (capacity: 11.3 L, Latchables, Rubbermaid Home Products, Fairlawn, OH) was filled with 1cm-thick layer of sterilized sand. Distilled water was added to the level that the sand was oversaturated and a thin film of free water was maintained. Three plastic plates were placed on top of the sand on which the treatment Petri dishes were placed. Several holes were made into the lids of the Petri dishes using a soldering iron and placed one above another in a way that the airspace inside each dish was connected with the saturated airspace of the humidity chamber. The humidity chamber was covered with aluminum foil to help maintain the saturated relative humidity inside the chamber for the 6 d test period. Prior to this, the relative humidity inside the chamber was measured using Testo® 625 humidity meter (Testo Inc., Sparta, NJ) and was found to be in saturated condition. Preliminary tests were carried out to determine the relative humidity condition inside the chamber for 6 days in the similar set up. The relative humidity measured daily for 6 days indicated that there was a constant saturated humidity inside the chamber.

Statistical Analysis. Statistical analysis was performed using SAS 9.1 software (© 2002-2003 SAS Institute Inc., Cary, NC). Colony effects were determined using two sample t-tests. Analysis of variance (ANOVA) was performed to determine the effects of temperature, moisture and interaction effects using proc mixed model. Tukey-Kramer's honestly significant difference (HSD) procedure was used for post-ANOVA comparisons. All significant levels were determined at $\alpha = 0.05$.

Results

Temperature and wood moisture levels had significant and complementary roles in wood consumption by *C. formosanus*. In the no-choice test there was a significant colony effect ($T = -2.23$; df = 70; $P = 0.028$). In both the colonies, wood consumption by *C. formosanus*

significantly differed based on wood moisture ($F = 124.04$; df = 3, 59; $P < 0.0001$), temperature ($F = 28.77$; df = 2, 59; $P < 0.0001$), and wood moisture by temperature ($F = 9.68$; df = 6, 59; $P < 0.0001$). In general, consumption of high moisture content (125-150%) wood was significantly higher than low moisture content and dry wood at all three temperatures in both the colonies (Fig. 3.2A, B). In colony 2, consumption of medium moisture content wood was also significantly higher at all three temperatures as compared to low moisture and dry wood; however, in colony 1, it was significantly higher at 28°C only. High moisture content wood consumption at 35°C or 28°C was significantly higher than at 19°C. Similarly, consumption of dry and low moisture content wood was substantially low at all three temperatures (Fig. 3.2A, B).

In the choice test, colony had no significant effect ($T = -1.56$; df = 70; $P = 0.122$) and the data from both the colonies were pooled for analysis. As in the no-choice test, wood consumption by *C. formosanus* differed significantly based on initial wood moisture ($F = 64.08$; df = 3, 59; $P = 0.0001$), temperature ($F = 23.73$; df = 2, 59; $P = 0.0001$) and wood moisture by temperature ($F = 7.65$; df = 6, 59; $P = 0.0001$) (Fig. 3.3). Consumption of high moisture content wood was significantly higher than low moisture content and dry wood at 28°C and 35°C. At 19°C, although the consumption of high and medium moisture wood was higher than dry and low moisture wood, the difference was not statistically significant (Fig. 3.3).

There was no termite mortality during the test period in the choice test but termite mortality was observed in the no-choice test. In the no-choice test, there was no significant difference on mortality based on colony ($T = 1.05$; df = 70; $P = 0.297$) but significant differences in mortality was observed based on wood moisture ($F = 57.98$; df = 2, 59; $P = 0.0001$), temperature ($F = 68.10$; df = 2, 59; $P = 0.0001$) and wood moisture by temperature ($F = 20.01$;
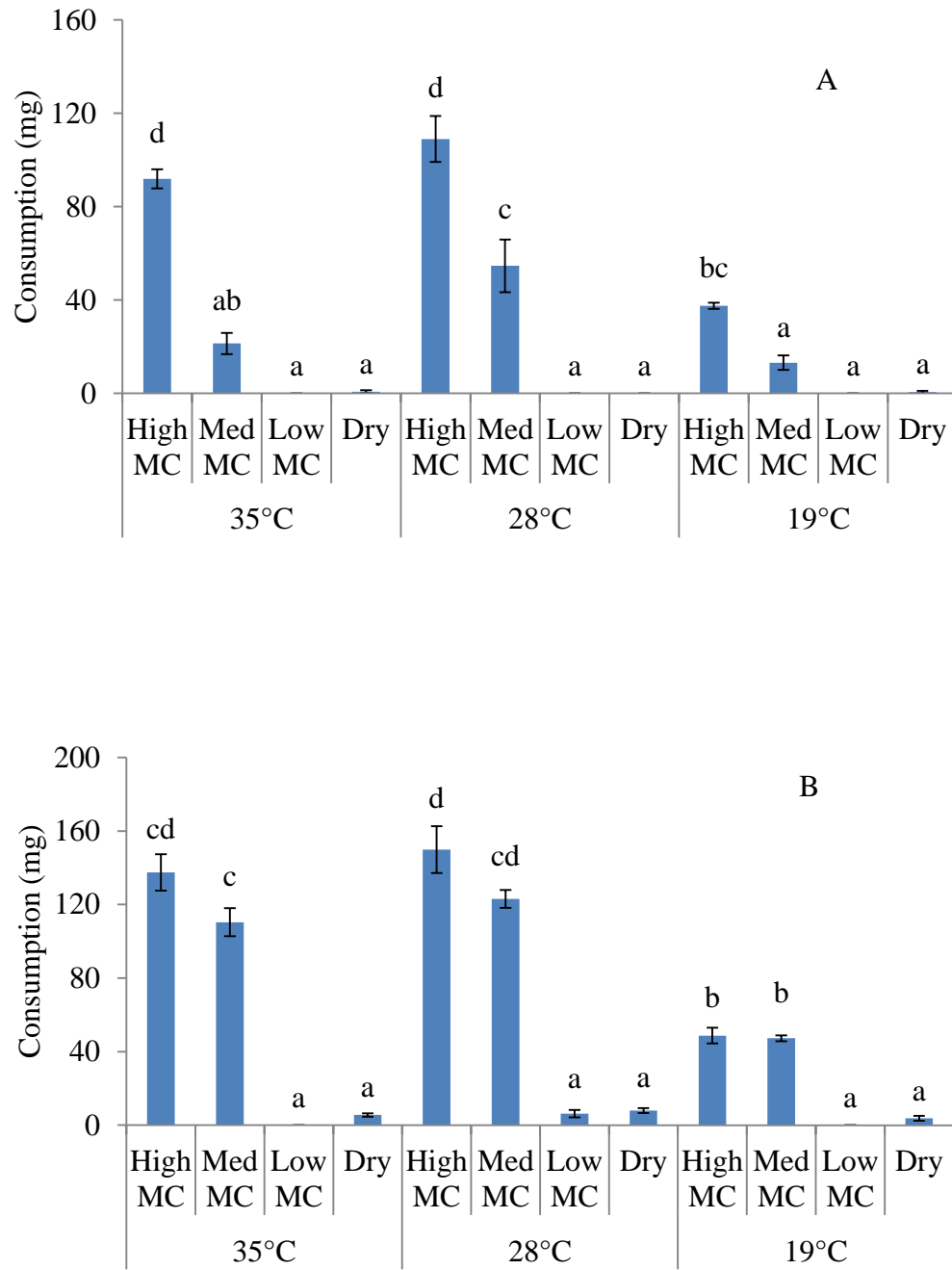
Figure 3.2. Mean ± SEM consumption of wood by *C. formosanus* in no-choice test. (A) Colony 1, (B) Colony 2. Means with the same letter are not significantly different ($P > 0.05$) using Tukey's mean separation.
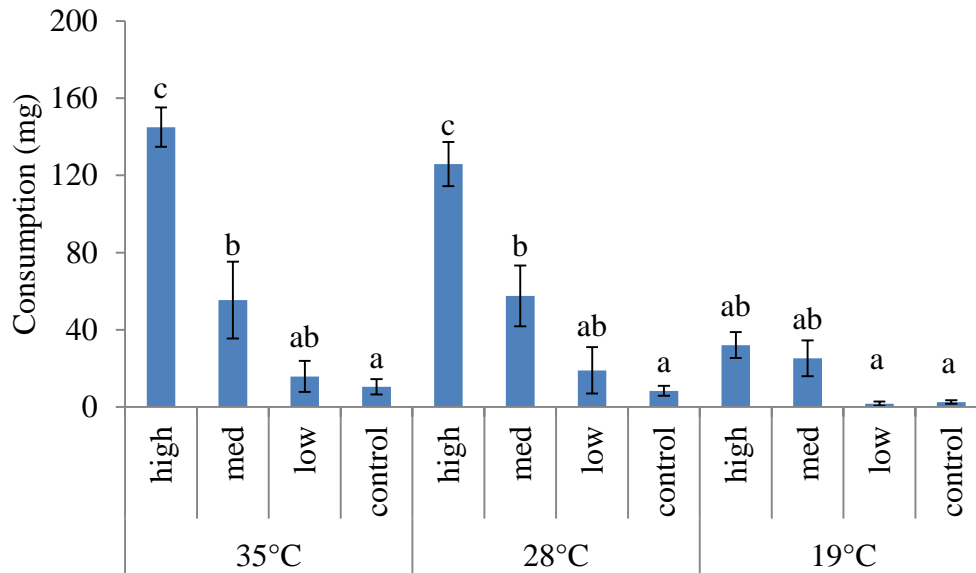
Figure 3.3. Mean ± SEM consumption of wood by *C. formosanus* in choice test. Means with the same letter are not significantly different ($P > 0.05$) using Tukey's mean separation.

df = 2, 59; $P = 0.0001$). Significantly higher mortality was observed on dry or low moisture content wood blocks than on medium or high moisture content wood blocks at 28°C or 35°C (Fig. 3.4). Mortality at 35°C was almost 100%, which was significantly higher than at 28°C. At 19°C, however, termite mortality was very low irrespective of the wood moisture content (Fig. 3.4).

Discussion

Preference for high moisture content wood by termites may be due to the wood fiber softness and ease to masticate (Delaplane and La Fage 1989). However, this does not imply that *C. formosanus* always prefer soft wood species over hard wood species as there are quite a few hard wood species that are equally preferred by this termite (Morales-Ramos and Rojas 2001). For a given wood species (whether hard or soft wood), in addition to making the wood fibers

soft, high moisture content is an added advantage for desiccation-prone termites since it is a source of free
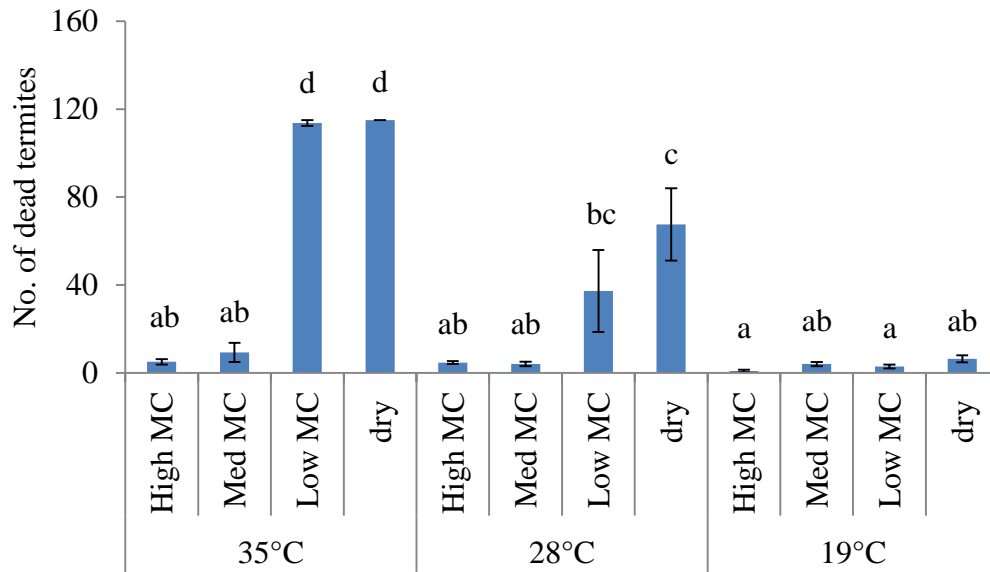
Figure 3.4. Mean ± SEM mortality of by *C. formosanus* in no-choice test. Means with the same letter are not significantly different ($P > 0.05$) using Tukey's mean separation.

water. In the choice tests, although termites contributed to enhance the process of equilibrium among the wood blocks having different levels of moisture (Delaplane and La Fage 1989, Gallaghar and Jones 2010), they were found to feed most heavily on the first attacked food. Termites preferred the highest initial moisture content wood blocks early when the wood moisture levels were more discrete, and then they continued to feed on those initially preferred blocks. This is consistent with the findings by Delaplane and La Fage (1989) who reported that *C. formosanus* preferred wood blocks having the highest initial moisture content offered (97%). With Japanese red pine, however, Nakayama et al. (2005) reported that medium moisture content (79-103%) wood was preferred over high moisture content (133-191%) wood by *C. formosanus*.

Wood species and possible other factors may have played a role in determining wood block preference in their experiment.

Of the temperatures tested, 28°C can be regarded as the optimum temperature for feeding because at the higher temperature (35°C), although the feeding was not significantly different than that at 28°C on high moisture content wood, a substantially high mortality was obtained when the wood moisture levels decreased. On the other hand, at the lower temperature (19°C), no substantial feeding occurred at any wood moisture level. Therefore, based on total overall feeding and termite survival, we conclude that 28°C temperature and high moisture wood is the best combination for feeding for *C. formosanus* among all the treatment combinations of this study. Fei and Henderson (2002) reported that *C. formosanus* survival was higher at 30°C than at 25°C or 33°C. The desert dampwood termite, *Paraneotermes simplicicornis* (Banks) also had the highest wood consumption at 28°C when they were exposed to various temperatures ranging from 16°C to 36°C (Haverty and Nutting 1974). In the case of the temperate inhabiting western subterranean termite, *Reticulitermes hesperus* Banks, however, the optimum temperature for feeding was reported to be 21°C (Smith and Rust 1993). Smith and Rust (1993) further reported that temperatures greater than 26°C produced very high mortality in *R. hesperus* in days. The desert subterranean termite, *Heterotermes aureus* (Snyder) was reported to have the highest consumption at 36°C (Haverty and Nutting 1974).

It is obvious from the present results that a high relative humidity environment alone is not adequate for sustained feeding by Formosan subterranean termites. For sustained feeding, the termites either need to be in contact with a moist substrate (soil) or the moisture content of wood needs to be well above the fiber saturation point which is defined as the moisture content of wood at which the cell walls are saturated with bound water and the cell cavities contain no free

water (Babiak and Kudela 1995). Most wood species have a fiber saturation point from 25% to 30%, in which moisture becomes largely inaccessible to the degrading organisms (Morrell 2002). This indicates that the wooden structures that are not in connection with outside water sources may not be at high risk of infestation from *C. formosanus* even if the atmospheric relative humidity is at saturation. Requirements of wood moisture above the fiber saturation point was also reported for the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), which could not survive on wood with moisture content ≤ 24% even if the relative humidity was at saturation (McManamy et al. 2008).

Very low mortality at 19°C in the no-choice test, irrespective of the wood moisture level, could be attributed to the low activities and reduced physiological mechanisms of the termites, which probably allowed them to live longer. On the other hand, a substantially high mortality at 28°C or 35°C on the termites exposed to only low moisture or dry wood blocks but not on those exposed to medium or high moisture wood blocks indicates that *C. formosanus* cannot survive on low moisture wood even if the ambient temperature is favorable for consumption. These results suggest that *C. formosanus* searches for high moisture content wood and ultimately congregates on it. Smythe and Carter (1970) suggested that *C. formosanus* could be forced to consume more wood of less preferred species in no-choice tests than in multiple choice tests, however, our results suggest that they could not be forced to feed on the low moisture content wood (at least for southern yellow pine wood) and therefore, have a critical requirement for wood moisture above a certain threshold level.

Monitoring and baiting systems can possibly be improved by exploiting termite behavior as affected by temperature and wood moisture. To make the monitoring device more effective, provisions should be in place that attract termites and keep them for prolonged periods of time.

Although numerous factors such as wood species and hardness, presence of secondary metabolites, presence or absence of fungi, etc. play a role in wood consumption by termites (Smythe et al. 1971, Carter and Smythe 1974, Nagnan and Clement 1990), our concern here is with the moisture content of wood. For this, initial moisture content of the monitoring/baiting stake should be higher. Possibly a continuous drip of water could be provisioned in dry areas to maintain a high moisture level. Similarly, increasing the temperature around the monitors/baits would be desirable if the soil temperature is below 28°C. Plastic mulches could be used since they increase soil temperature and at the same time preserve moisture (Ramakrishna et al. 2006, Locher et al. 2005).

References

Babiak, M., and J. Kudela. 1995. A contribution to the definition of the fiber saturation point. Wood Sc. Technol. 29: 217-226.

Behr, E. A., C. T. Behr, and L. F. Wilson. 1972. Influence of wood hardness on feeding by the eastern subterranean termite, *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 65: 457-460.

Carter, F. L., and R. V. Smythe. 1974. Feeding and survival responses of *Reticulitermes flavipes* (Kollar) to extractives of woods from 11 coniferous genera. Holzforschung 28: 41-45.

Collins, M. S. 1969. Water relations in termites, pp. 433-435. *In* K. Krishna and F. M. Weesner (eds.), Biology of termites, vol. I. Academic, New York.

Delaplane, K. S., and J. P. La Fage. 1989. Foraging tenacity of *Reticulitermes flavipes* and *Coptotermes formosanus* (Isoptera: Rhinotermitidae). Sociobiology 16: 183-189.

Fei, H., and G. Henderson. 2002. Formosan subterranean termite (Isoptera: Rhinotermitidae) wood consumption and worker survival as affected by temperature and soldier proportion. Environ. Entomol. 31: 509-514.

Gallaghar, N. T., and S.C. Jones. 2010. Moisture augmentation of food items by *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Sociobiology 55: 735-748.

Gautam, B. K., and G. Henderson. 2011. Effects of sand moisture level on food consumption and distribution of Formosan subterranean termites (Isoptera: Rhinotermitidae) with different soldier proportions. J. Entomol. Sci. 46: 1-13.

Haverty, M. I., and W. I. Nutting. 1974. Natural wood-consumption rates and survival of a dry-wood and a subterranean termite at constant temperatures. Ann. Entomol. Soc. Am. 67: 153-157.

Imamura, Y., and Y. Fujii. 1995. Analysis of feeding activities of termites by AE monitoring of infested wood (in Japanese). Wood Preservation 21: 11-19

Locher, J., A. Ombòdi, T. Kassai, and J. Dimèny. 2005. Influence of coloured mulches on soil temperature and yield of sweet pepper. Eur. J. Hortic. Sci. 70: 135-141.

McManamy, K., P. G. Koehler, D. D. Branscome, and R. M. Pereira. 2008. Wood moisture content affects the survival of eastern subterranean termites (Isoptera: Rhinotermitidae), under saturated relative humidity conditions. Sociobiology 52: 145–156.

Moore, B. P. 1969. Biochemical studies in termites, pp. 407–432. *In* K. Krishna and F. M. Weesner (eds.), Biology of termites, vol. I. Academic, New York.

Morales-Ramos, J. A., and M. G. Rojas. 2001. Nutritional ecology of the Formosan subterranean termite (Isoptera: Rhinotermitidae): Feeding response to commercial wood species. J. Econ. Entomol. 94: 516-523.

Morrell, J. J. 2002. Wood-based building components: what have we learned? Int. Biodeter. Biodegr. 49: 253-258.

Nagnan, P., and J. L. Clement. 1990. Terpenes from the maritime pine *Pinus pinaster*: toxins for subterranean termites of the genus *Reticulitermes* (Isoptera: Rhinotermitidae). Biochem. Syst. Ecol. 18: 13-16.

Nakayama, T., T. Yoshimura, and Y. Imamura. 2004. The optimum temperature-humidity combination for the feeding activities of Japanese subterranean termites. J. Wood Sci. 50: 530-34.

Nakayama, T., T. Yoshimura, and Y. Imamura. 2005. Feeding activities of *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* (Kolbe) as affected by moisture content of wood. J. Wood Sci. 51: 60-65.

Pearce, M. J. 1997. Termite biology and behavior, pp. 53–55. *In* M. J. Pearce (ed.), Termites: biology and pest management. CAB International, Wallingford, United Kingdom.

Ramakrishna, A., H. M. Tam, S. P. Wani, and T. D. Long. 2006. Effect of mulch on soil temperature, moisture, weed infestation and yield of groundnut in northern Vietnam. Field Crops Res. 95: 115-125.

Smith, J. L., and M. K. Rust. 1993. Effect of relative humidity and temperature on the survival of *Reticulitermes hesperus* (Isoptera: Rhinotermitidae). Sociobiology 21: 217–224.

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. Am. 63: 841-847.

Smythe, R. V., and L. H. Williams. 1972. Feeding and survival of two subterranean termite species at constant temperatures. Ann. Entomol. Soc. Am. 65: 226–229.

Smythe, R. V., F. L. Carter, and C. C. Baxter. 1971. Influence of wood decay on feeding and survival of the eastern subterranean termite *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 64: 59-62.

Sponsler, R. C., and A. G. Appel. 1990. Aspects of the water relations of the Formosan and eastern subterranean termites (Isoptera: Rhinotermitidae). Environ. Entomol. 19: 15-20.

CHAPTER 4

RELATIVE HUMIDITY PREFERENCE AND SURVIVAL OF STARVED FORMOSAN SUBTERRANEAN TERMITES (ISOPTERA: RHINOTERMITIDAE) AT VARIOUS TEMPERATURE AND RELATIVE HUMIDITY CONDITIONS[3]

Introduction

The invasion of a new habitat by an introduced species may depend on a number of factors such as the suitability of the abiotic environment (Blackburn and Duncan 2001), the ability of the species to adapt to the novel environment (Sax and Brown 2000) and the interaction between the invader and the recipient community (Vitousek et al. 1996, Holway 1998). Invading social insects are among the most harmful organisms, which disrupt indigenous communities, impact large geographic areas and are usually expensive to manage (Holway 1998).

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is one such invasive social insect and has made its home in most of the states of the southern United States (Woodson et al. 2001). Both biotic and abiotic factors play a role in the successful establishment of this species. Of the abiotic factors, moisture and temperature play a vital role in determining which areas are the most suitable for this invasive insect (Emerson 1955, Rudolph et al. 1990). Some studies (Delaplane and La Fage 1989, Fei and Henderson 2002, Nakayama et al. 2004, 2005, Gautam and Henderson 2011a, b) have shown that moisture and temperature are important determining factors for feeding preference and survival of *C. formosanus*. The workers and soldiers, which comprise a large proportion of the colony's population, have a soft integument that makes them extremely vulnerable to desiccation (Moore 1969). Therefore, the availability of moisture may be an essential precondition for the establishment of *C. formosanus* into an area.

Moisture studies on subterranean termites have mostly been focused on understanding the feeding activities of termites at different temperatures, substrate moisture and relative humidity (RH) conditions. For example, Yusuf et al. (2000) reported that the optimum RH for the activity of *C. formosanus* was > 70% at a constant temperature of 28°C. Similarly, Nakayama et al. (2004) reported the optimum RH conditions for feeding to be 90% for *C. formosanus* and 70-

56

90% for *Reticulitermes speratus* (Kolbe) at 30°C. These authors provided moistened food in their experiments. Providing moist food means termites would be able to obtain moisture from the food source even if the ambient RH condition was recorded to be very low. It is probable that moisture emitted from moist substrate or food contributes to the micro-atmospheric RH, increasing the RH around the termite's body. Sponsler and Appel (1990) reported that substrates such as termite nest materials that have a moisture content > 16% usually have their interstitial spaces at or near saturated RH levels. Therefore, a true RH study is probably not possible when moist food is provided. In other words, stable and defined RH may only be possible when bioassays are conducted in the absence of moist food (starvation condition) and/or moist substrate. Although termites may have plenty of food in nature, it is not uncommon for foraging groups to become isolated from their food sources and face periods of starvation during the course of food search, or due to various reasons like natural calamities and human activities (Husby 1980, Forschler and Henderson 1995, Strack and Myles 1997).   Here, we attempted to assess the preference for atmospheric RH at a constant temperature and the impact of various RH and temperature levels in the survival of groups of *C. formosanus* under starvation conditions. This study will be of practical use to obtain knowledge on how long termites will survive while searching for food in a heterogeneous environment, for example, in housing structures where apparently no tubing connection with the soil or moist food source remain established.

Materials and Methods

Termites. Formosan subterranean termites were collected from Brechtel Park and along the flood walls on Tchoupitoulas Street in New Orleans, Louisiana in April and May 2008 using milk crate traps according to the methods described in Gautam and Henderson (2011a). The

termites were maintained in the laboratory in Rubbermaid® trash cans (121 L, Roughneck) with wet wood under high RH for a maximum of 3 months before they were used for the tests.

RH Preference Trials. Experimental chambers for RH preference trials were constructed by connecting five round plastic containers (inside dimensions: 10.3 cm diam by 12.1 cm deep; ©Pioneer Plastics Inc., North Dixon, KY) with small clear vinyl tubes (4 cm long, 0.64 cm inside diam, Watts Co., North Andover, MA) in a linear arrangement. A small PVC coupling (LASCO 3.81 cm diam by 6.99 cm tall) was attached at the base inside each container with an adhesive sealant (Loctite®, Hankel Consumer Adhesives Inc., Avon, OH) instant crazy glue, which served as a stand for an open Petri dish (100 mm by 15 mm). The vinyl tubes were inserted into the snugly fitting holes made by a soldering iron on the container and also the Petri dish placed on the PVC stand inside the container so that termites could easily have access from one Petri dish to another among the five containers (Fig. 4.1). The test containers were partially filled with either silica gel or distilled water or a series of salt solutions so as to maintain a humidity gradient of 9-98% in the test arena. Saturated salt solutions were prepared according to the methods of Winston and Bates (1960). Previous studies have shown that the use of saturated salt solutions to maintain a particular RH in a closed container had no adverse odor effects on insects including termites (Smith and Rust 1993, Steidle and Reinhard 2003, Walters and Mackay 2003). The subsequent RH provided by the salt solutions in the middle 3 containers, were as follows: - $MgCl_2.6H_2O$ (33%), $Mg (NO_3)_2.6H_2O$ (53%) and NaCl (75%). The first container was partially filled with dry silica gel which provided the lowest RH (9%) and the fifth was partially filled with distilled water that provided an RH near saturation (98%). Each saturated solution was prepared by dissolving the appropriate amount of salt in boiling water and adding more salt as the solution cooled down so that some undissolved salt crystals remained in the solution. This

solution was poured in the container and the container left to equilibrate for one day to insure the

desired RH in the chamber. On the second day, the RH in each chamber was measured using a

Testo$^®$ 625 humidity meter (Testo Inc., Sparta, NJ), and 50 termites (42 workers and 8 soldiers;

workers were externally undifferentiated individuals developed to at least the third instar) were

placed in the Petri dish of each container (total of 250 termites in each trial). The Petri dishes

contained a monolayer of autoclaved and oven-dried sand to facilitate the termite movement as

our preliminary tests showed that termites moved faster and easier in the presence of sand than

on a smooth dish surface. The containers were immediately closed and left undisturbed at ~

26°C. As the level of the salt solution was below the Petri dish level, there was no contact of

termites with salt solutions. The numbers and locations of living and dead termites were recorded

after 1, 2, 4 and 12 h. Six replications were conducted using termite groups from 2 colonies. To

reduce the effects of directional bias, replicates were placed facing in different directions in the
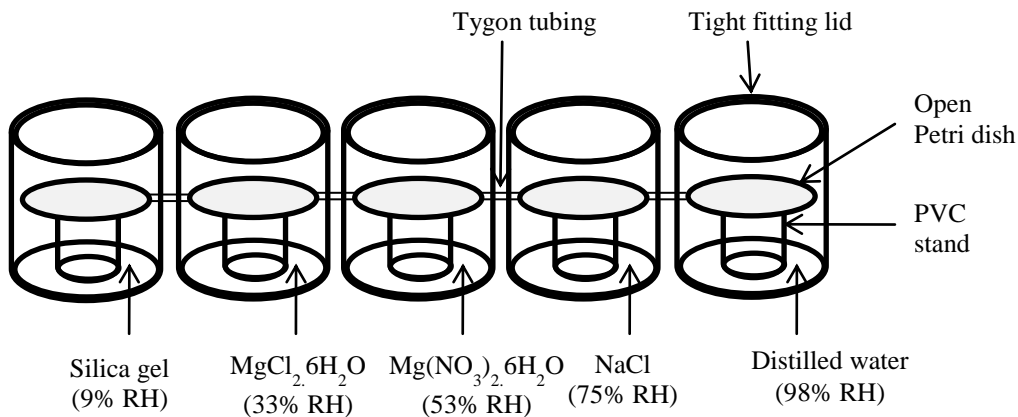
laboratory.

Figure 4.1. Diagrammatic representation of the humidity gradient arena established for the relative humidity preference trials in the laboratory.

Survival Trials. Test chambers for survival trials were constructed using the same procedures as described in the preference trials except that the containers were not connected to each other and no holes were present in the containers or Petri dishes. Individual containers were partially filled with the aforementioned saturated solutions including dry silica gel and left to equilibrate for 1 d. There were a total of 15 combinatorial treatments of 5 RH levels (9%, 33%, 53%, 75% and 98%) and 3 temperature levels (20°C, 28°C and 36°C; maintained in 3 incubators). After 1 d of equilibration, 50 termites (42 workers and 8 soldiers; workers were externally undifferentiated individuals developed to at least the third instar) were placed in the open Petri dish in each container and the containers were placed in 3 incubators set to the 3 different temperatures. Trials were replicated 3 times with termite groups collected from a single colony. Dead and living termites were recorded after 1, 2, 4, 12, 18, 24 h and every 12 h interval thereafter up to 180 h. Fungus growth on the dead termites was also recorded.

Statistical Analysis. In the RH preference tests, the proportion of living termites in each RH chamber at various time periods was recorded. Dead termites were not counted so that the proportion of living termites in all RH chambers always summed to one (or 100%). This type of data is called compositional data and contains only relative information. If one component increases, others must decrease, hence they are not independent. One way of dealing with such data is the transformation of raw proportions to "centered logratio" (Aitchison 1986). The centered log ratios are not constrained to sum to one (or 100%) and can therefore be analyzed using standard statistical techniques (Pawlowsky-Glahn and Egozcue 2006). Here, the transformed data were analyzed using repeated measures ANOVA with RH as between subject effect and time as within subject effect. Since termites were used from two colonies, the colony effect was analyzed using a two sample t-test.

60

The survival data were arcsine of the square root transformed and analyzed using repeated measures analysis where RH and temperatures were the between subject effects and time as within subject effect. Analyses were performed using generalized linear model in SAS software ([©]2002-2003 SAS Institute Inc. Cary, NC).

Results

RH Preference. Colony had no significant effect on termite aggregation among the various RH chambers (two sample t-test: $P = 0.99$). There was a significant interaction effect of RH by time ($F_{12, 75} = 12.32$, $P < .0001$). Similarly, there was a significant between subject effect (effect of RH: $F_{4, 75} = 53.28$, $P < .0001$) but there was no within subject effect (effect of time: $F_{3, 75} = 0.0$, P = 1). After 1 h, a significantly greater percentage of the released termites aggregated to the highest RH chamber (~ 46%) compared to any of the lower RH chambers. As more and more termites moved to the highest RH chamber, ~ 97% of the termites aggregated in the highest RH chamber after 12 h, which was ~ 85% of the total released termites (~ 12% termites died during 12 h period, all in the ≤ 75% RH chambers). The percentage of termites aggregated in the 4 lower RH chambers did not differ significantly among each other at any observation periods except at the 4 h observation period where the percentage of termites in the 35% RH chamber was significantly greater (~ 6%) than in the 74% RH chamber (~ 3%) (Fig. 4.2A-D).

Survival. Survival of starved *C. formosanus* in the laboratory test arena was impacted significantly by the interaction of temperature and RH over time ($F_{144, 540} = 23.17$, $P < .0001$), by temperature over time ($F_{36, 540} = 101.13$, $P < .0001$) and by RH over time ($F_{72, 540} = 25.69$, $P < .0001$). Separate repeated measures ANOVA conducted for each RH solution revealed that in the 9% RH chamber the survival of the termites at 2 h post exposure declined significantly at 36°C (74.67% survival) compared with those at 20°C or 28°C (100% survival in both). At 4 h period,
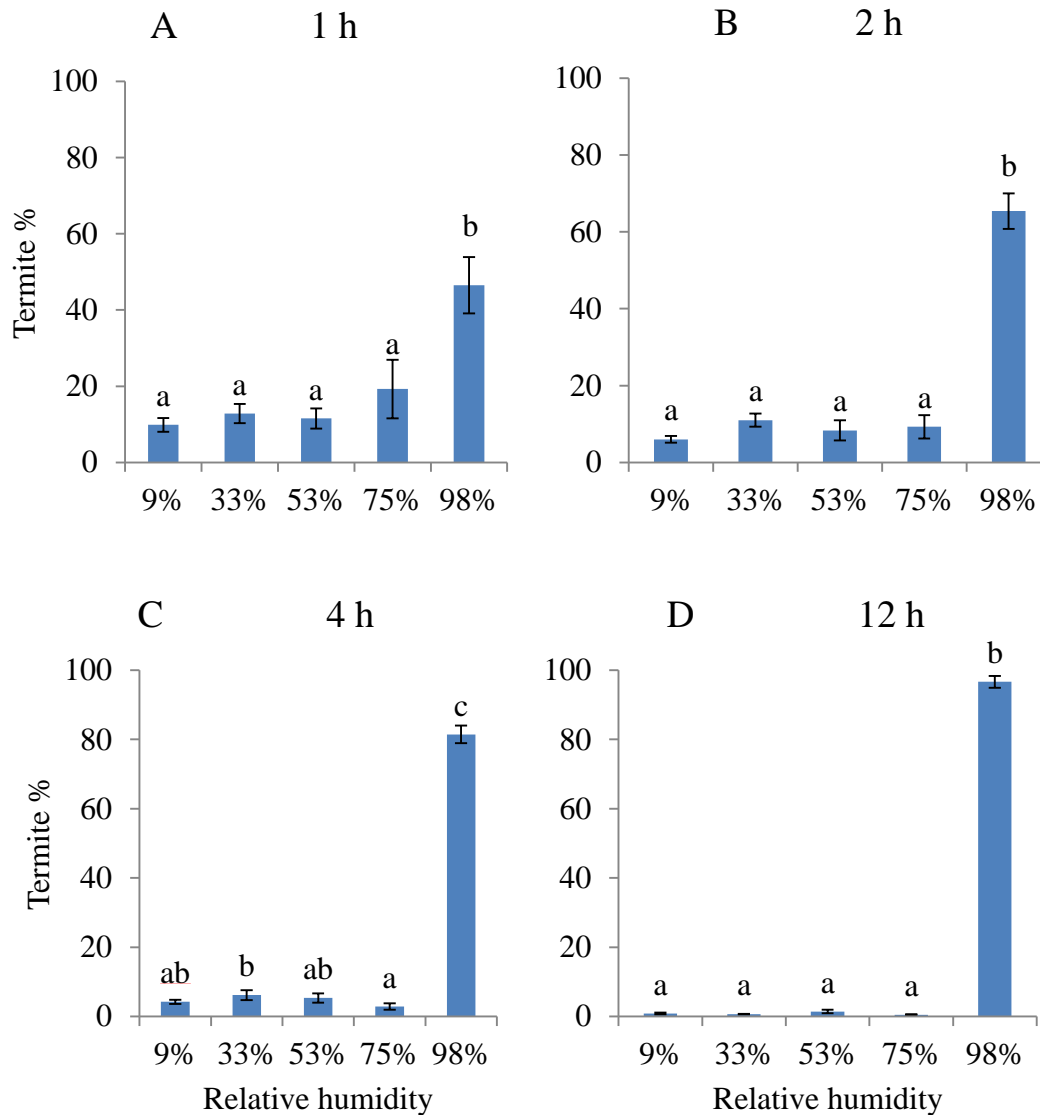
Figure 4.2(A-D). Mean % (± SEM) of live *C. formosanus* aggregated in various relative humidity chambers observed at 4 different time periods after release at a constant temperature of ~26°C.

there was ~ 5% survival at 36°C but the survival did not decline at 20°C or 28°C. At 28°C,

survival was reduced significantly at 12 h post exposure (8.6%) compared to that at 20°C

(97.3%). About one third of *C. formosanus* survived (32.6% survival) for 48 h at 20°C but this

was reduced to ~ 7% at 60 h post exposure at this temperature (Fig. 4.3A).

Termites exposed to the 33% RH chamber survived relatively longer than in the 9% RH chamber. At 4 h post exposure, the survival was reduced significantly at 36°C (63%) compared to 20°C or 28°C (100% in both). The survival at 28°C was reduced significantly at 18 h post exposure (4.6%) whereas at 20°C, > 98% termites were alive. At 60 h post exposure, ~ 25% of the termites were alive at 20°C (Fig. 4.3B).

In the 53% RH chamber, 87% of the termites were alive at 4 h post exposure at 36°C which was significantly lower than the 100% survival at 20°C or 28°C. Most of the termites died at 36°C at 12 h period (2% survival). The same level of reduction in survival (2% survival) was observed at 36 h of exposure at 28°C, whereas > 90% survival was observed during this period at 20°C. The survival reduced to 7% at 96 h of exposure at 20°C (Fig. 4.3C).

At 75% RH, termites survived relatively longer than in the lower RH chambers at all the three temperatures. At 36°C, ~27% termites survived at 12 h post exposure but almost all the termites died at 24 h post exposure which was significanlty different comapred to the survival at 28°C and 20°C (78.66% and 99.33% survival, respectively). At 28°C, the survival reduced to < 10% at 48 h post exposure whereas the survival was still > 97% at 20°C. There was > 40% survival at 108 h post exposure at 20°C, but it was reduced to 11.33% at 132 h of exposure (Fig. 4.3D).

When the RH was near saturation (98%), termites survived the longest compared to all other RH chambers at all the three temperatures. There was no significant difference in survival at 12 h post exposure among the three temperatures. But at 18 h post exposure, the survival was significantly lower at 28°C and 36°C (95.33% and 93.33%, respectively) than at 20°C (100%). The survival was reduced to 30% at 60 h post exposure at 36°C but survivals remained significanlty higher at 20°C and 28°C during this period (95.33% and 91.33%, respectively); all

the termites died at 84 h post exposure at 36°C. After 120 h of exposure, although a higher trend of survival was observed at 28°C (80%), the difference was not statistically significant compared with that at 20°C (55% survival) (Fig. 4.3E).

Fungi attack was not observed on dead termite bodies in any of the RH chambers placed at 20°C, or in RH chambers at or below 75% at all the three temperatures. However, dead termites at 98% RH chambers placed at 28°C and 36°C incubators were covered by fungal growth (data not shown).
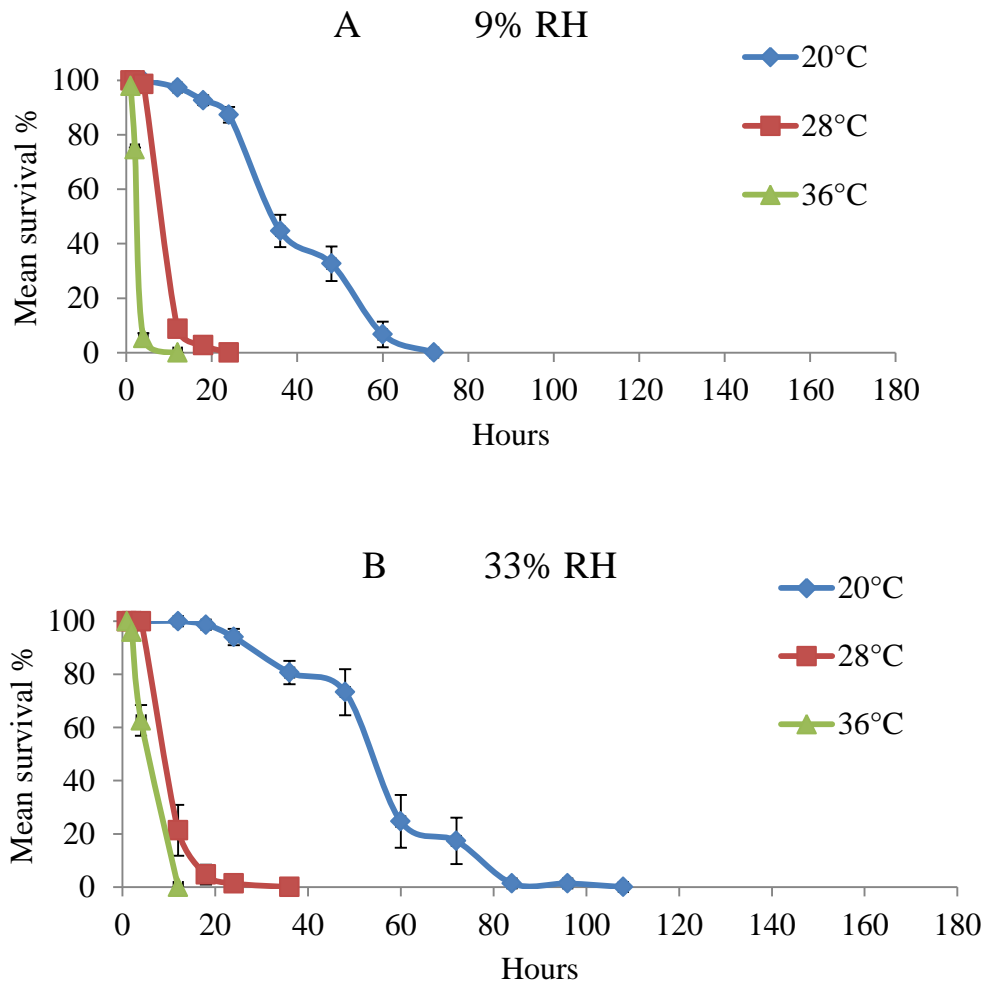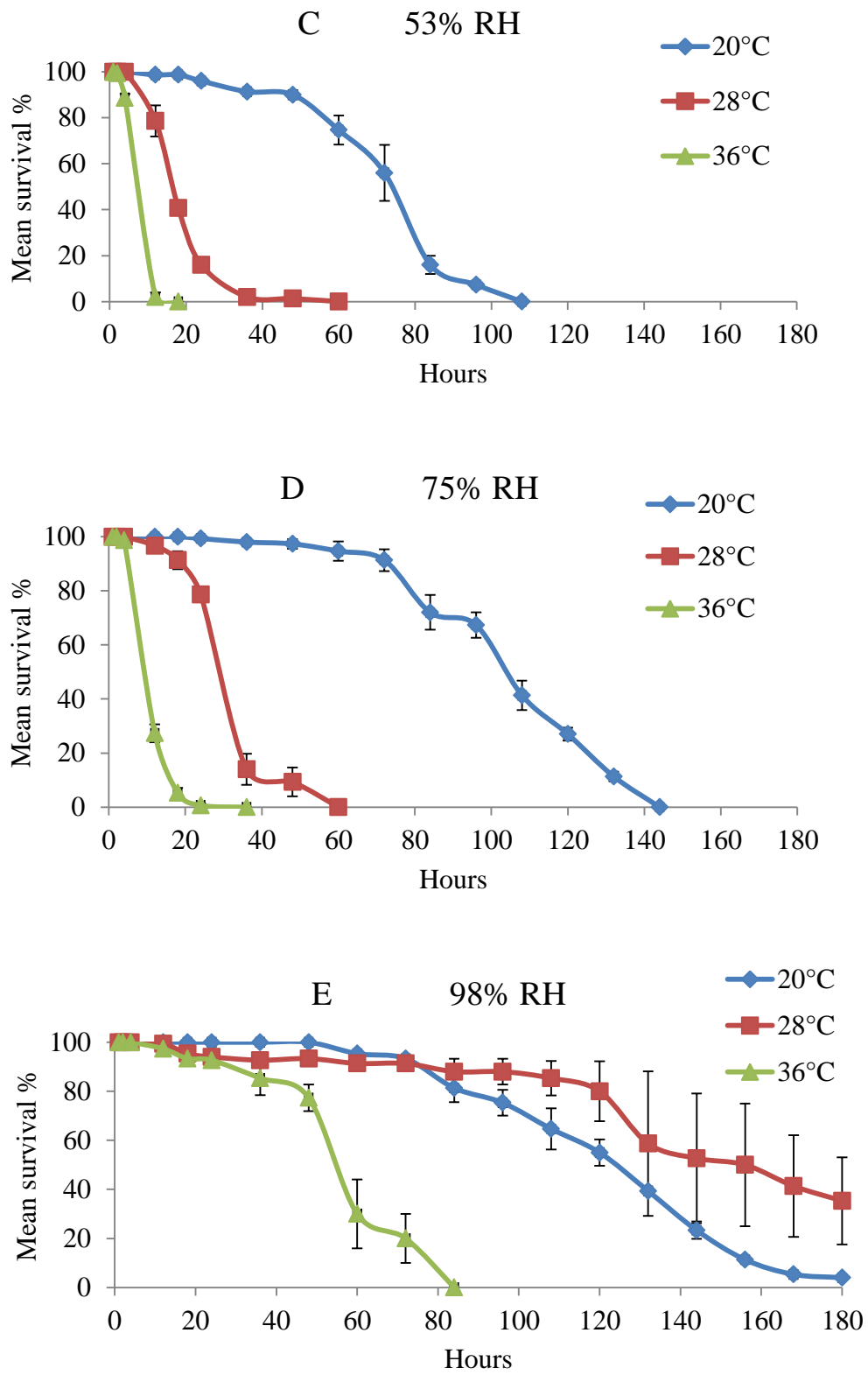

Figure 4.3(A-E). Mean survival % (± SEM) of *C. formosanus* exposed to various temperature and relative humidity combinations observed at different time periods.

(Figure 4.3 contd.)

Discussion

The results from this study supported our hypothesis that *C. formosanus* workers and soldiers aggregate to the highest RH area. Individual workers and soldiers preferentially selected the highest RH chamber in the RH gradiant arena, and at the group level, survival of *C. formosanus* was greatest at the near saturated RH environment. In our previous study (Gautam and Henderson 2011a), we reported that *C. formosanus* randomly aggregated to one of the chambers that had high RH (> 98%) and moist sand substrate irrespective of the sand moisture levels unless the sand was dry or saturated. However, when it comes to wood consumption, high RH alone was not enough, rather they were found to preferentially select the wood having the highest initial moisture levels (Delaplane and La Fage 1989, Gautam and Henderson 2011b). These studies suggest that substrate moisture level and wood moisture level distinctly influence *C. formosanus* biology. In the present experiment, there was no food source or moist substrate in the bioassay arena (a monolayer of dry sand in the RH preference test arena was intended just to facilitate termite walking), which means termites were influenced by environmental RH only and they chose the aggregation site based on RH preference.

Termites are known to employ various behavioral mechanisms such as avoidance (Steward 1981, 1982, Cabrera and Rust 1996, Gautam and Henderson 2011a) and aggregation (Collins 1969, Minnick et al. 1973, Steward 1982) to minimize the effects of desiccation. Here we suggest that the avoidance behavior by Formosan subterranean termites was observed as they avoided low RH chambers. Strack and Myles (1997) suggested that foraging groups of *R. flavipes* avoided the exposure of adverse temperatures by moving downward in the ground where temperatures were more favorable. Similarly, Hu and Song (2007) demonstrated the behavioral avaoidance of adverse temperature in foraging groups of *C. formosanus*. In the present

66

preference test, the temperature was constant, therefore the high RH alone served as the cue for *C. formosanus* aggregation.

Cuticular water loss represents the most important potential source of body fluid leakage in terrestrial insects (Edney 1977, Hadley 1985). Water loss is enhanced by the large body surface area to mass ratio of these small arthropods (Edney 1977, Hadley 1994). Insects with soft cuticle, such as workers and soldiers of subterranean termites, are extremely susceptible to desication because they lose moisture rapidly through the highly permeable integument (Collins 1969, Moore 1969, Sponsler and Appel 1990). The epicuticular permeability is affected by environmental factors such as temperature and RH. Temperature alters the phase of the lipid component of the epicuticular wax layer and RH influences the saturation deficit, or potential for water loss (Edney 1977). We did not measure, however, how much impact was on RH in the closed container by the water loss from termites' body. We assume that since the RH chamber was fairly big, the water loss might not have increased the RH level significantly. Survival of groups of termites exposed to various temperatures and/or RH conditions is closely correlated with their distribution and habitat patterns characteristic of that species. In this study, *C. formosanus* showed a tendency of higher survival at high atmospheric RH (98%) and medium temperature (28^oC). These environmental conditions commonly prevail in most of Louisiana. Southern parts of Louisiana, including cities like New Orleans and Baton Rouge, have mean monthly temperatures around 28 ± 2^oC from May to September. Even in the condition where soil moisture content is low, it should be sufficient enough to make the RH in the soil pores near saturation making the substrate suitable for *C. formosanus* (Gautam and Henderson 2011a). We believe that such a favorable habitat in most part of Louisiana is one of the important reasons of a dramatic increase of *C. formosanus* infestation in the state (La Fage 1987, Henderson 2001).

Collins (1991) reported that survival time for species of Rhinotermitidae exposed to 0-4% RH and 34°C could be measured in minutes or hours. The present results showed a similar trend in which < 5% *C. formosanus* survived more than 4 h at 9% RH and 36°C. The level of desiccation tolerance varies even among species of Rhinotermitidae. For instance, Khan (1980) reported that *Coptotermes niger* Snyder and *R. speratus* were more susceptible to desiccation than *Heterotermes indicola* (Wasmann) and *Reticulitermes tibialis* Banks. He also reported that termites of the same species but of the different geographical locations showed differences in their resistance to desiccation. Temperature plays an inherent role to either enhance or delay the rate of desiccation. In the present study, the fastest rate of mortality due to desiccation was observed in termites exposed at high temperature (36°C) followed by medium (28°C) and then low temperature (20°C) at a given RH condition. Survival of the temperate species like *Reticulitermes hesperus* Banks was highest in cool temperatures (16 – 21°C) and high RH (> 99%) (Smith and Rust 1993). Unlike subterranean termites, drywood termites in the genus *Cryptotermes* Banks have been reported to have an extreme desiccation tolerance capacity (Minnick et al. 1973, Steward 1981, 1982, Rudolph et al. 1990, Collins 1991).

In the present survival tests, although termites were starved, starvation does not seem to be the leading cause of mortality. In preliminary studies, where groups of *C. formosanus* were released in moist sand substrate but no food source, not a single termite died of starvation when observed after 2 weeks period. Song et al. (2006) reported < 10% mortality on starved *C. formosanus* at 10 d. It was obvious that the leading cause of mortality on termites exposed to low RH chambers (≤ 75%) was desiccation whereas a combination of factors, including attack of pathogenic fungi, desiccation and starvation may have contributed for the death of termites exposed to 98% RH chambers. We assume that starvation may have reduced the resistance of

termites to pathogenic fungi attack and also to desiccation tolerance. Rudolph et al. (1990) suggested that passive water uptake by an insect body from the atmosphere is not possible unless the atmosphere is damp. He demonstrated that *C. formosanus* lost up to 10% of its body mass within 1 d even when placed at atmospheric RH close to the saturation point, i.e. 98%. He therefore concluded that atmospheric RH is only a minor and mostly indirect importance for the water balance and the survival of subterranean termites. Our present results showing the mortality of ~ 50% termites at 7 d even when placed at environmental conditions of 98% RH and 28°C indicated that *C. formosanus* needs additional sources of water for their normal activities and survival.

Our results showed that termites were not attacked by pathogenic fungi at low temperature (20°C) irrespective of the RH levels, or ≤ 75% RH levels irrespective of the temperatures, possibly because these environmental conditions were not favorable for the pathogenic fungi to grow and proliferate (Gillespie and Crawford 1986, Hywel-Jones and Gillespie 1990, Rath 2000). However, pathogenic fungi attack may have enhanced the termite mortality at high temperatures and high RH conditions. Studies such as Higa (1981) and Jayasimha and Henderson (2007) reported the possibility of high mortalities in termite colonies maintained in the laboratory due to attack of pathogenic fungi. In nature, however, *C. formosanus* may use naphthalene to fumigate its nest which inhibits the growth of the pathogenic fungi (Chen et al. 1998).

Results herein suggest that starvation is not the major cause of death of foraging groups of *C. formosanus* when they are isolated from a moist food source and/or moist substrate, rather they will die from desiccation if the atmospheric RH is low and desiccation and pathogenic fungi attack if the atmospheric RH is close to saturation point before they are directly affected by

starvation. We suspect that the low movement (or activities) of termites in a confined situation in the laboratory arena may also have weakened their natural immune power against the causal mortality factors.

References

Aitchison, J. 1986. The Statistical Analysis of Compositional Data. Chapman and Hall, New York.

Blackburn, T. M., and R. P. Duncan. 2001. Determinants of establishment success in introduced birds. Nature 414: 195–197.

Cabrera, B. J., and M. K. Rust. 1996. The behavioral responses to light and thermal gradients by the western drywood termite (Isoptera: Kalotermitidae). Environ. Entomol. 25: 436-445.

Chen, J., G. Henderson, C. C. Grimm, S. W. Lloyd, and R. A. Laine. 1998. Termites fumigate their nests with naphthalene. Nature 392: 558-559.

Collins, M. S. 1969. Water relations in termites, pp. 433-458. *In* K. Krishna and F. M. Weesner (eds.), Biology of termites, vol.1. Academic, New York.

Collins, M. S. 1991. Physical factors affecting termite distribution. Sociobiology 19: 283–286.

Delaplane, K. S., and J. P. La Fage. 1989. Preference for moist wood by the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 82: 95-100.

Edney, E. B. 1977. Water balance in land arthropods. Zoophysiology and Ecology, vol. 9. Springer, Berlin.

Emerson, A. E. 1955. Geographical origins and dispersion of termite genera. Fieldiana Zool. 37: 465-519.

Fei, H., and G. Henderson. 2002. Formosan subterranean termite (Isoptera: Rhinotermitidae) wood consumption and worker survival as affected by temperature and soldier proportion. Environ. Entomol. 31: 509-514

Forschler, B. T., and G. Henderson. 1995. Subterranean termite behavioral reaction to water and survival of inundation: implications for field populations. Environ. Entomol. 24: 1592–1597

Gautam, B. K., and G. Henderson. 2011a. Effects of sand moisture level on food consumption and distribution of Formosan subterranean termites (Isoptera: Rhinotermitidae) with different soldier proportions. J. Entomol. Sci. 46: 1-13.

Gautam, B. K., and G. Henderson. 2011b. Wood consumption by Formosan subterranean termites (Isoptera: Rhinotermitidae) as affected by wood moisture content and temperature. Ann. Entomol. Soc. Am. 104: 459-464.

Gillespie, A. T., and E. Crawford. 1986. Effect of water activity on conidial germination and mycelial growth of *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces* spp. and *Verticillium lecanii*. *In* R. A. Samson, J. M. Vlak, and D. Peters (eds.), Fundamental and applied aspects of invertebrate pathology. Society of Invertebrate Pathology, Wageningen, The Netherlands. 254 pp.

Hadley, N. F. 1985. The Adaptive Role of Lipids in Biological Systems. Wiley, New York.

Hadley, N. F. 1994. Water Relations of Terrestrial Arthropods. Academic Press, San Diego.

Henderson, G. 2001. Practical consideration of the Formosan subterranean termite in Louisiana: a 50-year-old problem. Sociobiology 37: 281-392.

Higa, S. Y. 1981. Flight, colony foundation, and development of the gonads of the primary reproductives of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. Ph.D. dissertation. University of Hawaii, Honolulu, HI.

Holway, D. A. 1998. Factors governing the rate of invasion: a natural experiment using Argentine ants. Oecologia 115: 206–212.

Hu, X.P., and D. Song. 2007. Behavioral reactions of two subterranean termites (Isoptera: Rhinotemitidae) to instant freezing or chilling temperatures – cold avoidance and survival strategy. Environ. Entomol. 36: 1450-1456.

Husby, W. D. 1980. Biological studies on *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) in southern Ontario. M.S. thesis, University of Guelph, Guelph.

Hywel-Jones, N. L., and A. T. Gillespie. 1990. Effects of temperature on spore germination in *Metarhizium anisopliae* and *Beauveria bassiana*. Mycol. Res. 94: 389–392.

Jayasimha, P., and G. Henderson. 2007. Fungi isolated from integument and guts of *Coptotermes formosanus* and their antagonistic effect on *Gleophyllum trabeum*. Ann. Entomol. Soc. Am. 100: 703–710.

Khan, M. A. 1980. Effect of relative humidity on survival of termites under starvation conditions. Zeitschrift fur Angewandte Zoologie 67: 133-178.

La Fage, J. P. 1987. Practical considerations of the Formosan subterranean termite in Louisiana: a 30-year old problem, pp. 37-42. *In* M. Tamashiro and N.-Y. Su (eds.), The Biology and Control of the Formosan Subterranean Termite. College Trop. Ag. Human Resources, Univ. of Hawaii, Honolulu.

Minnick, D. R., S. H. Kerr, and R. C. Wilkinson. 1973. Humidity behavior of the drywood termite *Cryptotermes brevis.* Environ. Entomol. 2: 597-601.

Moore, B. P. 1969. Biochemical studies in termites, pp. 407-432. *In* K. Krishna and F. M. Weesner (eds.), Biology of Termites, Vol. 1. Academic, New York.

Nakayama, T., T. Yoshimura, and Y. Imamura. 2004. The optimum temperature-humidity combination for the feeding activities of Japanese subterranean termites. J. Wood Sci. 50: 530-34.

Nakayama, T., T. Yoshimura, and Y. Imamura. 2005. Feeding activities of *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* (Kolbe) as affected by moisture content of wood. J. Wood Sci. 51: 60-65.

Pawlowsky-Glahn, V., and J. Egozcue. 2006. Compositional data and their analysis: an introduction, pp. 1-10. *In* A. Buccianti, G. Mateu-Figueras, and V. Pawlowsky-Glahn, (eds.), Compositional Data Analysis in the Geosciences: From Theory to Practice. Geological Society, Special Publications, London.

Rath, A. C. 2000. The use of entomopathogenic fungi for control of termites. Biocontrol sci. techn. 10: 563 – 581.

Rudolph, D., B. Glocke, and S. Rathenow. 1990. On the role of different humidity parameters for the survival, distribution and ecology of various termite species. Sociobiology 17: 129-140.

Sax, D. F., and J. H. Brown. 2000. The Paradox of Invasion. Global Ecol. Biogeogr. 9: 363–371.

Smith, J. L., and M. K. Rust. 1993. Effect of relative humidity and temperature on the survival of *Reticulitermes hesperus* (Isoptera, Rhinotermitidae). Sociobiology 21: 217-224.

Song, D., Hu, X. P., and N.-Y. Su. 2006. Survivorship, cannibalism, body weight loss, necrophagy, and entombment in laboratory groups of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, under starvation. Sociobiology 47: 27-39.

Sponsler, R. C., and A. G. Appel. 1990. Aspects of the water relations of the Formosan and eastern subterranean termites (Isoptera; Rhinotermitidae). Environ. Entomol. 19, 15–20.

Steidle, J. L. M., and J. Reinhard. **2003.** Low humidity as a cue for habitat preference in the parasitoid *Lariophagus distinguendus*. Biocontrol 48:169-175.

Steward, R. C. 1981. The temperature preferences of building- damaging dry-wood termites (*Cyptotermes*: Isoptera). J. Therm. Biol. 6: 153-160.

Steward, R. C. 1982. Comparison of the behavioral and physiological responses to humidity of five species of dry-wood termites, *Cryptotermes* species. Physiol. Entomol. 7: 71-82.

Strack, B. H., and T. G. Myles. 1997. Behavioral responses of the eastern subterranean termite to falling temperatures (Isoptera: Rhinotermitidae). Proc. Entomol. Soc. Ont. 128: 13–17

Vitousek, P. M., C. M. D'Antonio, L. L. Loope, and R. Westbrooks. 1996. Biological invasions as global environmental change. Am. Sci. 84: 468–478.

Walters, A. C., and D. A. Mackay. 2003. An experimental study of the relative humidity preference and survival of the Argentine ant, *Linepithema humile* (Hymenoptera, Formicidae): comparisons with a native *Iridomyrmex* species in South Australia, Insectes Soc. **50**: 355–360.

Winston, P. W., and D. H. Bates. 1960. Saturated solutions for the control of humidity in biological research. Ecology 41: 232–237.

Woodson, W. D., B. A. Wiltz, and A. R. Lax. 2001. Current distribution of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the United States. Sociobiology 37: 661-671.

Yusuf, S., Y. Yanase, Y. Sawasa, Y. Jufii, T. Yoshimura, and Y. Imamura. 2000. Evaluation of termites feeding activities by acoustic emission (AE under various relative humidity (RH) conditions), pp. 173-178. *In* Proceedings of the Third International Wood Science Symposium, November 1-2, Uji.

CHAPTER 5

ESCAPE BEHAVIOR OF THE FORMOSAN SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE) IN RESPONSE TO DISTURBANCE[4]

---

[4] Reprinted by permission of "Journal of Insect Behavior"

Introduction

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is one of the most destructive structural pests in the United States (Su and Scheffrahn 1990). This termite lives a cryptic lifestyle where workers and soldiers forage through tunnels and galleries originating from their nests. Popular control practices involve the use of non-repellent termiticides and baiting systems (Henderson 2001). Although baiting is the most environmental friendly way of controlling termites, about two thirds of the treatments by pest control companies rely on the use of liquid insecticides in soil (Curl 2004). The success of baiting system is more variable than that of the liquid soil termiticides (Forschler and Ryder 1996, Su and Scheffrahn 1996, Lewis 1997). Baiting systems depend on the exploitation of foraging behavior of subterranean termites where a subset of individuals from a colony feed on the cellulosic food material impregnated with slow-acting toxicants and introduce the toxicants to the colony (Traniello and Thorne 1994, Grace et al. 1996, Pawson and Gold 1996, Su and Scheffrahn 1998). The goal of a baiting system is to eliminate the entire termite colony from an area with the least possible cost and harm to non-target organisms in the environment (Su and Scheffrahn 1998).

Termite baiting protocols involve an inevitable degree of disturbance during periodic checking of monitors and/or replacing with baits. There have been mixed reports about the impacts of disturbances on the subterranean termites. Noirot and Darlington (2000) reported that termites usually respond to a disturbance by leaving the food source or the nest. However, others have reported that the escape was usually temporary and the time taken to return to the disturbed area varied depending on the termite species, type of disturbance, soldier proportions and environmental factors such as temperature (Hu et al. 2003, Schwinghammer and Houseman 2006). Hu et al. (2003) applied a vibrational stimulus on a group of *C. formosanus* without

exposing them to outside air movement, which may not be comparable to the actual disturbance encountered in the field while checking monitors or replacing with baits. With increased investments in the development of termite baits, it is crucial to know whether the disturbance commonly encountered during checking/replacement of monitors/baits have impacts on the termites' revisiting the disturbed site. Knowledge regarding this, even in laboratory conditions, would add to our understanding of *C. formosanus* behavior and gives us an idea on how to increase the effectiveness of the termite baiting system.

Here we conducted a laboratory study to investigate the effects of disturbance on the escape behavior of *C. formosanus*. The experimental design is an attempt to simulate a situation in the field where foraging termites encounter only one food source in a relatively large area, especially likely when foragers cover a long distance in search of food. We tried to simulate the disturbance with what is commonly encountered during a monitoring and baiting process. A digital video camera was used to track the behavioral response of termites every second after disturbance. We used wet and dry sand in the peripheral chambers to see whether favorable versus unfavorable substrates on the outer periphery impacted time-lines to stay away from the already attacked but disturbed food source.

Materials and Methods

Termites. Formosan subterranean termites were collected from Brechtel Park in New Orleans, Louisiana in August 2008 using milk crate traps as described in Gautam and Henderson (2011). The termites were maintained in the laboratory for a maximum of one month before they were used for the bioassays. Healthy looking and active termites were selected for the experiment.

Preparation of Experimental Arena. The experimental arena was constructed from 5 equal sized round acrylic containers (size: 5.08 by 3.63 cm, Pioneer Plastics Inc, North Dixon, KY) where one central container was connected to 4 peripheral containers with small clear vinyl tubes (4 cm long, 0.95 cm outside diameter, 0.64 cm inside diameter, Watts Co., North Andover, MA) arranged at equal intervals around the central container (Fig. 5.1). The peripheral chambers consisted of a thin layer (12 g) of either moist sand (15% moisture w: w) or dry sand whereas the central chamber contained two moistened filter paper disks (4.25 cm diameter, Whatman[®]) and a moist wood block (1.9 × 1.9 × 0.9 cm Southern yellow pine, *Pinus* sp.) that was soaked in deionized water for three days before using for the tests. The moist wood block and the filter papers provided enough moisture and relative humidity for the termites throughout the 3 d test period. The arena was affixed to the lab bench surface with instant crazy glue and transparent tape to reduce vibration disturbance to peripheral chambers when applying a disturbance to the central chamber because our intention was to create a disturbance in the central chamber only and not in the peripheral chambers.
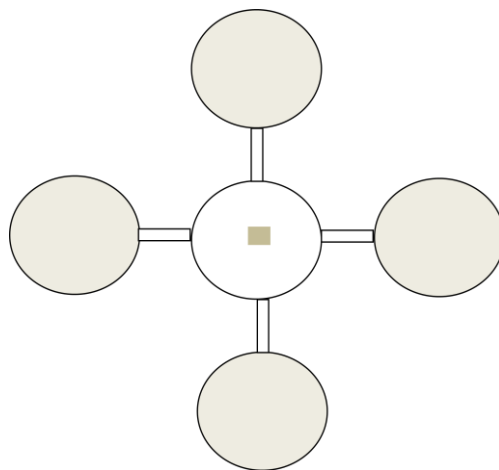

Figure 5.1. Experimental arena: The central chamber (disturbed chamber) containing a moist wood and two filter papers is connected to 4 peripheral chambers containing 12 g of either moist sand (15% moisture w: w) or dry sand with 4 cm long clear vinyl tube around the periphery at equal intervals.

A Group of 150 *C. formosanus* individuals (120 workers and 30 soldiers; workers were externally undifferentiated individuals developed to at least the third instar) was introduced into the central chamber and left for acclimation for 24 hours. During this period termites would move freely from one chamber to another but finally the majority of them settled in the food containing central chamber. After the acclimation and immediately before disturbance, the numbers of termites present in the central chambers (hereafter called 'initial termites') were recorded to compare them with the respective numbers after disturbance. Termites were counted from still images from the video recording.

Application of Disturbance. A disturbance was created in the central chamber as described: the lid of the central chamber was opened, the wood block was lifted with a pair of stiff forceps and termites were shaken off the wood block by tapping against the upper edge of the central chamber. A 2 s puff of canned air (Stoner Gust Easy Duster, Quarryville, PA) was directed to the central chamber from a height of 50 cm at an angle of 90°. The wood block was placed back into its original position, 1 ml deionized water was added and the container was covered with the lid. Caution was taken not to inflict injury to the termites and not to throw them out of the container during the entire process. The disturbance applied in the central chamber had no noticeable effect to the peripheral chambers. We believe that this type of disturbance resembled the disturbance experienced during the actual replacement of monitors with baits in termite baiting systems.

Video Recording. Termite activities before and after disturbance were video recorded as in Nagendra et al. (2010). Prior to disturbance, a digital video camcorder (model ZR10A, Canon Inc., Tokyo Japan) was set above the arena on a tripod. The area recorded by video camera included the central disturbance chamber and the tubes leading to the four peripheral chambers.

The videotaping started two minutes before the application of disturbance and continued for one hour after the disturbance and was saved onto a miniDV tape. The video was then transferred to Windows Movie Maker (version 2.1.4026.0, Microsoft Co., Redmond WA) software and reviewed manually. Control groups were videotaped after acclimatization but without any disturbance. Both worker and soldier movements from and to the central chamber were recorded. The time of the movement, duration of the movement and the direction of the movement were quantified.

To minimize variation, a prepared arena was disqualified if the number of initial termites in the central chamber was < 60% of the released population. Ultimately, there were 14 replicates for wet sand arenas and 9 replicates for dry sand arenas. The data were standardized by converting the numbers into percentages of the initial termites in the central chamber (also called percentages of the disturbed termites). Percentages of the initial termites were calculated by counting the number of termites in the central chamber at each time interval after disturbance and dividing the number with the number of initial termites in the central chamber. The variables measured after disturbance included the percentage of initial termites that escaped, time to escape, percentage of escapees that returned to the disturbed area and time to return. A trend line (curve) of mean percentage of initial termites in the central chamber over time was drawn and the lowest point on the line was the maximum percentage of escapees (evacuees) and the highest point after that was the maximum percentage of post-disturbance returnees. These lowest and highest points on the line were also the time to evacuate from the disturbed area and time to return to the disturbed area, respectively.

Statistical Analysis. Data were analyzed using SAS software (©2002-2003 SAS Institute Inc., Cary, NC), which can handle unequal replications very well. All normally distributed data

were analyzed using proc mixed model and type III sums of squares were used to get appropriate statistics. Time to escape from the disturbed area and time to return to the disturbed area were not normally distributed, so, they were assigned ranks and analyzed using Kruskal-Wallis ANOVA tests. Tukey-Kramer's HSD tests were used to compare post ANOVA results and the significance was determined using $\alpha < 0.05$.

Results

Immediately following opening of the lid, both workers and the soldiers displayed unorganized and undirected movements. Removing of the wood block, dropping of termites back to the chamber by tapping against the chamber edge and application of the air burst created a larger disturbance in the central chamber. Workers exhibited accelerated movements away from the source of disturbance, whereas soldiers moved with jaws open, heads turning left and right and mostly remained congregated in the central part of the chamber. A few workers made back-stepping movements in response to the sudden disturbance. After a few seconds (3-4 s), the termites moved through the tubes into the peripheral chambers, with the tubes often having a momentary heavy traffic of mostly outgoing but one or two incoming termites.

Mean response curves in dry and wet sand arenas for control and disturbed groups are shown in Fig. 5.2A (response in seconds) and B (response in minutes). There were no escape and return responses in control groups. Although termites were moving from and to the central chamber, the number of termites in the central chamber in control groups was fairly uniform during the 1 h period, and never went below 96% of the initial termites. The response curves in Fig. 5.2A demonstrate an obvious escape response in disturbed groups. The time taken to escape the maximum number of termites from the disturbed chamber ranged from 20 s to 210 s, the mode being 30 s, followed by gradual return to the disturbed chamber. The return time was
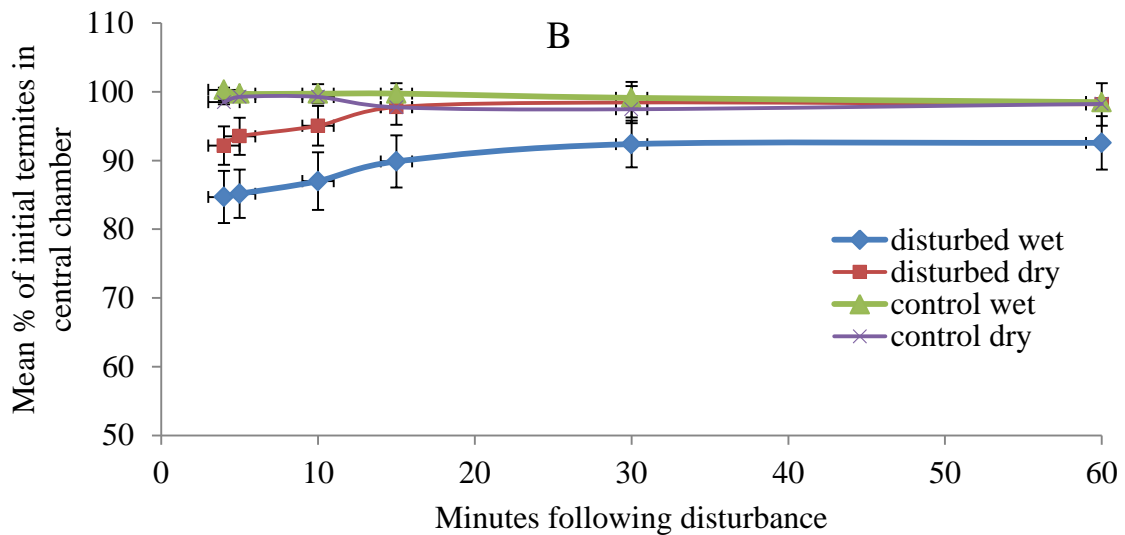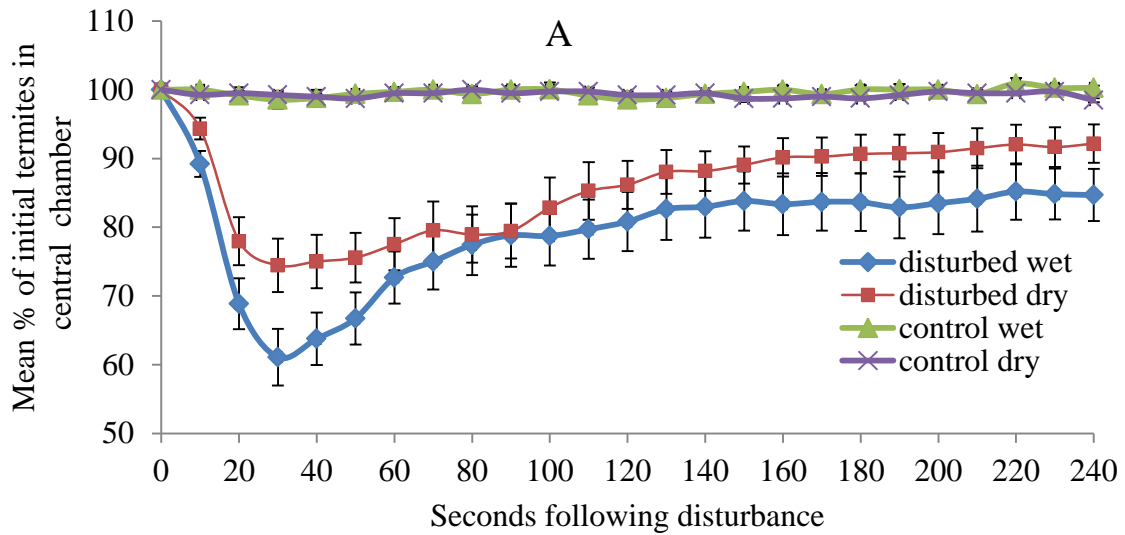
80

Figure 5.2(A, B). Mean (± SEM) response curves showing escape behavior by undisturbed (control) and disturbed groups of *C. formosanus* workers and soldiers in wet and dry sand experimental arenas at time intervals: (A) from 0 to 240 s post-disturbance. (B) from 4 to 60 min post-disturbance. Data represent the number of termites in the central chamber (disturbed chamber) standardized as a percentage of initial termites (initial termites = number of termites in the central chamber just before disturbance).

longer than the evacuation time which ranged from a few minutes to close to an hour in some replicates. Within the first 15 min of disturbance, > 80% of the escaped termites returned to the disturbed central chamber making the population of the central chamber > 90% of the initial termites (Fig. 5.2B). After 15 min, there was a very slow but steady return of termites. Eventually, by the end of 1 h, some replicates witnessed even more termites returned to the central chamber than originally escaped due to some peripherally located termites at the time of the disturbance.

A significantly lower mean percentage of initial termites escaped from the disturbed chamber in dry sand arenas (30.55%) compared with that in wet sand arenas (43.28%). However, the mean percentage of escapees that returned to the disturbed central chamber in dry sand arenas was not significantly different compared with that in wet sand arenas (109.44% and 106.14%, respectively). There were no significant differences in time to evacuate and time to return between these two sand moisture conditions (Table 5.1).

Table 5.1. Mean percentage (± SEM) and ANOVA results comparing disturbed group of *C. formosanus* that initially escaped, percentage of escapees that returned, time to escape and time to return in dry sand and wet sand experimental arenas after a disturbance was created in the central chamber.

Factor	Sand type		ANOVA results
	Dry sand	Wet sand	
Percentage of disturbed escaped	30.55 ± 3.13a	43.28 ± 4.40b	$F = 4.41$; df $= 1, 21$; $P = 0.04$
Percentage of escapees returned	109.44 ± 12.85a	106.14 ± 8.95a	$F = 0.05$; df $= 1, 21$; $P = 0.82$
Time to escape (s)	56.66 ± 13.54a	50.71 ± 12.81a	$\chi^2 = 0.16$; df $= 1$; $P = 0.68$
Time to return (min)	32.51 ± 7.67a	31.64 ± 6.51a	$\chi^2 = 0.05$; df $= 1$; $P = 0.81$

Numbers followed by the same letter are not significantly different at $\alpha = 0.05$ (Tukey's HSD). Comparisons are made within the same row.

In general, termites escaped from the disturbed central chamber through all four tubes. In the wet sand arenas, the numbers of escapees through the four tubes were more or less uniform, whereas in the dry sand arenas a significantly greater number of termites escaped through one tube (north side tube) compared with another (west side tube) (Table 5.2). The first termite that moved out in response to the disturbance was a worker in all the replicates except one where a soldier escaped first followed by workers (data not shown). Fig. 5.3 shows the mean percentage of initial soldiers (or disturbed soldiers) in the central chamber following disturbance. In the dry sand arenas, the percentage of soldiers in the central chamber after disturbance was always higher than before disturbance, whereas in wet sand arenas a few soldiers were found to escape initially, followed by gradual return to the disturbed chamber.

Table 5.2. Mean number (± SEM) and ANOVA results comparing *C. formosanus* escaped from central chamber through the tubes located in four directions in dry sand and wet sand experimental arenas in 30 s[a] period after a disturbance was created in the central chamber[b].

Sand condition	Mean (± SEM) number of termites escaped in 30 s from four directions				ANOVA results
	East	North	South	West	
Dry sand	18.44 ± 2.41a	21.55 ± 2.63a	13.00 ± 2.29a	11.77 ± 1.69b	$F = 4.04$; df = 3, 32; P = 0.015
Wet sand	17.71 ± 1.55a	17.35 ± 1.43a	18.57 ± 2.47a	15.07 ± 2.60a	$F = 0.51$; df = 3, 52; P = 0.674

[a]The average time period required to escape the maximum number termites from the central chamber after disturbance was 30 s.
[b]Numbers followed by the same letter are not significantly different at α = 0.05 (Tukey's HSD). Comparisons are made within the same row.
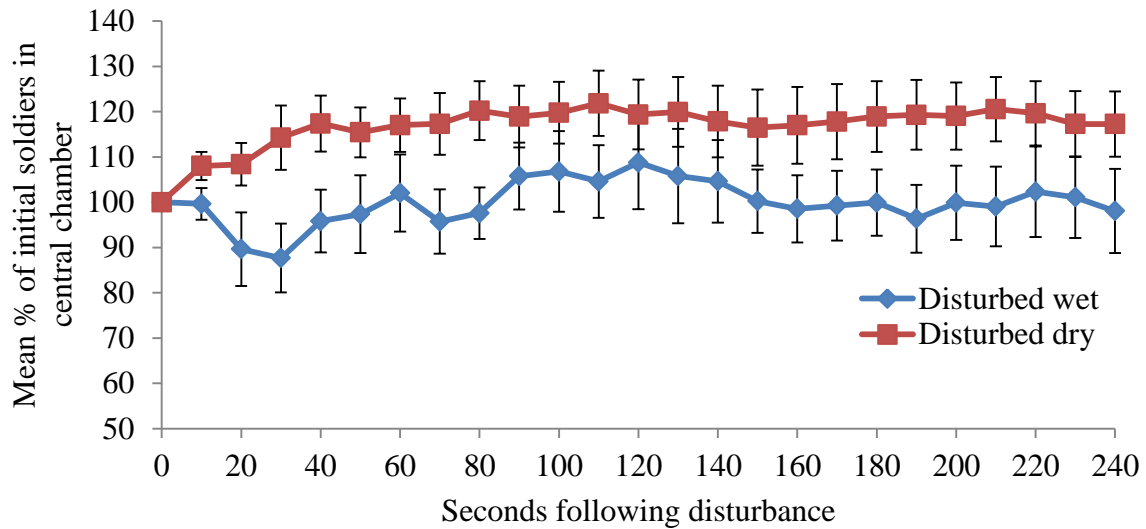
Figure 5.3. Mean (± SEM) response curves showing escape behavior by disturbed groups of *C. formosanus* soldiers in wet and dry sand experimental arenas at time intervals from 0 to 240 s. Data represent the number of soldiers in the disturbed central chamber (disturbed chamber) standardized as a percentage of initial soldiers (initial soldiers = number of soldiers in the central chamber just before disturbance).

Discussion

Following disturbance termites responded in a manner that indicated a stereotypic alarm response. Stuart (1963) described two types of alarm in termites: 'general alarm' where a large number of individuals are affected at the same time and 'specific alarm' where one or a few individuals are affected. General alarm behaviors include stoppage of normal activity, increase in excitation level, head bumping, bunching together or seeking cracks and crevices and pheromone trail laying, whereas specific alarm behaviors include trail following, recruitment and preparing for defense (Stuart 1967). In our study, *C. formosanus* first displayed general alarm behavior in response to the disturbance. This behavior appeared to facilitate their escape through the tubes into the peripheral chambers. Excited termites may have deposited trail pheromones while escaping through the tubes.

The partial escape (average: 31% and 43% of the initial termites escaped in dry sand and wet sand arenas, respectively) was followed by an immediate but gradual return to the disturbed area (average: 32 min and 31 min took to return to the disturbed chamber in dry and wet sand arenas, respectively). Hu et al. (2003) reported that *C. formosanus* and *Reticulitermes flavipes* (Kollar) showed only momentary escape (average time taken to return to the disturbed area: 160 s for *C. formosanus* and 226 s for *R. flavipes*) when disturbed by a vibrational stimulus. In their experiment, they did not remove the food source and knock the termites off the food source, and did not introduce air bursts, a series of activities that termites might encounter during checking and replacing of monitors/baits in a termite baiting system. Interestingly, the present results showed that even a large level of disturbance does not cause *C. formosanus* to abandon the attacked food source for more than an hour. The return time for *R. flavipes* disturbed by puffs of air was reported to be ≈ 3 h. (Schwinghammer and Houseman 2006) and for *Microcerotermes crassus* Snyder disturbed by knocking them out of wood was reported to be more than 1 day (Wong and Lee 2010). The return of the termites to the central chamber could be explained in the context of the specific alarm behavior. As noted by Stuart (1963) in *Zootermopsis nevadensis* (Hagen) and Schwinghammer and Houseman (2006) in *R. flavipes*, we propose that general alarm in *C. formosanus* translated into specific alarm when the escaped termites encountered the undisturbed termites in peripheral chambers, prompting behaviors such as recruitment and preparation for defense.

We were particularly cautious not to injure termites that might lead to their death in the disturbed chamber. Therefore, none of the experimental chambers had dead termites. Previous studies such as Su et al. (1982), Fei and Henderson (2005) and Woodrow et al. (2008) have reported that the presence of dead termites either elicited an anti-feeding behavior or avoidance

behavior in *C. formosanus.* Wong and Lee (2010) found that food source disturbance and the presence of dead termites deterred *Microcerotermes crassus* only temporarily but old carcasses infected with fungi deterred them permanently. Campora and Grace (2007) reported that the avoidance of borate treated wood by *C. formosanus* was neither due to a necrophobic behavior nor a learned response, but probably due to a weak trail pheromone. Although there are mixed reports on whether *C. formosanus* permanently abandons the sites because of dead termites, all indications are that the death of termites will negatively influence the success of a baiting system on *C. formosanus*. Our results suggest that a certain degree of disturbance, which is usually inevitable during periodic checking of monitors and/or replacing with baits, does not cause *C. formosanus* to abandon the food source. In fact, there is a possibility that such a disturbance might even help to spread the toxicants in the termite population as different or sometimes more individuals may visit the site after the disturbance. In a field study in Africa, Eggleton et al. (2002) reported that lower termites like *Coptotermes sjostedti* Holmgren and *Neotermes* sp. (Kalotermitidae) were found more abundantly in more disturbed sites than in less or undisturbed sites.

It is likely that the disturbed termites escaped via the nearest exit discovered. Since all the four tubes were located in equal distance interval around the periphery and the escape time was a window of a very short period, probably the most efficient escape is to utilize all the available routes more or less equally. It is also likely that the alarmed termites randomly met with the peripheral tubes while moving away from the source of disturbance that eventually lead to escape through the tubes, where each tube had equal probability of receiving the escapees. Why a significantly higher number of termites escaped through one tube than the others in dry sand

86

arenas is unclear. Such a difference in escape pattern was not observed in wet sand arenas indicating that *C. formosanus* is more likely to aggregate under unfavorable conditions.

Substrate moisture is an important requirement for subterranean termites. Our previous study (Gautam and Henderson 2011) showed that dry or fully saturated sand moisture conditions are unfavorable for *C. formosanus.* In the present study, the escape of a significantly higher percentage of the initial termites in wet sand arenas than in the dry sand arenas could be explained by the unfavorable condition in the peripheral chambers in the dry sand arenas. This finding may be biologically relevant because it suggests that an unfavorable substrate on the outer periphery of the monitoring stake could increase the chance of higher bait consumption given that baiting station itself has sufficient moisture. Interestingly, the time taken to escape from the disturbed chamber, and later return to the disturbed chamber was not significantly different between wet and dry sand arenas indicating that the speed of response to alarm signal or disturbance and acclimation to said alarm or disturbance on *C. formosanus* appeared unaffected by the substrate moisture level as opposed to temperature effects as observed by Swinghammer and Houseman (2006).

Here *C. formosanus* workers played an important role in adjusting the foraging population in a stressful situation. In both wet and dry sand conditions, workers were the first to escape from most of the disturbed chambers indicating that workers move into action first by the alarm signal or disturbance. This finding is consistent with the findings by Hu et al. (2003) who reported that workers always escaped first in response to a vibrational stimulus in *C. formosanus* and *R. flavipes*, and Reinhard and Clement (2002) who reported that workers were the first to respond and be attracted to the alarm triggered by the squashed soldier heads in European species of the genus *Reticulitermes*. Reinhard and Clement (2002) concluded that the presence of

workers was crucial for alarm communication. The present findings showed that the disturbed soldiers did not escape in proportion to the workers, rather there was a modest increase in soldier number in the central chamber. Soldiers recruited for defense in the disturbed area.

References

Campora, C. E., and J. K. Grace. 2007. Foraging behavior of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in response to borate treated wood. International Research Group on Wood Preservation, Stockholm, Sweden. IRG Document No. IRG/WP 07-10605. 13 pp.

Curl, G. 2004. Pumped-up termite market. Pest control technol. 32: 26, 28, 33.

Eggleton, P., D. E. Bignell, S. Hauser, L. Dibog, L. Norgrove, and B. Madong. 2002. Termite diversity across an anthropogenic disturbance gradient in the humid forest zone of West Africa. Agric. Ecosyst. Environ. 90: 189–202.

Fei, H., and G. Henderson. 2005. Repellency of Formosan subterranean termites (Isoptera: Rhinotermitidae) to dead termites and attraction to 2-phenoxyethanol with and without nonrepellent insecticides. J. Agric. Urban Entomol. 23: 159-172.

Forschler, B. T., and J. C. Ryder. 1996. Subterranean termite, *Reticulitermes* spp. (Isoptera: Rhinotermitidae), colony response to baiting with hexaflumuron using a prototype commercial termite baiting system. J. Entomol. Sci. 31: 143-51.

Gautam, B. K., and G. Henderson. 2011. Effects of sand moisture level on food consumption and distribution of Formosan subterranean termites (Isoptera: Rhinotermitidae) with different soldier proportions. J. Entomol. Sci. 46: 1–13.

Grace, J. K., C. H. Tome, T. G. Shelton, and R. J. Oshiro. 1996. Baiting studies and considerations with *Coptotermes formosanus* (Isoptera: Rhinotermitidae) in Hawaii. Sociobiology 28: 511–520.

Henderson, G. 2001. Practical considerations of the Formosan subterranean termite in Louisiana: a 50-year-old problem. Sociobiology 37: 281-392.

Hu, X. P., A. G. Appel, and J. F. A. Traniello. 2003. Behavioral response of two subterranean termites (Isoptera: Rhinotermitidae) to vibrational stimuli. J. Insect Behav. 16: 703–715.

Lewis, V. R. 1997. Alternative control strategies for termites. J. Agr. Entomol. 14: 291–307.

Nagendra, N., B. K. Gautam and G. Henderson. 2010. Effects of oil-based paint marking on the foraging behavior of Formosan subterranean termites (Isoptera: Rhinotermitidae). Sociobiology 55: 369-378.

Noirot, C., and J. P. E. C. Darlington. 2000. Termite nests: architecture, regulation and defense, pp. 121–139. *In* T. Abe, D. E. Bignell and M. Higashi M. (eds.), Termites: Evolution, Sociality, Symbioses, Ecology. Kluwer Academic, Dordrecht.

Pawson, B. M., and R. E. Gold. 1996. Evaluation of baits for termites (Isoptera: Rhinotermitidae) in Texas. Sociobiology 28: 485–510.

Reinhard, J., and J. L. Clement. 2002. Alarm reaction of European *Reticulitermes* termites to soldier head capsule volatiles (Isoptera: Rhinotermitidae*).* J. Insect Behav. 15: 95-107.

Schwinghammer, M. A., and R. M. Houseman. 2006. Response of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) to disturbance in laboratory arenas at different temperatures and soldier proportions. J. Econ. Entomol. 99: 462-468.

Stuart, A. M. 1963. Studies on the communication of alarm in the termite *Zootermopsis nevadensis* (Hagen), Isoptera. Physiol. Zool. 36: 85-96.

Stuart, A. M. 1967. Alarm, defense, and construction behavior relationships in termites (Isoptera). Science 156: 1123-1125.

Su, N.-Y., and R. H. Scheffrahn. 1990. Economically important termites in the United States and their control. Sociobiology 17: 77-94.

Su, N.-Y., and R. H. Scheffrahn. 1996. A review of the evaluation criteria of bait-toxicant efficacy against field colonies of subterranean termites (Isoptera). Sociobiology 28: 521–530.

Su, N.-Y., and R. H. Scheffrahn. 1998. A review of subterranean termite control practices and prospects for integrated pest management programs. IPM Rev. 3: 1- 3.

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

Traniello, J. F. A., and B. L. Thorne. 1994. Termite baits in theory and practice, pp. 28–40. *In* W. H. Robinson (ed.), Proceedings of the National Conference on Urban Entomology, 20–22 February, Atlanta, GA.

Wong, N., and C.-Y. Lee. 2010. Effects of disturbance and the presence of termite and other invertebrate carcasses at feeding sites on the behavior of the subterranean termites *Microcerotermes crassus* (Isoptera: Termitidae). Sociobiology 55: 353-367.

Woodrow, R. J., T. G. Shelton, R. J. Oshiro, J. K. Grace, and T. L. Wagner. 2008. Effects of disturbance-induced trauma on foraging by subterranean termites (Isoptera: Rhinotermitidae). Sociobiology 52: 107-118.

CHAPTER 6

LABORATORY STUDY OF THE INFLUENCE OF SUBSTRATE TYPE AND TEMPERATURE ON THE EXPLORATORY TUNNELING BY THE FORMOSAN SUBTERRANEAN TERMITE

Introduction

Subterranean termites search for food by constructing a ramified tunnel and gallery system underneath or above the soil. Usually the tunnels radiate out from the nest and as they go farther away the main tunnels produce secondary and tertiary tunnels (Hedlund and Henderson 1999, Arab and Costa-Leonardo 2005). The efficiency of food search by subterranean termites therefore depends on the organization of the tunnel systems (Traniello and Robson 1995). Studies have shown that environmental factors such as soil texture, soil moisture, ambient temperature and the presence or absence of food sources influence the search tunnel formation by subterranean termites (King and Spink 1969, Smith and Rust 1991, Jouquet et al. 2002, Evans 2003, Su and Puche 2003, Fei and Henderson 2004, Arab and Costa-Leonardo 2005, Cornelius and Osbrink 2010, Gautam and Henderson 2011).

Hedlund and Henderson (1999) demonstrated that the presence and amount of food influenced the branching and total length of tunnel system constructed by *C. formosanus* and Reinhard et al. (1997) reported similar findings in *Reticulitermes santonensis* De Feytaud. On the other hand, studies such as Su et al. (1984) and Campora and Grace (2001) reported that food search by *C. formosanus* is systematic and not affected by food in the foraging arena. Nevertheless, these studies agree that the main and branched tunnels are organized in a way that they avoid searching the area that was already explored.

Although there is no debate on the requirement of substrate moisture in the foraging arena, there are still contradictory reports on the influence of a particular level of moisture in different substrates for search tunnel formation by subterranean termites. Su and Puche (2003) reported that early tunneling activity by *C. formosanus* was higher in high moisture sand than in low moisture sand when provided with a moisture gradient, but during the later part of the 10 d

experiment the difference was not significant. Evans (2003) reported the increased rate of tunneling in wet sand than in dry sand by *C. frenchi* Hill. Arab and Costa-Leonardo (2005) reported that *C. gestroi* (Wasmann) constructed more secondary tunnels at 15% moisture sand than in 5% moisture sand. In contrast, Potter et al. (2001) demonstrated from their field study that there was no difference in damage ratings between Sentricon® stations placed in wet soil or dry soil. One confounding factor here is the use of 'dry soil' in the experiment. It is difficult to determine whether the dry soil in these studies was completely dry or had low moisture levels, because the survival of subterranean termites is a major issue in a completely dry soil. When a wet food matrix is provided or when the dry soil is adjoined with wet soil then termites can relocate water to make the dry soil moist (Grub and Rudolph 1999, Gallaghar and Jones 2010). Like moisture, ambient temperature also influences the tunneling behavior of subterranean termites. For example, Arab and Costa-Leonardo (2005) reported that *C. gestroi* and *Heterotermes tenuis* (Hagen) constructed more secondary tunnels at 20°C and 25°C than at 15°C in sand.

Soil texture impacts subterranean termite foraging both by its particle size and its water holding properties. As the soil particles become coarser the water holding capacity of the soil decreases (Khaleel et al. 1981); as a result, relatively more water is available for termites. In sand, the moisture content as low as 4% by wt. provides sufficient moisture for *C. formosanus* to tunnel and aggregate normally (Gautam and Henderson 2011). Cornelius and Osbrink (2010) reported faster tunneling by *C. formosanus* in sand than in top soil and clay. In moist condition, however, *C. formosanus* preferred to aggregate in soil with finer particles than in soils with coarser particles and the opposite was found in dry condition (Cornelius and Osbrink 2010). *C. gestroi* excavated significantly more secondary tunnels in soil with uniform particle size of 2.0

mm than in soil with heterogenous particle size (0.002 - 4.00 mm) while *H. tenuis* could not even

penetrate the arenas filled with the latter soil type (Arab and Costa-Leonardo 2005). *C.*

formosanus and *Reticulitermes hesperus* Banks constructed different tunneling patterns based on

the particle size present in the soil (Smith and Rust 1991). In this study, first we aimed to test the

substrate preference between sand and sandy loam at two different moisture levels and second to

compare the rate and pattern of tunneling among sand, sandy loam and silt loam at two ambient

temperatures.

Materials and Methods

Termites. Formosan subterranean termites were collected from a heavily termite-infested

area of Brechtel Park in New Orleans, Louisiana in October 2010 using milk crate traps as

described in Gautam and Henderson (2011). The termites were maintained in the laboratory for

~3 months before they were used for the bioassays.

Preference Tests: Bioassay arenas were prepared according to Hedlund and Henderson

(1999) with some modification. Each arena consisted of two square Plexiglas® plates (33 by 33

by 0.3 cm) assembled together to make a tunneling chamber 0.3 cm thick. The upper and lower

plates were held together with test substrates sandwiched in the middle portion separated by 4

narrow edge strips (1.0 by 0.3 by 33 cm or 31 cm). An orifice (0.5 cm diam) was opened with a

soldering iron on the central point of the upper plate. The lower plate was divided into 4 equal

parts by 4 strips (1.0 by 0.3 by 14.0 cm). These divider strips were glued in a way that an open

space was created on the central point along the orifice of the upper plate and four equal gates

leading to four parts of the arena were formed. Each part of the arena was filled with one of the 4

treatment substrates: 15% moisture sandy loam, 5% moisture sandy loam, 15% moisture sand

and 5% moisture sand, and leveled with as uniform compactness as possible. A filter paper

(Whatman®, 4.2 cm diam) was placed at each corner as a food source and the upper and lower plates were clamped together with metal binder clips. A circular acrylic container (called release chamber; size: 5.08 by 3.63 cm, Pioneer Plastics Inc, North Dixon, KY) with the same sized hole on the bottom side was glued to the upper plate so that the two holes (holes of the upper plate and the release chamber) were aligned with each other and the termites from the release chamber could easily get access to the tunneling chamber (Fig. 6.1). We chose sand (fine construction sand, Louisiana Cement Products LLC, Baton Rouge, LA) and sandy loam (collected from backyard of the second author, St. Gabriel, LA) for the substrate preference study as sand represented the low organic matter substrate with uniformly coarser particles and sandy loam represented the high organic matter substrate with finer particle size (Table 6.1). Two moisture levels were chosen based on our experience on conducting the laboratory experiments. Five percent moisture represents the lower range of sand moisture for most laboratory experiment and 15% represents the upper range. Soil compaction was recorded by measuring the wet bulk density of test substrate in the foraging arena, which was as followed: Sand 2.33 g/cm^3, sandy loam 1.22 g/cm^3 and silt loam 1.32 g/cm^3.

One hundred termites (88 workers, 10 soldiers and 2 nymphs; workers were externally undifferentiated individuals developed to at least the third instar) were released in the releasing chamber which contained 10 g mixture of both the substrates (sandy loam and sand) moistened to 15% by wt. Nymphs were included in the tests since the field collected groups were composed of workers, soldiers and nymphs. The test was repeated 5 times.

Number of termites present in each substrate was recorded after 24 h. To record the number of termites present on both sides of the foraging chamber: the arena was placed on the scanner, the individuals were counted from the top whereas the bottom was scanned at the same

94

Fig. 6.1. Two dimensional experimental arena for substrate type preference tests

time so that duplication in counting was avoided. During the process, the arena disturbance was minimized. The scanned image transferred on computer was used to count the termites seen from the bottom part of the arena. Since the thickness of the substrate was only 0.3 cm, termites in the tunnels were visible either from the top or from the bottom of the foraging chamber. Termites not seen on the foraging chamber from either side were presumed to be present in the releasing chamber and were not included in the analysis. Length of the tunnels was measured with the help of a string overlaid on the tunnels. Only one measurement was taken of the tunnels that were visible from both the sides of the foraging chamber otherwise separate measurements were taken. Total length of the tunnels in each substrate was then calculated by adding the lengths of primary and branched tunnels (branched tunnels included both secondary and the tertiary tunnels).

Tunneling Tests: The bioassay arena consisted of a similar structure as described in the preference tests except that the release chamber was fitted to one edge of the square tunneling chamber, a filter paper placed on the opposite edge of the chamber and the foraging arena was filled with only one type of substrate, so, there were no dividers in the arena. One more substrate

95

type, silt loam (collected from Ben Hur Research Station, Baton Rouge, LA) was added for tunneling tests, which had higher organic matter than the sand but lower than the sandy loam and had the finest particle of all the three substrates (Table 6.1). Sand was moistened to 15% by wt. and sandy loam and silt loam to 30% by wt. These moisture levels were chosen to approximately equate the saturation of the substrates based on their moisture holding capacity, as sand gets saturated at ~28% moisture whereas the other two substrates get saturated at ~55%. Two temperature conditions, 22°C and 28°C were chosen because the former temperature represents the most common laboratory room temperature and the latter represents the temperature usually maintained in the incubator for most bioassays. The release chamber contained 10 g of the same substrate as in the foraging chamber.

One hundred termites (88 workers, 10 soldiers and 2 nymphs; workers were externally undifferentiated individuals developed to at least the third instar) were introduced in the release chamber and the arena was placed undisturbed either in the incubator set to 28°C or in the laboratory room having a temperature at 22°C. There were 3 replications for each temperature and substrate type. Tunneling was recorded after 14 h and 24 h of release. Different colored permanent marker pens were used to trace the tunnels made at each time period. The number of primary and branched tunnels was recorded and compared among the tested substrates. The total length of the tunnels in the arena was calculated as described in the preference tests.

Table 6.1. Characteristics of substrates used in experiments

Sample ID	Organic matter (%)	Particle size distribution (%)			Textural class
		Sand	Silt	Clay	
Ben Hur Soil	6.56	20.6	53.1	26.3	Silt Loam
St. Gabriel Soil	15.09	68.4	22.8	8.8	Sandy Loam
Sand	0.09	99.1	0.6	0.3	Sand

Statistical Analysis. Data analysis was done with SAS 9.1 software (©2002-2003 SAS Institute Inc., Cary, NC). Tunnel volumes and number of primary and branched tunnels were subjected to ANOVA using a generalized linear model. Location count data were square root transformed to improve normality and analyzed using ANOVA. Untransformed data are reported in the text. Multiple comparisons were done using Tukey's honestly significance difference at α = 0.05.

Results

In the preference tests, a significantly greater total length of tunnels was excavated in sand compared to sandy loam ($F = 35.26$; df = 1, 16; $P < 0.0001$) in the 24 h period. However, tunnel length was not significantly different between the two moisture levels ($F = 1.19$; df = 1, 16; $P = 0.29$) and there was no interaction effect of the substrates and the moisture levels ($F = 0.48$; df = 1, 16; $P = 0.50$) (Fig. 6.2). Likewise, after 24 h, a significantly greater proportion of released termites were recovered in sand than in the sandy loam ($F = 325.37$; df = 1, 16; $P < 0.0001$) but the moisture levels had no significant impact on the number of termites found on a given substrate type ($F = 0.69$; df = 1, 16; $P = 0.43$) and had no interaction effect of the substrate and moisture levels ($F = 0.88$; df = 1, 16; $P = 0.36$) (Fig. 6.3).

In the tunneling tests, substrate type had a significant impact on the length of tunnels at both time periods (14 h post-release: $F = 6.57$; df = 2, 12; $P = 0.0118$ and 24 h post-release: $F = 7.71$; df = 2, 12; $P = 0.0070$). The length of tunnels was not significantly different between 22°C and 28°C (14 h post-release: $F = 0.03$; df = 1, 12; $P = 0.8745$ and 24 h post-release: $F = 0.01$; df = 1, 12; $P = 0.9260$), however, there was a substrate type and temperature interaction effect at 24 h post-release (14 h post-release: $F = 3.77$; df = 2, 12; $P = 0.0538$ and 24 h post-release: $F = 5.28$; df = 2, 12; $P = 0.0227$). Means comparison showed that there was no difference in the
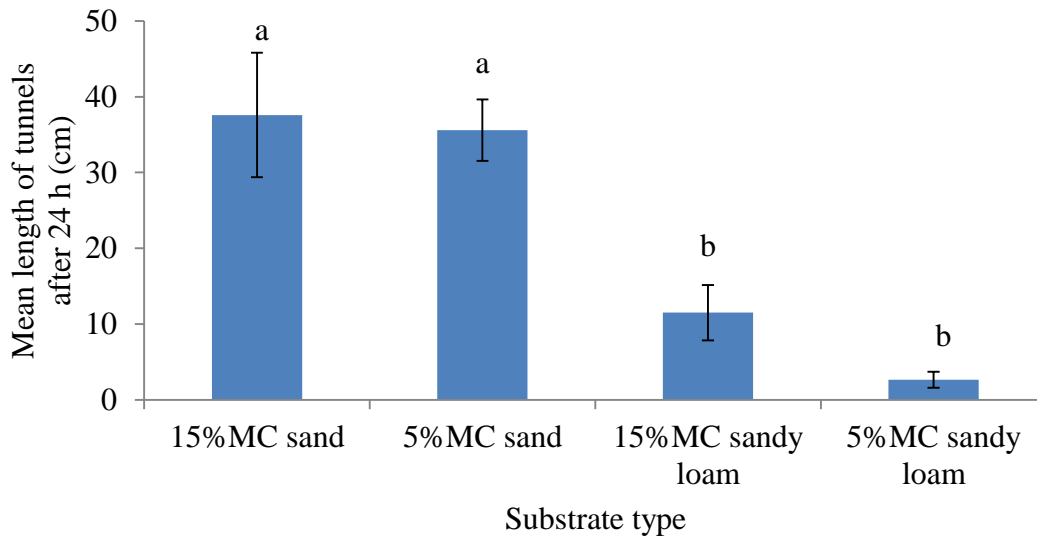
Figure 6.2. Mean length (± SEM) of tunnels excavated in two different substrates each with two moisture levels (MC) in the preference tests in 24 h period. Bars with different letters are significantly different (α < 0.05, Tukey's test) from each other.
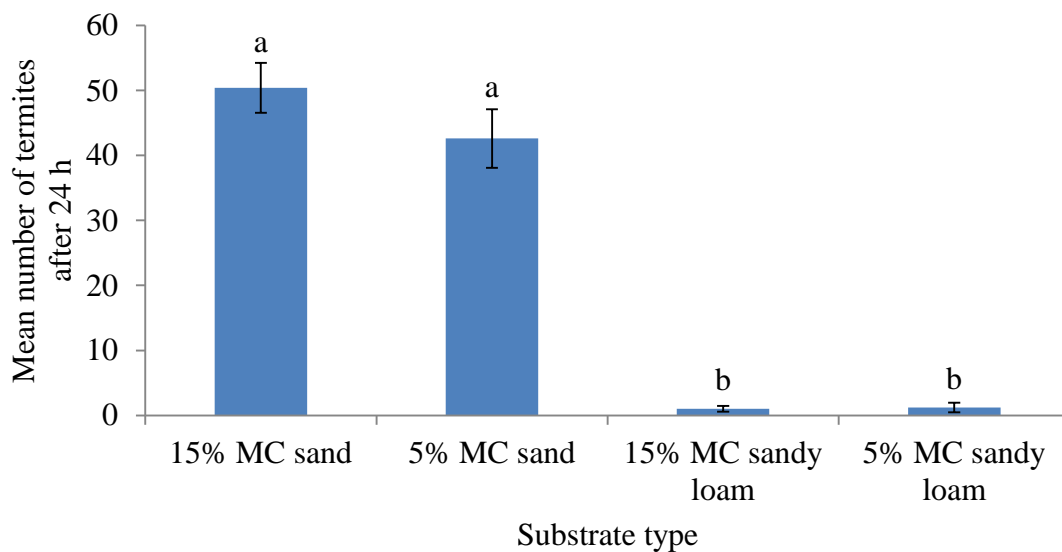

Figure 6.3. Mean number (± SEM) of termites recovered in two different substrates each with two moisture levels (MC) in the preference tests after 24 h period. Bars with different letters are significantly different (α < 0.05, Tukey's test) from each other.

length of tunnels among the substrates at 28°C but only at 22°C. At 14 h post-release, the length

of tunnels was significantly greater in sand than in sandy loam and after 24 h post-release it was

significantly greater in both the sand and the silt loam compared to sandy loam (Table 6.2).

Substrate type had a significant effect on the number of primary tunnels after 24 h of

release ($F = 7.75$; df $= 2$, 12; $P = 0.0069$), however, there was no temperature effect ($F = 1.33$; df

$= 1$, 12; $P = 0.2707$) and also no interaction effect ($F = 0.58$; df $= 1$, 12; $P = 0.5731$). Similarly,

substrate type effected the number of branched tunnels ($F = 10.07$; df $= 2$, 12; $P = 0.0027$) but

not the temperature ($F = 4.17$; df $= 1$, 12; $P = 0.638$) or the interaction of substrate and

temperature ($F = 3.31$; df $= 2$, 12; $P = 0.0717$). The number of branched tunnels was greater in

sand or silt loam than in the sandy loam at 22°C and the number of primary tunnels was greater

in sand compared to sandy loam but not the silt loam at 24 h post-release (Table 6.3).

Table 6.2. Mean length (± SEM) of tunnels excavated in 14 h and 24 h periods in three different substrates at two temperature conditions

Substrate	Mean length of tunnels in 14 h (cm)		Mean lengths of tunnels in 24 h (cm)	
	28°C	22°C	28°C	22°C
sand	80.00 ± 16.64^a	99.36 ± 7.62^a	104.83 ± 13.49^a	123.16 ± 9.09^a
sandy loam	66.83 ± 13.63^a	28.53 ± 10.25^b	86.73 ± 14.30^a	39.90 ± 13.73^b
silt loam	58.00 ± 2.51^a	72.33 ± 13.41^{ab}	72.33 ± 2.42^a	103.83 ± 18.33^a

Values with different letters in superscript within the same column are significantly different ($\alpha < 0.05$; Tukey's test).

Table 6.3. Mean number (± SEM) of primary and branched tunnels excavated in 24 h period in three different substrates at two temperature conditions

Substrate	Mean number of primary tunnels in 24 h		Mean number of branched tunnels in 24 h	
	28°C	22°C	28°C	22°C
sand	2.33 ± 0.33^a	3.33 ± 0.88^a	9.33 ± 3.33^a	18.00 ± 3.60^a
sandy loam	1.00 ± 0.00^a	1.00 ± 0.00^b	4.66 ± 1.20^a	1.66 ± 0.66^b
silt loam	2.00 ± 0.57^a	2.33 ± 0.33^{ab}	7.33 ± 0.33^a	13.66 ± 2.90^a

Values with different letters in superscript within the same column are significantly different ($\alpha < 0.05$; Tukey's test).

Discussion

It is generally agreed that substrate moisture is one of the critical factors that favor foraging activity of subterranean termites. However, there are variable suggestions when it comes to concluding what is the preferred level of moisture for optimum tunneling activity (Su and Puche 2003, Gautam and Henderson 2011, Arab and Costa-Leonardo 2005, Cornelius and Osbrink 2010, Evans 2003). Su and Puche (2003) reported that *C. formosanus* tunneled faster in higher moisture sand than in lower moisture sand for the middle 3-4 days of the 10 d test period; tunneling rate did not differ the rest of the days. Evans (2003) reported higher tunneling rate in wet sand than in dry sand by *C. frenchi*, but did not determine the exact moisture content of the wet sand. The present data shows that the total tunnel length excavated by *C. formosanus* does not differ between 5% and 15% moisture sand. Our earlier study on sand moisture preference also showed that *C. formosanus* did not preferentially select a particular moisture level among a range of moisture levels from 4 to 24% (Gautam and Henderson 2011). This suggests that *C. formosanus* readily gets moisture required for its normal tunneling activities from sand that has as low as 4% moisture. In sandy loam, *C. formosanus* constructed very short tunnels initially but later stopped and very few termites were recovered at the end of the test period. Both less

moisture availability and finer particle size could have contributed to make this substrate less preferable over sand.

The rate of tunneling and the spatial dispersion of tunnel webs were influenced by the substrate type and ambient temperature. A greater total length of tunnel network in sand than in sandy loam at 12 h or 24 h post-release could primarily be due to the uniformly coarser particle size present in the sand. Termites construct tunnels by picking up soil particles and depositing them elsewhere to clear the way for tunnels. Cornelius (2005) reported that tunnel construction by *C. formosanus* was faster in uniformly coarse sand (particle size: 0.59-0.84 mm) than in the sand with varied particle size (0.30-2.0 mm). Although coarse particles would usually be faster to excavate, very coarse particles also could be impenetrable for termites. Ebeling and Pence (1957) reported that *R. hesperus* was unable to penetrate the sand particle size that ranged from 1.2-1.7 mm diameter and Tamashiro et al. (1991) reported that sand particle size ranging from 1.7 – 2.4 mm diameter was impenetrable to *C. formosanus*. As termites pick up individual particles while excavating tunnels, they would have to do more back and forth movement to displace the same volume of substrate in finer particles thus requiring more efforts and time to do the same job. Li and Su (2009) suggested that termites can pick up 3-4 particles (0.300 – 0.355 mm) at a time depending upon the size. However, this may not necessarily mean that termites always pick several fine particles from silt or clay to make the same bulk as would have been from a single particle of coarse sand of maximum size they can grasp, and sometimes finer particles may drop on the way requiring another trip thus delaying the excavation work. Our observation where longer tunnels were recorded in sand than in the sandy loam or silt loam demonstrates that *C. formosanus* excavates faster in uniform coarse particles than the substrate having finer particle size.

An efficient search tunnel system would be the one that minimizes the energy expenditure in tunnel excavation and that would probably be achieved by minimizing the total length of tunnels from origin to the first food source. Primary tunnels mainly determine how far the foraging area is covered from the origin and the organization of branching system determines how efficient the food search system foraging groups of subterranean termites employ (Hedlund and Henderson 1999). Construction of a larger number of branched tunnels (secondary, tertiary and quaternary tunnels) helps to exploit the maximum area of the arena. In the present experiment, *C. formosanus* built more extensive tunnel webs in sand than in sandy loam as evident from higher number of primary as well as secondary tunnels in both the temperature conditions, although the difference was statistically significant only at 22°C. Arab and Costa-Leonardo (2005) reported that *C. gestroi* and *H. tenuis* constructed more secondary tunnels at 20°C and 25°C than at 15°C in sand. Probably 15°C is very low temperature for normal activities for the termites. It would be interesting to see how *C. formosanus* tunneling would be impacted at 15°C. Surprisingly, silt loam, which has a lower proportion of sand than the sandy loam, had greater number of branched tunnels and greater total length of tunnels. We do not think moisture was a limiting factor in sandy loam or silt loam because the moisture content of these substrates was 30% whereas that of the sand was only 15%. We assume that, in addition to particle size and soil moisture, there may be some other factors that influence the speed of excavation. Organic matter content was exceptionally high in the sandy loam and pH was slightly higher, however, it is unknown whether these factors have a role to lessen the tunneling activities by *C. formosanus*. An increase in tunneling activity by *C. formosanus* has direct biological relevance as it will increase the likelihood of finding a monitor or bait placed in the nearby areas of infested structures.

References

Arab, A., and, A. M. Costa-Leonardo. 2005. Effect of biotic and abiotic factors on the tunneling behavior of *Coptotermes gestroi* and *Heterotermes tenuis* (Isoptera: Rhinotermitidae). Behav. Processes 70: 32-40.

Campora, C. E., and J. K. Grace. 2001. Tunnel orientation and search pattern sequence of the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 94: 1193-1199.

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

Cornelius, M. L., and W. L. A. Osbrink. 2010. Effect of soil type and moisture availability on the foraging behavior of the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 103: 799-807.

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.

Evans, T. A. 2003. The influence of soil heterogeneity on exploratory tunneling by the subterranean termite *Coptotermes frenchi* (Isoptera: Rhinotermitidae). Bull. Entomol. Res. 93: 413-423.

Fei, H., and G. Henderson. 2004. Effects of temperature, directional aspects, light conditions and termite species on subterranean termite activity (Isoptera: Rhinotermitidae). Environ. Entomol. 33: 242-246.

Gallaghar, N. T., and S. C. Jones. 2010. Moisture augmentation of food items by *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Sociobiology 55: 735-748.

Gautam, B. K., and G. Henderson. 2011. Effects of sand moisture level on food consumption and distribution of Formosan subterranean termites (Isoptera: Rhinotermitidae) with different soldier proportions. J. Entomol. Sci. 46: 1-13.

Grube, S., and D. Rudolph. 1999. Water supply during building activities in the subterranean termite *Reticulitermes santonensis* de Feytaud (Isoptera, Rhinotermitidae). Insectes Soc. 46: 192-193.

Hedlund, J., and G. Henderson. 1999. Effect of available food size on search tunnel formation by the Formosan subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 92: 610-616.

Jouquet, P., M. Lepage, and B. Velde. 2002. Termite soil preferences and particle selections: strategies related to ecological requirements, Insectes Soc. 49: 1–7.

Khaleel, K. A., K. R. Reddy, and M. R. Overcash. 1981. Changes in soil physical properties due to organic waste applications: A review. J. Environ. Qual. 10: 133-141.

King, E. G., and W. S. Spink. 1969. Foraging galleries of the Formosan subterranean termite, *Coptotermes formosanus,* in Louisiana. Ann. Entomol. Soc. Am. 62: 536-542.

Li, H.-F., and N.-Y. Su. 2009. Buccal manipulation of sand particles during tunnel excavation of the Formosan subterranean termite (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 102: 333-338.

Potter, M. F., E. A. Eliason, K. Davis, and R. T. Bessin. 2001. Managing subterranean termites (Isoptera: Rhinotermitidae) in the Midwest with hexaflumuron bait and placement considerations around structures. Sociobiology 38: 565-58.

Reinhard, J., H. Hertel, and M. Kaib. 1997. Systematic search for food in the subterranean termite *Reticulitermes santonensis* De Feytaud (Isoptera, Rhinotermitidae). Insectes Soc. 44: 147-158.

Smith, J. L., and M. K. Rust. 1991. Factors affecting the tunneling behavior of the western subterranean termite, *Reticulitermes hesperus* Banks, USDA Forest Service. Gen. Tech. Rep. 1: 28–33.

Su, N.-Y., and H. Puche. 2003. Tunneling activity of subterranean termites (Isoptera: Rhinotermitidae) in sand with moisture gradients. J. Econ. Entomol. 96: 88-93.

Su, N.-Y., M. Tamashiro, J. R. Yates, and M. I. Haverty. 1984. Foraging behavior of the Formosan subterranean termite (Isoptera: Rhinotermitidae). Environ. Entomol. 13: 1466–1470.

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

Traniello, J.F.A. and S. Robson, 1995. Trail and territorial pheromones in the social insects. In: *Chemical Ecology of Insects* (W.J. Bell and R. Carde, Eds.), Chapman and Hall publishers, Oxford. pp. 241–285.

CHAPTER 7

SUMMARY AND CONCLUSION

Subterranean termites are some of the most destructive structural insects causing billions of dollars of economic loss in the United States. The Formosan subterranean termite is an introduced species in the United States that has surpassed the damage potential to its native counterpart, *Reticulitermes* spp. in some of the southern states, including Louisiana. Owing to their great economic importance, various control strategies are in place targeting subterranean termites. Naturally, efforts are underway to develop less invasive, more economical and more environmental friendly control approaches. Baiting systems and spot treatment (or minimum treatment) by nonrepellent delayed action termititcides are some of the strategies to eliminate the whole colony from an area. However, to achieve a desired success from these control strategies require an in-depth understanding of foraging behavior and the food transfer system of these social insects. Subterranean termite foraging behavior is impacted by an array of biotic and abiotic factors. Of the abiotic factors, moisture and temperature play a vital role in influencing their foraging behavior and distribution which ultimately determine their survival. The importance of moisture and temperature is attributable mainly to these insects' soft cuticle with poor water retaining properties that renders them extremely vulnerable to desiccation. Because of this compelling link between moisture and temperature and their survival, knowledge of water and temperature relations of subterranean termites is indispensible to optimizing control strategies. This dissertation research is one such study that aimed to aid in the understanding of *C. formosanus* foraging behavior in relation to soil or substrate moisture, micro-environmental relative humidity and ambient temperature.

Formosan subterranean termites were tested under laboratory conditions to determine preferences among different sand moisture levels in a choice test. Foraging behavior, rate of filter paper consumption and distribution patterns of the termites were studied among 3 soldier

106

ratios: low (4% soldier), normal (16% soldier) and high (32% soldier) and 7 sand moisture

gradients (4, 8, 12, 16, 20, 24 and 28% wt/wt) in a circular 3-dimensional arena. Sand moisture

had a significant effect on termite distribution and filter paper consumption, whereas soldier

proportions had no effect. Treatment means comparisons indicated that there were no significant

differences in termite distribution or consumption among moisture levels ranging from 4 - 24%;

however, significant differences were obtained when termite distribution or consumption on

these moisture levels were compared with that of the saturated sand (28% moisture). Termites

also were not present in the center release chamber (which had dry sand and no food) at any

observation point. Within the range of 4 -24%, we found a very uneven distribution pattern

where 70 - 80% of the total released termites aggregated in 1 of the 6 moisture chambers. Filter

paper consumption corresponded with the aggregation sites. Importantly, among the 27

replicates no particular moisture chamber was consistently chosen for aggregation indicating that

Formosan subterranean termites probably can adapt to a range of substrate moisture levels in

nature provided other conditions are suitable.

   Food moisture level may impact subterranean termite foraging behavior in a different

way than the soil moisture level. To understand this, the relationship between the moisture

content (MC) of wood blocks and the feeding preference of *C formosanus* at three ambient

temperatures (19, 28, and 35°C) was investigated with multiple-choice as well as no-choice

bioassays. In multiple-choice tests, when four wood blocks with initial MC as dry (0-3%), low

(22-24%), medium (70-90%), and high (125-150%) were exposed to workers and soldiers, the

highest consumption was found in the high MC wood blocks at all three temperatures, although

high MC wood consumption at 19 ^{o}C was significantly lower than that at 28 or 35 ^{o}C. The

preference for high MC wood blocks was detectable within the first few hours and continued for

the duration of the test. In no-choice tests, the highest wood consumption also was obtained on the high MC wood blocks at all three temperatures. However, in the no-choice tests, significant mortality was observed with termites exposed to dry or low MC wood blocks. Termite mortality was found to be higher with higher ambient temperature. Sustained feeding was not observed on wood blocks with ≤ 24% MC. Temperature played a vital and complementary role for wood consumption at all moisture levels.

Relative humidity and temperature mainly determine the water loss from an organism. Foraging groups of *C. formosanus*, which are extremely susceptible to desiccation, were tested for their relative humidity (RH) preference in a humidity gradient arena in the laboratory at a constant temperature of 26°C. Five RH levels (9%, 33%, 53%, 75% and 98%) were maintained in the test arena comprising of a series of closed containers using dry silica gel, saturated salt solutions or distilled water alone. Termites gradually aggregated to the highest RH chamber in the arena. After 1 h, a greater percentage of termites (~ 46%) aggregated to the highest RH chamber (98%) compared to the lower RH chambers (≤ 75%). After 12 h, > 97% of the termites aggregated to the 98% RH chamber. In survival tests, where termites were exposed to 15 combinatorial treatments of 5 RH levels (9%, 33%, 53%, 75% and 98%) and 3 temperatures (20°C, 28°C and 36°C) for a week, the survival was significantly influenced by RH, temperature and their interaction. Higher mortality was observed in termites exposed to ≤ 75% RH chambers compared to 98% RH chamber at the three temperatures and significantly lower survival was found at 36°C than at 28°C or 20°C.

The practical aspect of understanding the foraging behavior in relation to moisture and temperature is the optimization of control strategies. Termite baiting system are the most environmental friendly control approaches which rely on periodic checking of monitors and

replacement with baits, so, involves an inevitable disturbance. The role of disturbance in baiting activities has been noted as a confounding factor for the success of baiting systems. To understand whether disturbance lead to food (or bait) abandonment, and whether wet versus dry substrate in the periphery of the food source influence their escape behavior, Formosan subterranean termites were tested in a suitably designed laboratory arena. Four peripheral chambers connected to the central food chamber through tubes served as escape routes from which escape patterns, escape and return times and differences in soldier and worker responses were recorded. We used two sand conditions: moist and dry to test whether favorable versus unfavorable conditions change their escape behavior. The post disturbance responses were video recorded for one hour. *C. formosanus* quickly escaped from the central chamber when disturbed. The time taken to reach the maximum number of termites that escaped ranged from 20 s to 210 s followed by a gradual return to the disturbed chamber. The majority of the escapees returned to the disturbed area within a few minutes after evacuation in both dry and wet sand conditions. A significantly higher percentage of termites escaped in wet sand arenas (43%) than in the dry sand arenas (31%). Our experiments demonstrate that *C. formosanus* is highly unlikely to abandon bait stations for extensive periods of time as a result of mechanical disturbances regardless of habitat quality.

Finally, to understand the exploratory tunneling behavior in various soil types, soil moisture levels and ambient temperatures, *C. formosanus* were examined in the laboratory using two-dimensional foraging arenas. In choice arenas consisting of two substrate types having two moisture levels each, and conducted at a constant temperature of 22°C, significantly greater proportion of termites aggregated in sand than in sandy loam and so was the tunnel length excavated. In a given substrate, termite aggregation or tunnel length did not differ between 5%

and 15% moisture levels. In no-choice tests, where three different substrates (sand, sandy loam and silt loam) were tested at two temperature conditions (22^oC and 28^oC), significantly greater tunnel length was excavated in sand than in either sandy loam or silt loam at 22^oC. *C. formosanus* constructed a significantly lower number of primary tunnels in sandy loam than in sand and a lower number of branched tunnels were excavated than either in sand or silt loam. No significant difference in tunnel length or number of primary or branched tunnels was found between the two temperature conditions.

APPENDIX

LETTER OF PERMISSION

Request for a reprint permission to Journal of Entomological Science

Gautam, Bal K.
Sent: Thursday, August 18, 2011 1:50 PM
To: wgardner@uga.edu

Wayne A. Gardner
Editor
Journal of Entomological Science

Subject: Request for a repring permission

Dear Dr. Gardner,

The following article
"Gautam, B. K. and G. Henderson. 2011. Effects of sand moisture level on food consumption and distribution of Formosan subterranean termites (Isoptera: Rhinotermitidae) with different soldier proportions" has been published in the Journal of Entomological Science (vol. 46(1): 1-13). The data and information from this article will also be published as a chapter of my dissertation for Louisiana State University. This dissertation will be submitted to the graduate school this fall. Could I request a letter or email stating that Journal of Entomological Science grants me the permission to reprint the materials from this article for my dissertation?
Thank you,
Bal

Bal K. Gautam
PhD Candidate
404 Life Sciences Building
Dept. of Entomology, LSU
Baton Rouge, LA 70803 USA
Phone: 225-578-1830
Cell phone: 225-439-7193
Email: bgautam@agcenter.lsu.edu

Reprint Permission From Journal of Entomological Science

From: Wayne A Gardner [wgardner@uga.edu]
Sent: Thursday, August 18, 2011 2:39 PM
To: Gautam, Bal K.
Subject: Reprint Permission

This hereby grants permission to B. K. Gautam to reprint the article, "Effects of sand moisture level on food consumption and distribution of Formosan subterranean termites (Isoptera: Rhinotermitidae) with different soldier proportions," by B.K Gautam and G. Henderson that appeared in the January issue of the Journal of Entomological Science (vol 46, pages 1-13) in 2011. I understand that this reprinting will appear as part of Mr. Gautam's dissertation that will be submitted the Graduate School at Louisiana State University in fulfillment of the requirements for the Ph.D. in Entomology.

If I can be on any further assistance, please contact me.

Wayne A. Gardner, Editor
Journal of Entomological Science
UGA Griffin Campus – Cowart Building
1109 Experiment Street
Griffin, GA 30223 USA
770-228-7341 voice
770-228-7287 facsimile

Reprint Permission From Journal of Insect Behavior

Please include the following copyright citation referencing the publication in which the material was originally published. Where wording is within brackets, please include verbatim.
"With kind permission from Springer Science+Business Media: <book/journal title, chapter/article title, volume, year of publication, page, name(s) of author(s), figure number(s), and any original (first) copyright notice displayed with material>."

Warranties: Springer Science + Business Media makes no representations or warranties with respect to the licensed material.

Indemnity
You hereby indemnify and agree to hold harmless Springer Science + Business Media and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

No Transfer of License
This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without Springer Science + Business Media's written permission.

No Amendment Except in Writing
This license may not be amended except in a writing signed by both parties (or, in the case of Springer Science + Business Media, by CCC on Springer Science + Business Media's behalf).

Objection to Contrary Terms
Springer Science + Business Media hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and Springer Science + Business Media (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

Jurisdiction
All disputes that may arise in connection with this present License, or the breach thereof, shall be settled exclusively by the country's law in which the work was originally published.

Other terms and conditions:

v1.2

Gratis licenses (referencing $0 in the Total field) are free. Please retain this printable license for your reference. No payment is required.

If you would like to pay for this license now, please remit this license along with your payment made payable to "COPYRIGHT CLEARANCE CENTER" otherwise you will be invoiced within 48 hours of the license date. Payment should be in the form of a check or money order referencing your account number and this invoice number RLNK11039024.
Once you receive your invoice for this order, you may pay your invoice by credit card. Please follow instructions provided at that time.

Make Payment To:
Copyright Clearance Center
Dept 001
P.O. Box 843006
Boston, MA 02284-3006

For suggestions or comments regarding this order, contact Rightslink Customer Support: **customercare@copyright.com** or **+1-877-622-5543 (toll free in the US) or +1-978-646-2777.**

Request for a reprint permission to Entomological Society of America

From: **Bal Gautam** <bgauta3@tigers.lsu.edu>
Date: Thu, Aug 18, 2011 at 11:43 AM
Subject: Request for a reprint permission
To: pubs@entsoc.org

Subject: Request for a reprint permission
Journal Title: Annals of the Entomological Society of America

Dear Editor,
The following article
"Gautam, B. K., and G. Henderson. 2011. Wood consumption by Formosan subterranean termites (Isoptera: Rhinotermitidae) as affected by wood moisture content and temperature" has been published in the Annals of the Entomological Society of America (vol. 104(3): 459-464). The data and information from this article will also be published as a chapter of my dissertation for Louisiana State University. This dissertation will be submitted to the graduate school this fall. Could I request a letter or email stating that Entomological Society of America grants me the permission to reprint the materials from this article for my dissertation?
Thank you,
Bal

Bal K. Gautam
PhD Candidate
404 Life Sciences Building
Dept. of Entomology, LSU
Baton Rouge, LA 70803 USA
Phone: 225-578-1830
Cell phone: 225-439-7193
bgauta3@lsu.edu

Reprint Permission from the Entomological Society of America

From: **Alan Kahan** <akahan@entsoc.org>
Date: Tue, Sep 13, 2011 at 7:43 AM
Subject: RE: Request for a reprint permission
To: bgauta3@lsu.edu

September 13, 2011

Bal K. Gautam
PhD Candidate
404 Life Sciences Building
Dept. of Entomology, LSU
Baton Rouge, LA 70803 USA

Dear Bal Gautam,

The Entomological Society of America grants you permission to include the article cited below as part of your Ph.D. dissertation for Louisiana State University.

Gautam, B. K., and G. Henderson. 2011. Wood consumption by Formosan subterranean termites (Isoptera: Rhinotermitidae) as affected by wood moisture content and temperature. Annals of the Entomological Society of America 104(3): 459-464.

Please provide proper credit.

Best wishes,

Alan Kahan
Director of Communications & Publications
Entomological Society of America
10001 Derekwood Lane, Suite 100
Lanham, MD 20706-4876
Phone: 301-731-4535 ext. 3020
Fax: 301-731-4538
akahan@entsoc.org

Request for a reprint permission to Journal of Entomological Society of America

From: **Bal Gautam** <bgauta3@tigers.lsu.edu>
Date: Thu, Aug 18, 2011 at 12:15 PM
Subject: Request for a reprint permission of Manuscript EN-11-062 Version2
To: pubs@entsoc.org

Subject: Request for a reprint permission
Journal Title: Environmental Entomology

Dear Editor,
The following article
"Gautam, B. K., and G. Henderson. Relative humidity preference and survival of starved Formosan subterranean termites (Isoptera: Rhionotermitidae) at various temperature and relative humidity conditions (manuscript EN-11-062 Version 2)" has been accepted for publication in Environmental Entomology on July 9, 2011. The data and information from this article will also be published as a chapter of my dissertation for Louisiana State University. This dissertation will be submitted to the graduate school this fall. Could I request a letter or email stating that Entomological Society of America grants me the permission to reprint the materials from this article for my dissertation?
Thank you,
Bal

Bal K. Gautam
PhD Candidate
404 Life Sciences Building
Dept. of Entomology, LSU
Baton Rouge, LA 70803 USA
Phone: 225-578-1830
Cell phone: 225-439-7193
bgauta3@lsu.edu

Reprint Permission from the Entomological Society of America

August 26, 2011

Bal K. Gautam
PhD Candidate
404 Life Sciences Building
Dept. of Entomology, LSU
Baton Rouge, LA 70803 USA

Dear Bal Gautam,

The Entomological Society of America grants you permission to include the article cited below as part of your Ph.D. dissertation for Louisiana State University.

Gautam, B. K., and G. Henderson. 2011. Relative humidity preference and survival of starved Formosan subterranean termites (Isoptera: Rhionotermitidae) at various temperature and relative humidity conditions. Environmental Entomology, in press.

Please provide proper credit.

Best wishes,

Alan Kahan
Director of Communications & Publications
Entomological Society of America
10001 Derekwood Lane, Suite 100
Lanham, MD 20706-4876
Phone: 301-731-4535 ext. 3020
Fax: 301-731-4538
akahan@entsoc.org

VITA

Bal Krishna Gautam was born and raised in a rural area of Parbat district of the Himalayan country Nepal. The family later migrated to the southern part of the country, Chitwan, where he obtained his undergraduate degree in agricultural science from the Institute of Agriculture and Animal Science, Rampur Campus. He then joined Nepal Agriculture Research Council as a Research Officer for one year before switching his career to Plant Protection Officer in the Department of Agriculture where he worked several years and earned invaluable hands-on experience in insect pest management in both urban and agricultural settings. In the meantime, Mr. Gautam received a competitive scholarship from the Indian Council for Cultural Research to pursue a master's degree in Entomology from G. B. Pant University of Agriculture and Technology, India. After receiving a master's degree in 2001, he again joined the Department job and undertook higher level responsibilities. Suddenly, his life took a turn when he decided to pursue a doctorate degree from the United States. He left his job and moved to the United States with wife Meena and 4 year old daughter Kusum. In January 2007, Mr. Gautam enrolled into a doctorate program under the supervision of Dr. Gregg Henderson in the Department of Entomology at Louisiana State University and Agricultural and Mechanical College.