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Effects of spinosad on Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae)

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EFFECTS OF SPINOSAD ON FORMOSAN SUBTERRANEAN TERMITE, *COPTOTERMES
FORMOSANUS* SHIRAKI (ISOPTERA: RHINOTERMITIDAE)

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
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Dependra Bhatta
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TABLE OF CONTENTS

ACKNOWLEDGEMENTS	ii
LIST OF FIGURES	v
ABSTRACT	vii
CHAPTER 1. INTRODUCTION	1
1.1 Termites	1
1.2 Formosan Subterranean Termites	2
1.2.1 Distribution	2
1.2.2 Economic Importance	2
1.2.3 Control Measures	3
1.2.3.1 Physical Measures	3
1.2.3.2 Biological Measures	3
1.2.3.3 Chemical Measures	4
1.3 References	6
CHAPTER 2. TOXICITY AND REPELLENCY OF SPINOSAD AND SPINETORAM ON FORMOSAN SUBTERRANEAN TERMITES, <i>COPTOTERMES</i> <i>FORMOSANUS</i> SHIRAKI (ISOPTERA: RHINOTERMITIDAE)	13
2.1 Introduction	13
2.2 Materials and Methods	14
2.2.1 Termites	14
2.2.2 Chemicals	15
2.2.3 No Choice Bioassay	15
2.2.4 Two-choice Bioassay	17
2.2.5 Multiple-choice Bioassay	18
2.2.6 Statistical Analysis	19
2.3 Results	20
2.3.1 No Choice Bioassay	20
2.3.2 Two-choice Bioassay	22
2.3.3 Multiple-choice Bioassay	25
2.4 Discussion	25
2.5 References	29
CHAPTER 3. AN ETHOGRAM ANALYSIS OF FORMOSAN SUBTERRANEAN TERMITES, <i>COPTOTERMES FORMOSANUS</i> SHIRAKI (ISOPTERA: RHINOTERMITIDAE) TREATED WITH A SUBLETHAL DOSE OF SPINOSAD	33
3.1 Introduction	33
3.2 Materials and Methods	34
3.2.1 Termites	34
3.2.2 Chemical	34
3.2.3 Bioassay	34

3.2.4 Statistical Analysis	37
3.3 Results	37
3.4 Discussion	41
3.5 References	43
CHAPTER 4. HORIZONTAL TRANSFER OF SPINOSAD IN <i>COPTOTERMES</i> <i>FORMOSANUS</i> (ISOPTERA: RHINOTERMITIDAE).....	47
4.1 Introduction	47
4.2 Materials and Methods	48
4.2.1 Termites	48
4.2.2 Termiticides	48
4.2.3 Recipient Termites.....	48
4.2.4 Substrate	49
4.2.5 Donor Termites.....	49
4.2.6 Mixing Donor and Recipient Termites.....	50
4.2.7 Statistical Analysis	50
4.3 Results	50
4.4 Discussion	55
4.5 References	58
CHAPTER 5. SUMMARY AND CONCLUSION	62
THE VITA	64

LIST OF FIGURES

Figure 2.01. No choice bioassay in Entrust, Tracer and Radiant at 1, 25, and 50ppm with an untreated balsa wood at center in (A) Sand, (B) Soil and (C) Filter paper.....	17
Figure 2.02. Two-choice bioassay with distilled water and chemical treated choice chambers.....	18
Figure 2.03. Multiple-choice bioassay with 1ppm, 25ppm, 50ppm, control and dry release chamber choices.	19
Figure 2.04. Cumulative mean percent mortality (\pm SEM) of <i>Coptotermes formosanus</i> in (A) Sand, (B) Soil and (C) Filter paper. Different letters within each time frame indicate significant differences ($P < 0.05$). C=Control; E=Entrust; T=Tracer; R=Radiant; 1=1ppm; 25=25ppm; 50=50ppm.	21
Figure 2.05. Tunnel area (\pm SEM) made by termites in no choice test in (A) Sand and (B) Soil. Different letters within each time frame indicate significant differences ($P < 0.05$). C=Control; E=Entrust; T=Tracer; R=Radiant; 1=1ppm; 25=25ppm; 50=50ppm.....	22
Figure 2.06. Consumption (\pm SEM) in no choice test in (A) Sand, (B) Soil and (C) Filter paper. Different letters indicates significant difference ($P < 0.05$). C=Control; E=Entrust; T=Tracer; R=Radiant; 1=1ppm; 25=25ppm; 50=50ppm.....	22
Figure 2.07. Cumulative mean percent mortality (\pm SEM) of <i>Coptotermes formosanus</i> in two-choice test in (A) Entrust, (B) Tracer and (C) Radiant. m=minutes; h=hour/s.....	23
Figure 2.08. Mean percent termite (\pm SEM) present in either control or treated choice in two-choice test: (A) Entrust 1ppm, (B) Entrust 25ppm, (C) Entrust 50ppm, (D) Tracer 1ppm, (E) Tracer 25ppm, (F) Tracer 50ppm, (G) Radiant 1ppm, (H) Radiant 25ppm, and (I) Radiant 50ppm. Different letters within each time frame indicate significant differences ($P < 0.05$).	24
Figure 2.09. Cumulative mean percent termite mortality (\pm SEM) in multiple-choice test.	26
Figure 2.1. Mean percent termite (\pm SEM) present in multiple-choice test in (A) Entrust, (B) Tracer and (C) Radiant. Different letters indicates significant difference ($P < 0.05$). R.C=Release Chamber. Different letters within each time frame indicate significant differences ($P < 0.05$).	26

Figure 3.01. An experimental unit containing two marked termites (in circle) on Entrust 1ppm treated sand and filter paper for ethogram.....	36
Figure 3.02. Worker termite marked with blue oil-based paint on its dorsum of abdomen.	36
Figure 3.03. Mean percentage (\pm SE) of time budgeted by control and treated termites in: (A) Walking, (B) Antennal touching, (C) Moving, (D) Grooming, (E) Gnawing, (F) Shaking, (G) Digging, and (H) Sand depositing. Different letters indicate significant differences ($P<0.05$).	38
Figure 3.04. Mean percent mortality of control and Entrust 1ppm treated termites over 7 days.....	41
Figure 4.01. Transfer bioassay: (A) recipient termites, (B-C) donor termites, (D-E) exposure of donor termites with recipient termites.	51
Figure 4.02. Combined mean percent mortality (\pm SEM) of donor and recipient termites in soil and sand. Different letters within each time frame indicate significant differences ($P<0.05$).	52
Figure 4.03. Mean percent donor and recipient mortality (\pm SEM) in sand caused by termites exposed to control and (A) 1ppm, (B) 25ppm, and (C) 50ppm. Different letters within each time frame indicate significant differences ($P<0.05$).	53
Figure 4.04. Mean percent donor and recipient mortality (\pm SEM) in soil caused by termites exposed to control and (A) 1ppm, (B) 25ppm, and (C) 50ppm. Different letters within each time frame indicate significant differences ($P<0.05$).	54

ABSTRACT

Formosan subterranean termites, *Coptotermes formosanus*, are economically important structural pests of warm and humid regions. Different control measures have been developed to avoid the damage caused by them. Among the available measures, chemical treatments have predominated for the last five decades. In our study we tested the effect of spinosad, a biopesticide, on *C. formosanus*.

Spinosad is composed of bacterial metabolites that have been widely used to control various agriculture pests. It has been tested on Kalotermitidae and Termitidae; however, not on Rhinotermitidae, the most destructive of termite families. Both no choice and choice tests were conducted using three concentrations, 1ppm, 25ppm and 50ppm, of spinosyn products Entrust, Tracer and Radiant. In the no choice test in sand, more than 85% mortality was observed at 25 and 50ppm after 1 day exposure followed by 100% mortality at 7 days. Similarly, after 7 days at 25 and 50ppm in soil and filter paper, 100% mortality was observed. In the two-choice test, observations before the onset of termite mortality showed that none of the products or concentrations was repellent. Likewise, in the multiple-choice test, the preference of termites among 1ppm, 25ppm, 50ppm, control and release chamber in all 3 concentrations was somewhat random. The tunnel area in control and treated choices was not significantly different which supports the non repellent attribute of spinosyns on *C. formosanus*.

Ethogram analysis was conducted to observe the behavioral changes of termites using a sublethal dose of spinosad over 7 days. Behaviors including walking, antennal touching, moving, grooming, gnawing, shaking, digging and particle movement were observed in this test. Spinosad did change the duration and frequency of some major behaviors like walking, antennal touching, grooming and moving (other than walking). Treated termites allocated more time in grooming

and moving behaviors. Results of ethogram analysis suggest that social behaviors like grooming could be exploited for toxin transfer.

Horizontal transfer of spinosad was conducted in sand and soil at 1, 25 and 50ppm. Donor and recipient termites began to contact and groom each other immediately after releasing them together. Spinosad was more effectively transferred in sand than in soil. In sand at 25 and 50ppm, significantly high mortality of donors and recipients was observed after 7 days. Our laboratory study suggests that spinosad and spinetoram are effective against termites and further study is needed to address its effects against *C. formosanus* in field conditions.

CHAPTER 1. INTRODUCTION

1.1 Termites

In the history of social evolution, termites were the first animal to live together in an organized colony (Wang et al. 2015). Overlapping generations, cooperative care of young and division of labor are some of the shared characters. The eusocial insects show altruistic behaviors and sterility and thus seemingly contradict the natural selection theory (Hamilton 1972). In a termite colony, reproductives, workers and soldiers are morphologically different castes which are responsible for different tasks. Reproductives are further divided into primary and secondary reproductives. Primary reproductives are the alate termites that are responsible to establish a new colony, and the secondary reproductives act as a substitute for primary reproductives (Lee and Wood 1971; Luscher 1976). Workers are responsible for foraging, construction and caring of young ones, soldiers and reproductives (Noirot and Quennedey 1974). Meanwhile, soldiers defend the colony from predators (Deligne et al. 1981; Wells and Henderson 1993; Chen et al. 1999).

Termites are mainly found in humid tropical and subtropical climates, but some species occur in temperate regions (Emerson and Schmidt 1955; Araujo 1970; Eggleton 1999). They belong to the order Isoptera. However, on the basis of some similarities with early branching termites in the morphology and symbiotic gut flagellates of the woodroach, *Cryptocercus*, they are now considered in the Blattodea (Klass et al. 2008; Xiao et al. 2012). Isoptera has seven families: Mastotermitidae, Hodotermitidae, Termopsidae, Kalotermitidae, Rhinotermitidae, Serritermitidae, and Termitidae (Emerson and Schmidt 1955; Krishna 1970; and Eggleton 2001).

1.2 Formosan Subterranean Termites

1.2.1 Distribution

Formosan subterranean termites, *Coptotermes formosanus* Shiraki, belong to the family Rhinotermitidae. They are native to China and Taiwan (Kistner 1985; Li et al. 2009). After invading Japan in the 18th century, they were first documented in Hawaii and later distributed to the continental U.S. after the World War II (Su and Tamashiro 1987). By the transportation of infested wood, they disperse from one state to another (LaFage 1987). They are mainly found in the southern states of the U.S. namely Alabama, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee and Texas (Su and Tamashiro 1987; Atkinson et al. 1993; Haagsma et al. 1995; Woodson et al. 2001). In Louisiana, they were first documented in Lake Charles and New Orleans in 1966 (Spink 1967).

1.2.2 Economic Importance

Out of 3,000 termite species, 183 species are considered pests, but only 80 species can cause severe damage (Edwards and Mill 1986). Moreover, subterranean termites share 38 species considered severe pests with 18 species in the genus, *Coptotermes* (Rust and Su 2012). According to the data of 2010, the worldwide total estimated control and repair cost due to termites was \$ 40 billion per year and 80% of that cost was shared by subterranean termites (Rust and Su 2012). *C. formosanus* and *C. gestroi* are two species in the genus *Coptotermes* that are widely spread and considered two of the most economically important pests (Rust and Su 2012).

C. formosanus are thought to be ‘super termites’. A mature colony contains approximately 10^6 individuals that forage extensively over a range of 100 meters (Tamashiro et al. 1980) to cause excessive damage on wooden structures (Lai et al. 1983; Edwards and Mill

1986; Tamashiro et al. 1987) and non cellulosic materials like telephone cables (Henderson and Dunaway 1999). In the United States, the annual control and repair cost caused by *C. formosanus* exceeds more than 1 billion dollars (Suszkiw 2000; Pimentel et al. 2005; Paudel et al. 2010). *C. formosanus* in New Orleans, LA causes an annual damage control and repair cost of 300 million dollar, excluding the aesthetic losses (Suszkiw 1998; Lax and Osbrink 2003). In 2005, New Orleans was flooded due to the undermining of levees (Seed et al. 2006). Henderson (2008) reported that *C. formosanus* might have caused the levees to weak. Similarly, events of flooding due to *C. formosanus* were also reported in China (Gao 1985).

1.2.3 Control Measures

1.2.3.1 Physical Measures

In physical measure, physical barriers are made to prevent the foraging of termites in wooden structures. For example, the barriers can be made by the use of Termimesh, a stainless steel mesh (Grace et al. 1996) or hard crushed basaltic rock (Tamashiro et al. 1987; Yates et al. 2000).

1.2.3.2 Biological Measures

Biological control techniques suppress insect populations with little if any negative effects on human health and environment. Cost effectiveness and permanent remedial control of pests are some limiting parameters of these techniques. Introduction of natural enemies like predators, parasitoids and pathogens has effectively controlled many agricultural pests (Doutt 1967; Caltagirone and Doutt 1989). However, *C. formosanus* is cryptic in nature and has many social defense mechanisms that minimize the effects of predators, parasitoids and pathogens (Chen et al. 1998; Culliney and Grace 2000; Grace 2003). Soldier proportions are the highest in termites compared to other social insects (Oster & Wilson 1978) and defend nestmates from

predators (Deligne et al. 1981; Wells and Henderson 1993; Chen et al. 1999). Workers use their fecal material for colony construction which helps to maintain the temperature and humidity inside the nest (Wood 1988). Feces also encourage the growth of actinobacteria, and the secondary metabolites of actinobacteria discourage the growth of the entomopathogen *Metarhizium anisopliae* (Chouvenc et al. 2013). The replication and distribution of entomopathogens is decelerated by grooming (Yanagawa et al. 2010). Moreover, the compounds secreted in the saliva and guts of termites also inhibit the growth of harmful fungus (Chouvenc and Su 2010; Hamilton and Bulmer 2011). Due to social behavior, the success of entomopathogen against *C. formosanus* is limited to laboratory tests only (Verma et al. 2009).

1.2.3.3 Chemical Measures

Chemical measures are mainly comprised of wood treatment, baits and soil treatments. To get rid of fungal and insect infestations in wood, chemical treatments are mainly in practice. Wood treated with quaternary ammonia compounds is stable against *C. formosanus* (Terzi et al. 2011). Similarly, neurotoxin, metabolic inhibitors or chitin synthesis inhibitors are used in termite baits (Evans and Iqbal 2014). Chitin synthesis inhibitors like hexaflumuron, diflubenzuron, triflumuron, lufenuron and noviflumuron inhibit the molting process of termites and cause delayed mortality (Su and Scheffrahn 1993; 1996; Vahabzadeh et al. 2007; Xing et al. 2014). The chitin synthesis inhibitor, hexaflumuron, is one of the most successful among the available baits against *C. formosanus* (Evans and Iqbal 2014). Use of liquid termiticides is more prominent among termite control firms from the last 6 decades (Su and Scheffrahn 1990; Smith and Rust 1993; Curl 2004; Su 2011). More than 80% of the termite control firms still prefer soil treatment measures (Su 2011). During 1989-1995, an exclusion hypothesis was the idea behind treatments in which synthetic pyrethroids or organophosphates were used for soil treatments to

prevent infestation of the structures by repelling termites (Forschler 1994; 2009; Kuriachan and Gold 1998). Later, the exclusion hypothesis was transformed into the attrition hypothesis by the introduction of slow and non repellent termiticides (Forschler 2009). In the attrition hypothesis, termites can forage into treated areas due to the non repellent effect of the termiticides and transfer the toxin to healthy nestmates to cause colony mortality (Potter and Hillery 2002; Forschler 2009). However, the applications of synthetic termiticides also pose threats to non-target organisms (Beard 1974; Su and Scheffrahn 1998; Henderson 2001). Biopesticides could be a possible candidate for effective termite control without adverse effects on the environment or non target species.

Spinosad is the secondary metabolite of soil dwelling bacteria, *Saccharopolyspora spinosa*. The secondary metabolites spinosyn A ($C_{41}H_{65}NO_{10}$) and spinosyn D ($C_{42}H_{67}NO_{10}$), are combined in a ratio of 17:3 to form spinosad (Kirst et al. 1991). This naturally occurring product was found in 1982 in the U.S Virgin Islands, and the bacteria was identified in 1985 (Anonymous 2001). Spinetoram is the synthetic product of spinosad and known as the second generation of spinosad (Dripps et al 2008). The combination of spinosyn J (major component) and spinosyn L (minor component) after synthetic modification are used to make spinetoram (Dripps et al 2008). Spinosyn J and spinosyn L are also naturally occurring metabolites of *S. spinosa* (Dripps et al 2008).

Spinosad is effective against insects either by dermal contact or feeding or both routes of entry (Sparks et al. 1995). Spinosad does not have an effect on binding sites in which other insecticides act. Thus, it has a novel mode of action (Salgado 1998). It acts primarily on the binding site of nicotinic acetylcholine receptors (nAChRs) and secondarily as Gama amino butyric acid (GABA) neurotransmitter agonist (Salgado 1998). Spinosad and spinetoram has

same mode of action, however, spinetoram is more effective and stable even at low concentrations and has broad spectrum than spinosad (Palumbo and Richardson 2008).

The use of spinosad is increasing mainly for agricultural pests and it has been registered for more than 250 crops in the USA (Dow 2014). In soil, the half-life of spinosad in the presence of light is 9-10 days and in the absence of light, during aerobic soil metabolism, is 9-17 days (Thompson and Sparks 2002). Moreover, spinosad has a short half-life of <1 day in water medium (Thompson and Sparks 2002). In the laboratory, it is harmful at high levels to some beneficial insects (Williams et al. 2003). Toxicity of spinosad in the honey bee, *Apis mellifera*, is 11.5 ppm and in the lady beetle, *Hippodamia convergens* is >200 ppm (Thompson et al. 2000). Concerning the environmentally safe and insecticidal properties of spinosad, we wanted to test its termiticidal effect with my following research objectives:

1. To analyze toxicity and repellency of spinosad and spinetoram on the *C. formosanus*;
2. To analyze the behaviors of *C. formosanus* to sublethal doses of spinosad; and
3. To analyze the horizontal transfer of spinosad among nestmates.

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CHAPTER 2. TOXICITY AND REPELLENCY OF SPINOSAD AND SPINETORAM ON FORMOSAN SUBTERRANEAN TERMITES, *COPTOTERMES FORMOSANUS* SHIRAKI (ISOPTERA: RHINOTERMITIDAE)

2.1 Introduction

Formosan subterranean termites, *C. formosanus*, are the most important pest of the new millennium as they are more destructive and cause economic damage of more than 1 billion dollars per year in the USA (Hunter 2000; Suszkiw 2000; Paudel et al. 2010). They can damage anything that consists of cellulose like living trees, railway sleepers, paper, wooden houses, boats and ships (Lai et al. 1983; Edwards and Mill 1986; Tamashiro et al. 1987). In spite of various physical, biological and chemical means for termite control, it is a great challenge to get rid of them from structures and prevent their reentry. Though biological control techniques are expanding to control agricultural and structural pests (e.g., ants, cockroaches), they seem unsuccessful against termites in the field. For example it is not possible to attain an epizootic in a termite colony from a biological means like bacteria or fungal inoculum in the field because almost all nest members must come in contact with high concentrations of microbes (Grace 2003). As a result, chemical treatments have predominated for the last 6 decades (Smith and Rust 1993; Su and Scheffrahn 1990). Meanwhile, fast acting termiticides usually repel termites away from the treated site due to quick mass mortality (Su et al. 1982; Fei and Henderson 2005). With non repellent, slow acting termiticides, termites come in contact with the chemicals that result in high but scattered (spread over the environment) mortality (Su et al. 1982; Gahlhoff and Koehler 2001; Remmen and Su 2005a, b).

Spinosad is insecticidal metabolites derived from naturally occurring soil actinomycete bacteria, *Saccharopolyspora spinosa* (Mertz and Yao 1990). It was registered as an insecticide in 1997 in the USA. Spinosad neither appears to be toxic to mammals or birds nor is it

carcinogenic, teratogenic, mutagenic or neurotoxic (Anonymous 1996). Spinosad treatment on rats has an acute oral LD₅₀ >5000 mg/kg and an acute inhalation of LC₅₀ >5 mg/kg (Thompson and Sparks 2002).

Spinosad and spinetoram have a primary effect on the binding site of nicotinic acetylcholine receptors and a secondary effect as a GABA neurotransmitter agonist (Salgado 1998; Palumbo and Richardson 2008). They have a novel mode of action because they act on distinct receptor of nicotinic or GABA target sites different from the target site of other insecticides (Sparks et al. 2001; Palumbo and Richardson 2008; Orr et al. 2009). Spinosad and spinetoram have been widely used to control Lepidoptera, Diptera, Hymenoptera, Thysanoptera and some Coleoptera (Anonymous 2001; Thompson and Sparks 2002; Dripps et al. 2008). In addition, a few studies were done to test the effect of spinosad on Kalotermitidae and Termitidae. A lab study by Scheffrahn et al. (1997) showed that two different species of drywood termites, *Cryptotermes brevis* Walker and *Incisitermes snyderi* Light, actively foraged from the untreated area to spinosad treated area resulting in significantly high mortality. Spinosad applied in wood galleries in lab bioassays at 0.23% and 0.5% resulted in 98-100% mortality for drywood termites, *C. brevis* and *I. snyderi* (Scheffrahn and Thoms 1999). In a comparison of the different termiticides, on *Microtermes mucophagus* Desneux, the LC₅₀ of spinosad was low, ranging from 3.24-3.72 ppm (Iqbal and Saeed 2013). Interestingly, at present there is no published study on the effects of spinosad and spinetoram in Rhinotermitidae.

2.2 Materials and Methods

2.2.1 Termites

Formosan subterranean termites were collected from two different places (behind dumpster and Scout Island, Brechtel Park, New Orleans, LA, USA) by using milk crate traps as

described in Gautam and Henderson (2011). These colonies were maintained in Roughneck® trash cans with 98% Relative Humidity and moist wood in the Urban Entomology Laboratory at 27.5°C at Louisiana State University.

2.2.2 Chemicals

Three Dow AgroSciences products, Entrust® (a.i. 80%), Tracer® (a.i. 480g/liter) and Radiant® (a.i. 120g/liter) were provided by the Soybean Entomology Lab at LSU. Entrust and Tracer have spinosad as an active ingredient, whereas Radiant has spinetoram. Spinosad consists of spinosyn A ($C_{41}H_{65}NO_{10}$) and spinosyn D ($C_{42}H_{67}NO_{10}$) in the ratio of 17:3 (Kirst et al. 1991), whereas, spinetoram is the synthetic modification of spinosyn J and spinosyn L (Dripps et al. 2008). This semi-synthetic product has greater photostability and residual control (Dripps et al. 2008).

2.2.3 No Choice Bioassay

To test the termiticidal effect of the chemicals and their concentrations in 3 different substrates, a no choice test was conducted using Petri dishes (Falcon® 60X15mm, Becton Dickinson Labware, Franklin Lakes, NJ). One ppm, 25 ppm and 50 ppm of Entrust, Tracer and Radiant in soil, sand and filter paper were tested. Soil was collected from Ben Hur Research Station (from a barn area without any fertilizer or pesticide application) Baton Rouge, LA and a sample without any course or foreign materials was sent for texture analysis to the Coastal Wetlands Soils Characterization Lab at LSU. The textural class of analyzed soil was silty clay loam (17.1% sand, 55.5% silt and 27.4% clay) with pH 5.49. Sand was purchased from Louisiana Cement Products, LLC (Baton Rouge, LA). Sand and soil was autoclaved (12 cycles at 250°F sterilization temperature for 1 hour) and oven dried at 60°C for 24 hours. Filter papers (Whatman™ 55 mm diameter) were oven dried for 24 hours at 60°C and initial weight of

individual filter paper was measured. On the basis of our preliminary research, 3 different concentrations, 1 ppm, 25 ppm and 50 ppm of all three chemicals were tested. Since the products differed in percent active ingredient, each was prepared differently to attain the desired ppms on a wt/wt basis. For example, for sand treated with Entrust (80% a.i.) to attain 50ppm treatments with 10% moisture, 4.125 mg Entrust was added to 6 ml distilled water and this slurry was then added to 60 grams of sand. In sand this moisture level provided the termites with enough free water. For soil treatments 20% moisture was necessary. Using the same product and ppm for 60 g soil required 4.5 mg Entrust be added to 12 ml distilled water. All treated soil and sand treatments were then mixed gently in a sealed Ziploc bag (S.C. Johnson & Son, Racine, WI). For filter paper treatments, as a final example to achieve a 50ppm treatment Entrust, 0.5313 mg Entrust was added to 6 ml of water and then 0.5 ml of mixed solution was added to the filter paper. Five grams of treated sand or soil or one filter paper was placed uniformly in each experimental unit. At the center of each unit, a piece of untreated balsa wood (1x1x0.2 cm) was placed as a food source (additional food source in case of filter paper) (Figure 2.01, A-C). Balsa wood was oven dried for 24 hrs. at 60°C and weighed before and after the experiment. Each experimental unit contained 20 termites with 10% soldiers. A mature colony of *C. formosanus* bears 10% -15% soldier (Haverty 1977; Mao et al. 2005). Each treatment was replicated 6 times for each of two colonies for a total of 360 experimental units. The experimental units were sealed with Parafilm® to avoid moisture loss and placed in Parker Coliseum room number 144, LSU at room temperature $28 \pm 1.5^{\circ}\text{C}$ and $75 \pm 8\%$ R.H. Termite mortality was recorded at 1, 3, 7 and 14 days after treatment. Walking and moving (other than walking) behaviors of termites in control and treated dishes were also recorded. Termites were recorded as dead when they did not show any movement of appendages for 5 seconds. Tunnel area and food consumption were also

recorded at 14 days after treatment after dismantling the experimental units. Tunnel area was measured by the calibrated thin flexible wire of aluminum foil (Choice Heavy Aluminum Foil 18”X1000’, China). In sand and soil treatments, consumption was recorded from balsa wood only, but in the filter paper treatment, it was recorded from balsa wood and filter paper.

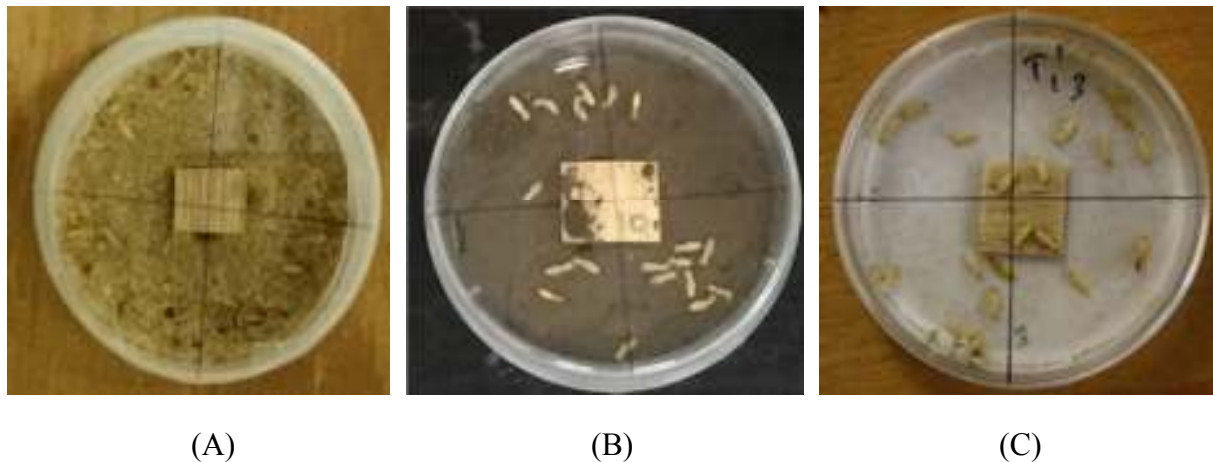


Figure 2.01. No choice bioassay in Entrust, Tracer and Radiant at 1, 25, and 50ppm with an untreated balsa wood at center in (A) Sand, (B) Soil and (C) Filter paper.

2.2.4 Two-choice Bioassay

Two equal sized round acrylic containers (size: 5.08x3.63 cm, Pioneer Plastics Inc., North Dixon, KY) were connected by a 2 cm long clear vinyl tube (0.95 cm outside diameter, 0.64 cm inside diameter, Watts Co., North Andover, MA) to form an experimental unit (Figure 2.02). In each experimental unit, one of the chambers received 0.5 ml distilled water and 5g untreated sand (10% moisture) and the other received either 0.5 ml distilled water and 5g control sand or 5g treated sand. Units containing a chemical treatment were the treated units. Similarly, in untreated units both chambers received only distilled water and sand. Three different chemical products (Entrust, Radiant and Tracer) with three concentrations (1ppm, 25pp, 50ppm) on wt/wt basis as described in the no choice test were used to treat sand. Termites from two different colonies were used. In each chamber, 25 termites including 3 soldiers were released for a total of

50 termites per experimental unit. Each treatment had 6 replications, three from each colony. The number of termites in treated and untreated chambers was recorded at 15 minutes, 30 minutes, 45 minutes, 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, 48 hours and 72 hours after treatment. Dead termites were counted and removed at each observation. For analysis, the live termites of the previous observation were considered as 100% as numbers alive were changed to a percentage. Common behaviors like walking and moving (other than walking) were also observed at each observational period. After 72 hours, experimental units were disassembled and tunnel area was measured as described in no choice tests.



Figure 2.02. Two-choice bioassay with distilled water and chemical treated choice chambers.

2.2.5 Multiple-choice Bioassay

In this test, termites were allowed to choose 1ppm, 25ppm, 50ppm, control or dry release chamber for foraging. A central round acrylic container (size: 5.08x3.63 cm, Pioneer Plastics Inc., North Dixon, KY) was connected by small clear vinyl tubes (2 cm long, 0.95 cm outside diameter, 0.64 cm inside diameter, Watts Co., North Andover, MA) with four same sized

containers equidistant from each other to make an experimental unit (Figure 2.03). In treated units, the central chamber received 5g of dry sand and the connected chambers contained 5g of moistened 1ppm, 25ppm, 50ppm and control sand. In control units, all connected chambers contained moist untreated sand with dry untreated sand in the center. Fifty termites with 10% soldiers were released in the central chamber. There were 12 replications, 6 from each colony, for control and treated group. The number of termites, mortality and tunnel area was recorded as in the two-choice bioassay.



Figure 2.03. Multiple-choice bioassay with 1ppm, 25ppm, 50ppm, control and dry release chamber choices.

2.2.6 Statistical Analysis

Colonies were considered as blocks in this experiment. PROC MIXED Repeated ANOVA was used to analyze mortality percentage and percentage of termites present in no choice and choice tests in SAS 9.4 (SAS Institute, Cary, NC, 2013). Tunnel area and food

consumption was analyzed by SAS PROC MIXED. Means were compared at $\alpha < 0.05$ by using Tukey's Honest Significant difference.

2.3 Results

2.3.1 No Choice Bioassay

In sand, termite mortality was significantly affected by chemical ($F_{3,109} = 104.44$; $P < 0.0001$), days after treatment (DAT) ($F_{3,330} = 546.60$; $P < 0.0001$) and chemical X DAT ($F_{9,330} = 63.21$; $P < 0.0001$). At 1 DAT, all three products at 25ppm and 50ppm caused more than 85% mortality followed by 100% mortality at 7 DAT. At 1 ppm at 7 DAT, Tracer was more effective than Entrust and Radiant. All termites were dead at 14 DAT in treated groups (Figure 2.04, A).

In soil, chemical ($F_{3,109} = 8.18$; $P < 0.0005$), DAT ($F_{3,330} = 791.92$; $P < 0.0001$) and chemical X DAT ($F_{9,330} = 9.72$; $P < 0.0001$) significantly affected the mortality. None of the products was effective in soil at 1 DAT; however, at 7 DAT, all three products at 25ppm and 50ppm were highly effective and caused 100% mortality. At 1ppm at 7 DAT, Tracer caused significantly higher mortality than Entrust and Radiant (Figure 2.04, B).

Similar to sand and soil, mortality in filter paper was significantly affected by chemical ($F_{3,109} = 6.55$; $P < 0.0021$), DAT ($F_{3,330} = 507.29$; $P < 0.0001$) and chemical X DAT ($F_{9,330} = 4.66$; $P < 0.0001$). Similar to soil, at 1 DAT, there was no difference in mortality between treatments and control. At 7 DAT all the products were effective causing significantly high mortality compared to controls. Moreover at 7 DAT, in 25ppm and 50ppm, 100% termite mortality was recorded. At 14 DAT all the products at 1ppm caused more than 70% mortality (Figure 2.04, C).

At 14 DAT, untreated chambers had significantly higher tunnel area in both sand and soil than did treated chambers (Figure 2.05). Similarly, consumption was also significantly higher in untreated chambers compared to treated chambers in sand, soil and filter paper, although there

was some variation in consumption among the treated chambers for soil and filter paper (Figure 2.06).

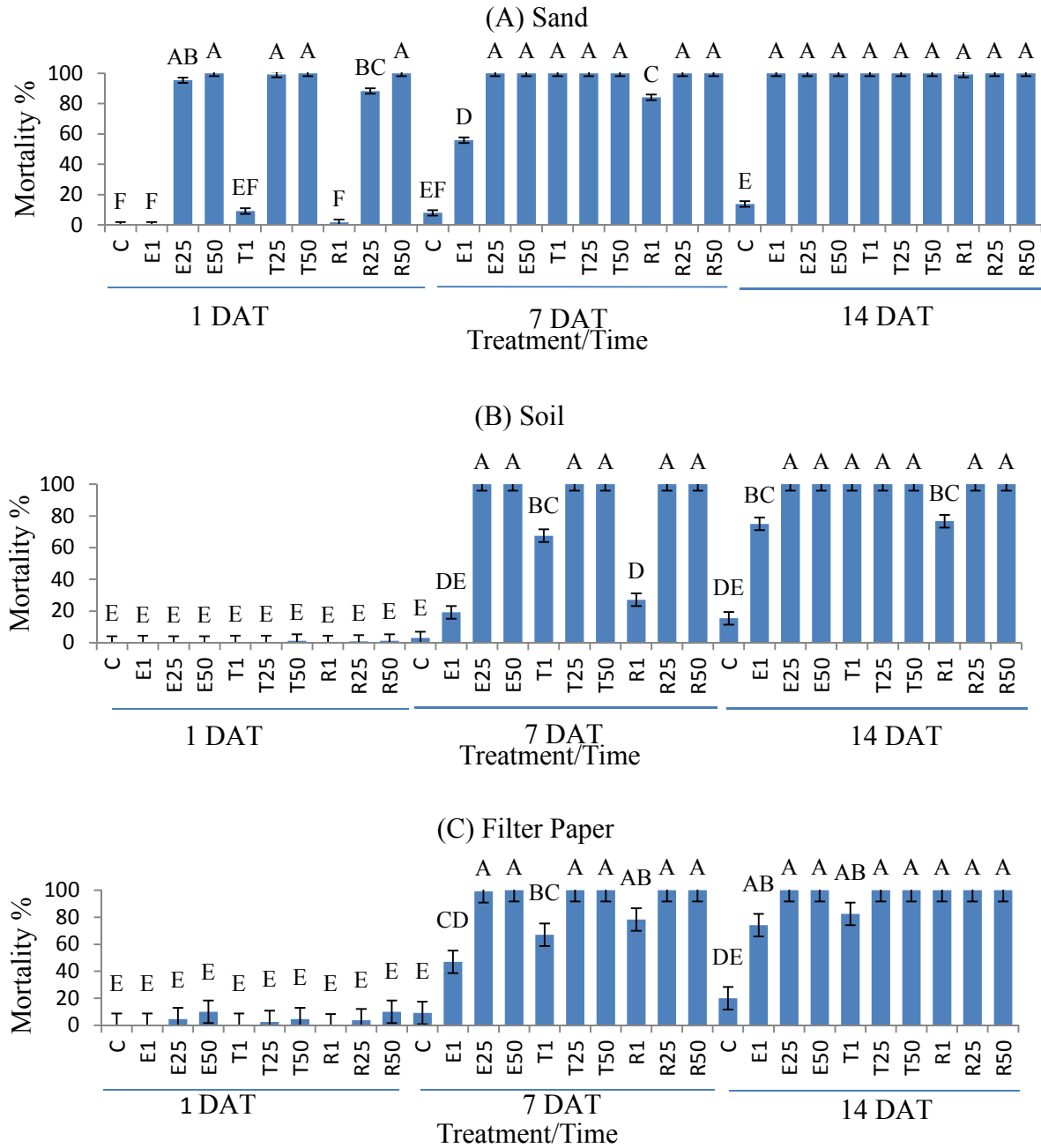


Figure 2.04. Cumulative mean percent mortality (\pm SEM) of *Coptotermes formosanus* in (A) Sand, (B) Soil and (C) Filter paper. Different letters within each time frame indicate significant differences ($P < 0.05$). C=Control; E=Entrust; T=Tracer; R=Radiant; 1=1ppm; 25=25ppm; 50=50ppm.

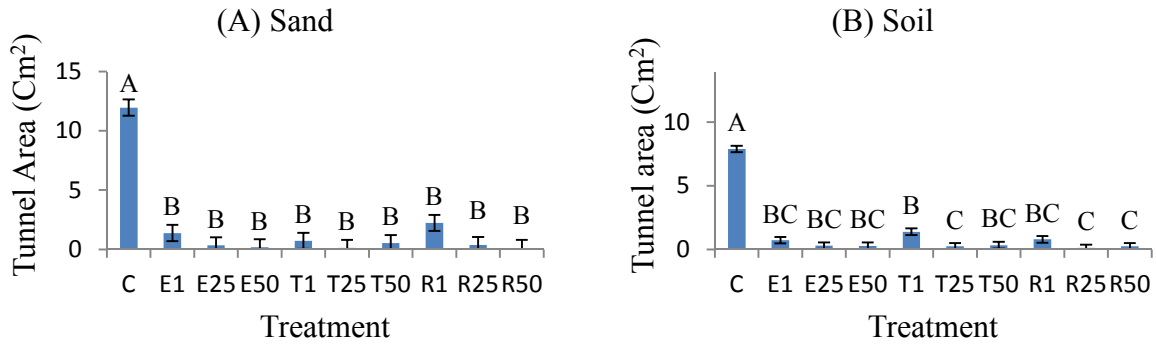


Figure 2.05. Tunnel area (\pm SEM) made by termites in no choice test in (A) Sand and (B) Soil. Different letters within each time frame indicate significant differences ($P < 0.05$). C=Control; E=Entrust; T=Tracer; R=Radiant; 1=1ppm; 25=25ppm; 50=50ppm.

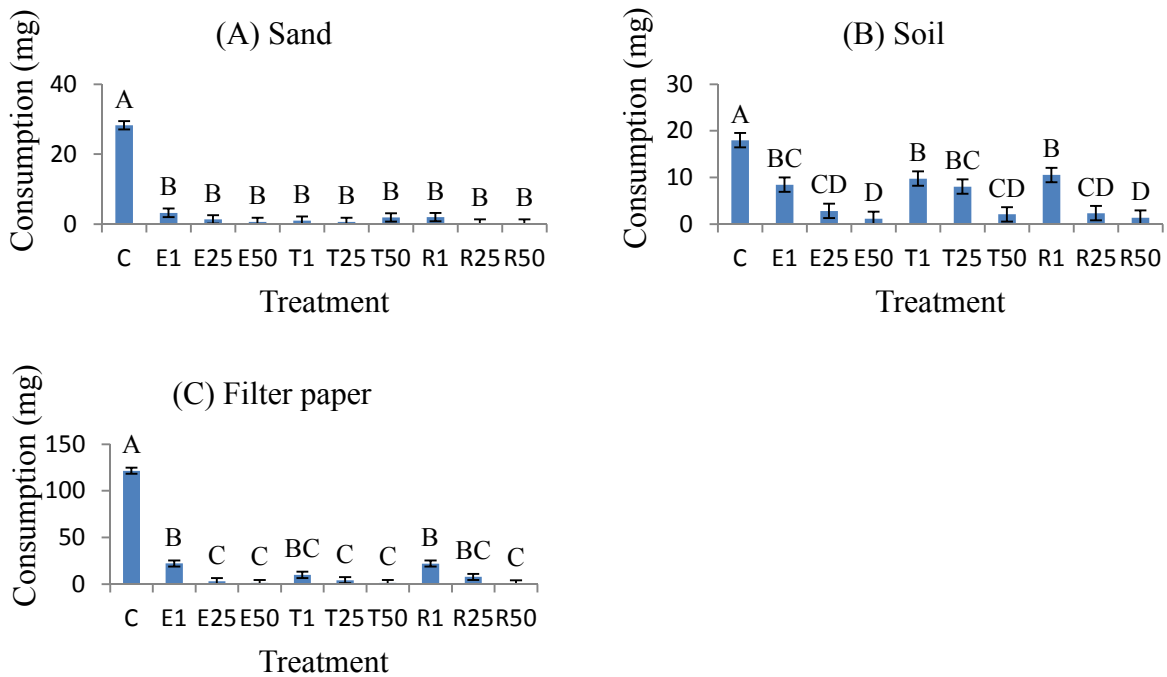


Figure 2.06. Consumption (\pm SEM) in no choice test in (A) Sand, (B) Soil and (C) Filter paper. Different letters indicates significant difference ($P < 0.05$). C=Control; E=Entrust; T=Tracer; R=Radiant; 1=1ppm; 25=25ppm; 50=50ppm.

2.3.2 Two-choice Bioassay

Termites in 25ppm and 50ppm treated units were as active as the termites in control units in walking behavior until the 6h observation in Entrust, and until the 3h observation in Tracer and Radiant when they started dying (Figure 2.07). At 1ppm in all products, there was no significant

difference in termites numbers between control and treated choices (Figure 2.08, A, D, G). The presence of termites in the chambers varied from one observation to another at 25 and 50ppm for all products (Figure 2.08, B, C, E, F, H, I). The tunnel area in treated chambers was not significantly different to untreated chambers at all concentrations for all products.

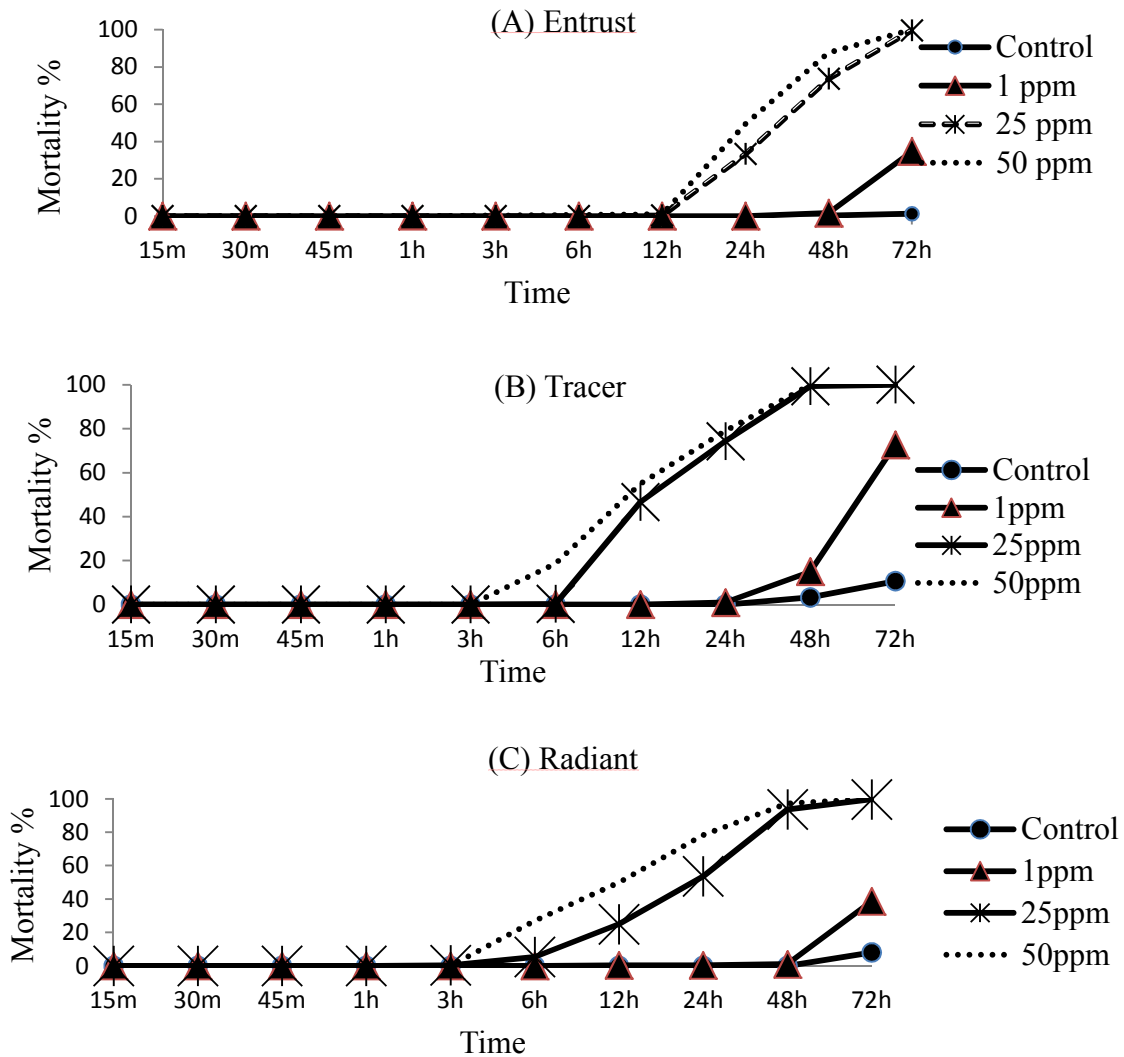


Figure 2.07. Cumulative mean percent mortality (\pm SEM) of *Coptotermes formosanus* in two-choice test in (A) Entrust, (B) Tracer and (C) Radiant. m=minutes; h=hour/s.

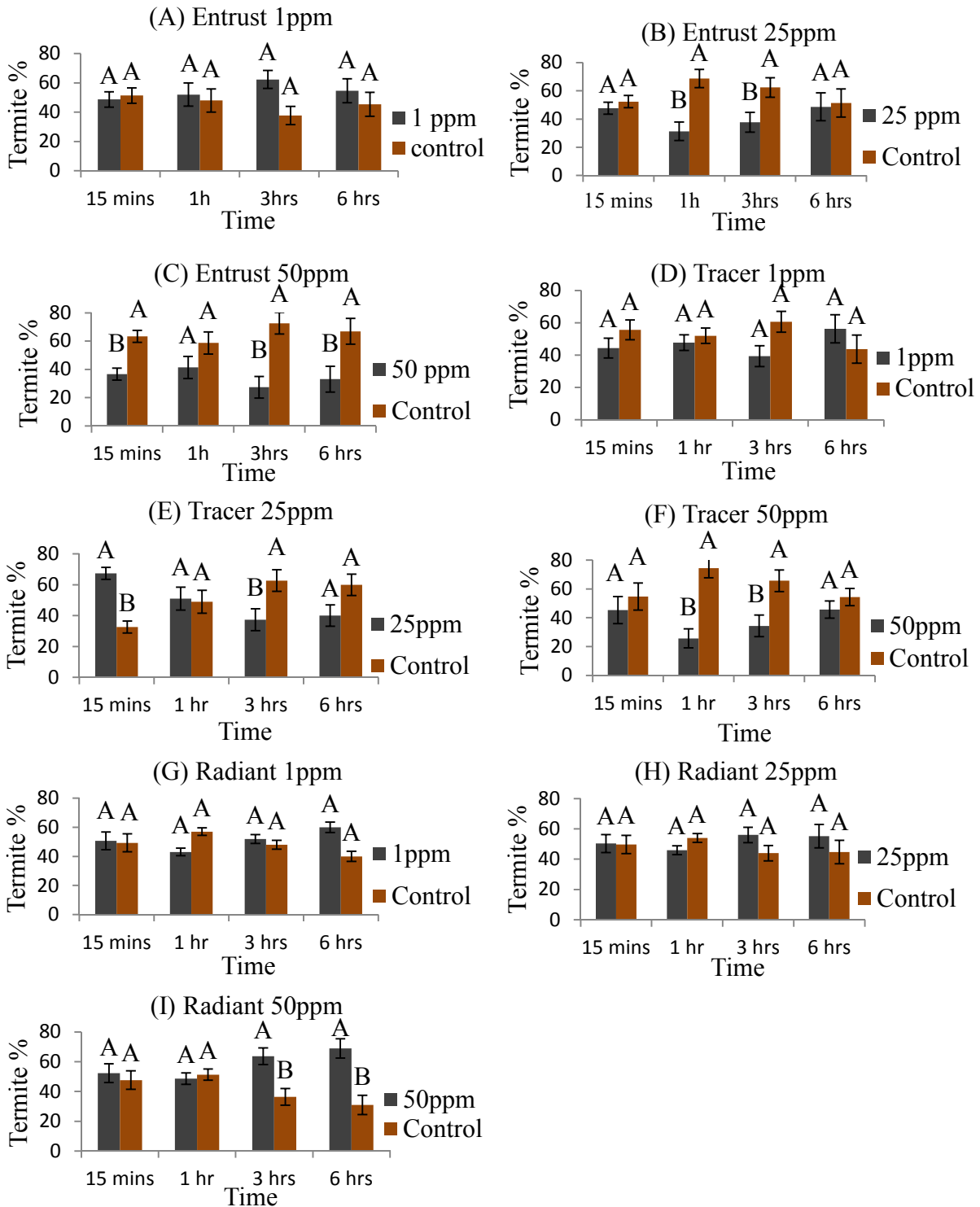


Figure 2.08. Mean percent termite (\pm SEM) present in either control or treated choice in two-choice test: (A) Entrust 1ppm, (B) Entrust 25ppm, (C) Entrust 50ppm, (D) Tracer 1ppm, (E) Tracer 25ppm, (F) Tracer 50ppm, (G) Radiant 1ppm, (H) Radiant 25ppm, and (I) Radiant 50ppm. Different letters within each time frame indicate significant differences ($P < 0.05$).

2.3.3 Multiple-choice Bioassay

Termites in treated units were actively walking similar to the termites in control units until 3h, at which time mortality started to occur (Figure 2.09). In Entrust, there was no significant difference in termite percentage among the 1ppm, 25ppm, 50ppm, untreated, and release chamber choices at any observations (Figure 2.1, A). Similarly, in Tracer at 15 minutes, significant differences among the choices were not found (Figure 2.1, B). The percentage of termites in the Tracer 50ppm choice decreased over the time, whereas in Radiant 50ppm, the percentage of termites decreased until 1h and then increased after 1h (Figure 2.1, C). In Radiant 25ppm, the percentage of termites also decreased over time. In Tracer and Radiant, for untreated chambers, the percentage of termites increased until 1h and decreased for the following observations. For the release chamber, the percentage of termites first decreased and then began to increase after 1h. Different from other observations, in Tracer and Radiant at 6h, the release chamber had significantly higher termite percentage than did other choices. The tunnel area among 1ppm, 25ppm, 50ppm and control chambers for all products was not significantly different.

2.4 Discussion

The results of the no choice test showed that mortality in sand required a shorter exposure period than soil and filter paper. Adsorption properties of the substrate can cause delayed or fast mortality. The soil adsorption coefficient value of spinosyn A in sand is $2,998 \text{ cm}^3/\text{g}$ that is very lower than in silt loam i.e. $145,350 \text{ cm}^3/\text{g}$ (Kollman 2003). Therefore, increased bioavailability of spinosyn A may have led for higher mortality in sand. Similar to our study, the study of Osbrink and Lax (2002), and Bobe et al. (1997) revealed that the bioavailability of fipronil was

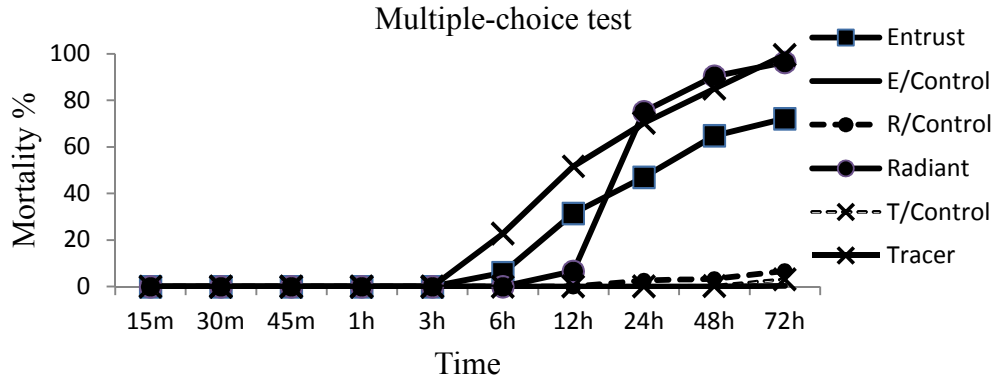


Figure 2.09. Cumulative mean percent termite mortality (\pm SEM) in multiple-choice test.

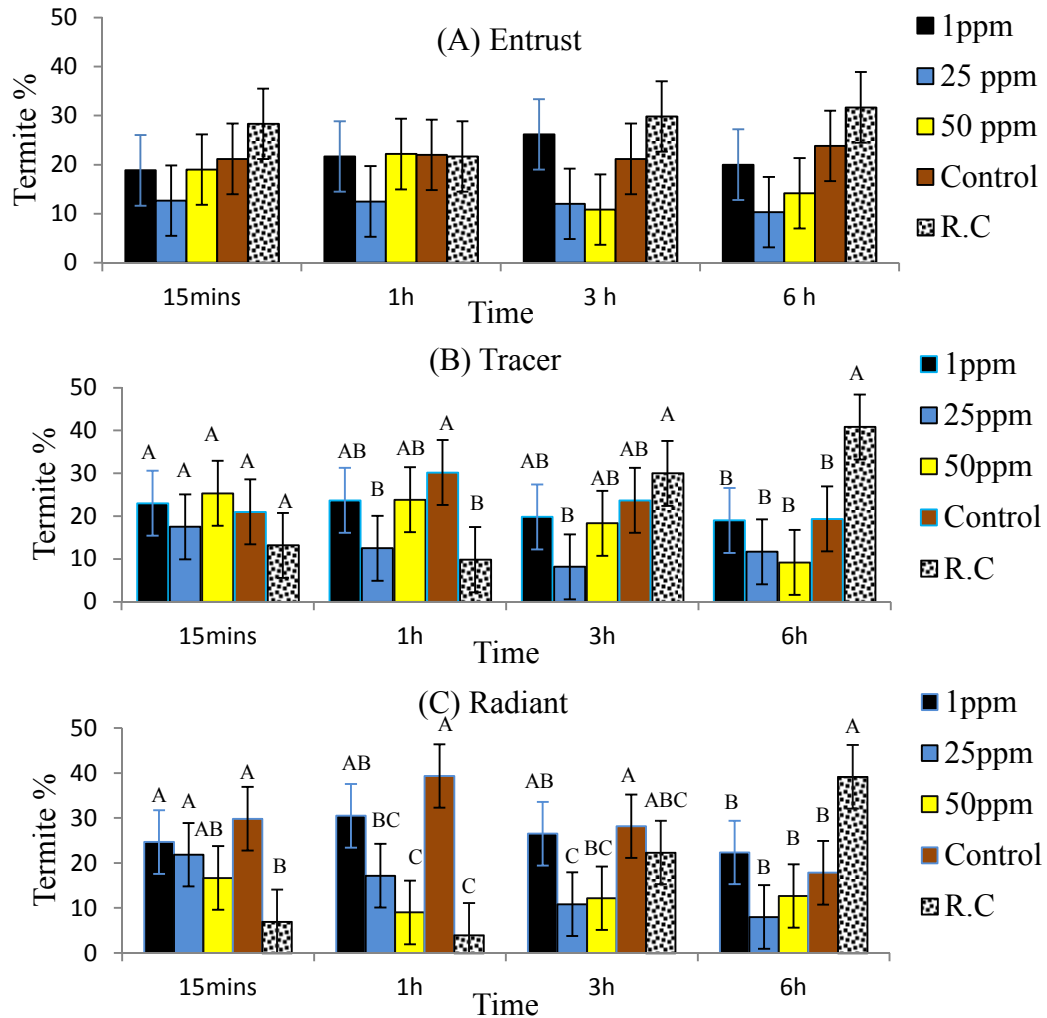


Figure 2.1. Mean percent termite (\pm SEM) present in multiple-choice test in (A) Entrust, (B) Tracer and (C) Radiant. Different letters indicates significant difference ($P < 0.05$). R.C.=Release Chamber. Different letters within each time frame indicate significant differences ($P < 0.05$).

higher in sand and caused higher mortality than in soil. Some termiticides like cypermethrin are more toxic in soils containing high clay (Smith and Rust 1993). Thus, termiticide binding capacity of the substrate can be a most important characteristic that defines the actual acceleration of chemical to reach the target site in termites (Forschler 2009). In addition to the bioavailability, concentration and exposure duration also affected termite mortality. Both 25 and 50ppm seemed equally effective in mortality, consumption and tunnel area in sand or soil or filter paper. As exposure period increased from 1 DAT to 7 DAT, the mortality in soil or filter paper rose from < 5% to 100% at 25 and 50ppm.

The toxicity level of chemicals also differs from species to species. Saljoqi et al. (2014) observed 91% mortality in *Heterotermes indicola* after 7 days in a no choice feeding test with Tracer (spinosad 240 SC) at 1.5ppm. Our results after 7 days with Tracer at 1ppm showed 67% mortality. The difference in mortality could be due to the difference in concentration or species or possibly a slower action of spinosad on *C. formosanus* than on *H. indicola*.

Termites may be intoxicated due to ingestion, contact or both. Spinosad and spinetoram can act by both routes of entry but ingestion is more effective to control most pests, especially lepidopterans (Anonymous 2001; Dripps et al. 2008; Palumbo and Richardson 2008). Cockroaches can acquire spinosad by contact and ingestion both routes acting on the nicotinic acetylcholine and GABA receptors (Watson 2001). However, the oral route seems to be more effective in administering the toxin to termites because while foraging on the treated area they can directly drink or feed from the treated substrate or move treated particles. Fipronil has the same level of effect by either of the routes (Forschler 2009). However, imidacloprid is more effective when administered through ingestion; oppositely, indoxacarb is more toxic on contact (Forschler 2009). In *Reticulitermes flavipes*, untreated termites can acquire imidacloprid by

grooming treated termites (Tomalski and Vargo 2004). At the time of grooming, termites first use their glossae then eat and excrete the foreign materials present on the surface of their nestmates (Yanagawa et al. 2009). From our study, it is very hard to surmise the route of entry of spinosad and spinetoram to the termites but they were acquired either through contact, orally or both.

Higher tunnel area and consumption in controls suggests that termites were more active in untreated than treated units. Treated areas did however exhibit some tunneling. Lab and field studies by Scheffrahn et al. (1997) described that two species of drywood termites, *Cryptotermes brevis* and *Incisitermes snyderi*, extend their foraging area to spinosad treated areas. Spinosad causes the dysfunction of mandibular muscles in insects (Anonymous 2001). Sublethal doses of Tracer (spinosad) affect the epithelial tissue of the midgut that ceases feeding in the cotton leafworm, *Spodoptera littoralis* (Abouelghar et al. 2013). Lower food consumption in treated containers compared to untreated ones could be due to the inactivation of mandibles or the deterioration of mid gut epithelium of treated termites. In field conditions, starved and intoxicated termites may still seek food and care from healthy individuals leading to transfer of spinosad to previously uncontaminated nestmates.

The uneven but non significant difference in numbers of termites in all chambers in the two-choice and the multiple-choice tests indicates that spinosad and spinetoram are non repellent to termites. Dead termites were found distributed among all of the chambers. The non significant tunnel area difference in the chambers also shows that termites were equally active in all choices. In the multiple-choice tests, the number of termites was increasing in the dry release chamber after 1 hour. Termites most likely entered the treated chambers and became symptomatic. Upon entry onto the dry sand, they would have difficulty walking and soon die.

Our no choice and choice tests revealed that spinosad and spinetoram are slow acting and non repellent to termites. However, it is still unknown whether spinosyns causes any abnormal behaviors or immediate changes in normal behaviors that could affect social interactions. Locomotion and grooming are important for toxin transfer; therefore, further investigation is necessary to address if behavior changes over time in different treatments of spinosyns.

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CHAPTER 3. AN ETHOGRAM ANALYSIS OF FORMOSAN SUBTERRANEAN TERMITES, *COPTOTERMES FORMOSANUS* SHIRAKI (ISOPTERA: RHINOTERMITIDAE) TREATED WITH A SUBLETHAL DOSE OF SPINOSAD

3.1 Introduction

Formosan subterranean termites, *C. formosanus*, mostly live in soil and expand their foraging area to man made structures. To control or keep them away from structures, liquid termiticides is most often applied (Gahlhoff and Koehler 2001, Anonymous 2008). Meanwhile, when social insects get danger signals, they often will not forage past the signal and thus can avoid injury or death (Leadbeater and Chittka 2009). For their effective control, non repellent and slow acting termiticides should be used so that the toxin can be carried by the foragers back to their colony where it can affect healthy nestmates (Su et al. 1982). Slow acting and non repellent termiticides like indoxacarb (Hu et al. 2005; Quarcoo et al. 2010), fipronil (Ibrahim et al. 2003) and imidacloprid (Shelton and Grace 2003) allow enough time for interactions between the intoxicated and healthy termites to cause colony mortality.

The results of my 2nd chapter support that 1, 25 and 50ppm of Entrust, Tracer and Radiant have termiticidal effects and are non repellent. However, the potentiality of spinosad for horizontal transfer is yet to be determined. The behavioral study of termites by Henderson (2003) on slow acting and non repellent termiticides fipronil (Termidor) and imidacloprid (Premise) showed that imidacloprid caused an almost immediate behavioral change, and it was concluded that the chance of horizontal transfer of fipronil was higher than imidacloprid.

Application of entomopathogenic fungus has also been proposed (Culliney and Grace 2000; Sun et al. 2002; Rath 2000; Verma et al. 2009) but commercially not available due to difficulties with repellency and sanitation maintenance in a termites colony (Chouvenc and Su 2010) that minimizes the chance of colony mortality (Grace 2003). Termite resistivity to

entomopathogenic fungus is also due to social behaviors like grooming and burying of the infested nestmates (Yanagawa and Shimizu 2005; Boucias et al. 1996; Shimizu and Yamaji 2003).

The chemicals that cause immediate mortality or immediate behavioral change might not be effective for colony mortality because that could impact negatively on foragers and their foraging behavior (Su et. al 1982). Therefore, it is essential to include a behavioral study of termites while measuring the efficacy of a termiticide. The interaction of termiticides and termites can help to determine the horizontal transfer capacity of a product. An ethogram is an analysis of behaviors over time. It can show how termites budget their time in different behaviors in different treatments. The purpose of this study was to observe how termite behaviors change depending on spinosad treatment.

3.2 Materials and Methods

3.2.1 Termites

Two different colonies of termites were collected from Brechtel Park, New Orleans, LA by using the structured crate technique as described in Gautam and Henderson (2011). Termites were maintained in the laboratory for about three months in high humidity with moist pine wood before the experiment.

3.2.2 Chemical

Termites behaviors were observed in 1ppm of Entrust® (a.i. 80%) and compared with untreated controls. Entrust contains spinosad as an active ingredient. For this experiment, Entrust was provided by Dr. Davis in the Soybean Entomology Lab, LSU.

3.2.3 Bioassay

The experiment was conducted in Petri dishes (Falcon® 60X15mm, Becton Dickinson Labware, Franklin Lakes, NJ) that contained filter paper (Whatman™ 4.25 cm diameter) and sand

(Louisiana Cement Products, LLC Baton Rouge, LA) (Figure 3.01). Both sand and filter paper were treated with Entrust in wt/wt basis to make 1ppm. For control units, distilled water, filter paper and sand was used. Saturated filter paper and 2g sand was placed in each Petri dish. Twenty termites were released in a Petri dish out of which 2 worker termites were marked with 2 different colored oil based paints (Zig® Painty® Kuretake Co. Ltd., Japan) on their dorsal surface as described in Nagendra et al. (2010) (Figure 3.02). There were six replications for treatment and control, three from each colony, for a total of 12 experimental units. A preliminary study was done to catalogue the behaviors analyzed. Behaviors were mutually exclusive and were catalogued as:

- Walking: Movement (directed or random)
- Antennal touching : Touching other termites with antennae or being touched by the antennae of other termites
- Grooming: Cleaning of nestmates or being cleaned by nestmates (mutual grooming) or cleaning itself (self-grooming)
- Gnawing : Scraping filter paper with mandibles
- Shaking : Vibration of body
- Digging : Digging into sand
- Particle movement : Grabbing and transferring sand particles or tiny units of filter paper
- Moving: Movement (other than walking) of body parts when termite is either at rest or laying on its dorsal surface with moving appendages

In this experiment, behaviors of each marked termite were recorded for each second for 120 seconds for a total of 48 minutes per day from day 1 to 7 days after treatment.



Figure 3.01. An experimental unit containing two marked termites (in circle) on Entrust 1ppm treated sand and filter paper for ethogram.



Figure 3.02. Worker termite marked with blue oil-based paint on its dorsum of abdomen.

3.2.4 Statistical Analysis

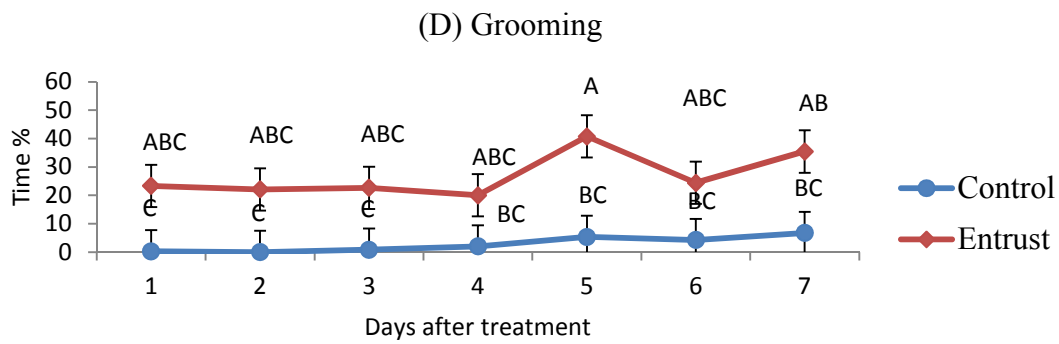
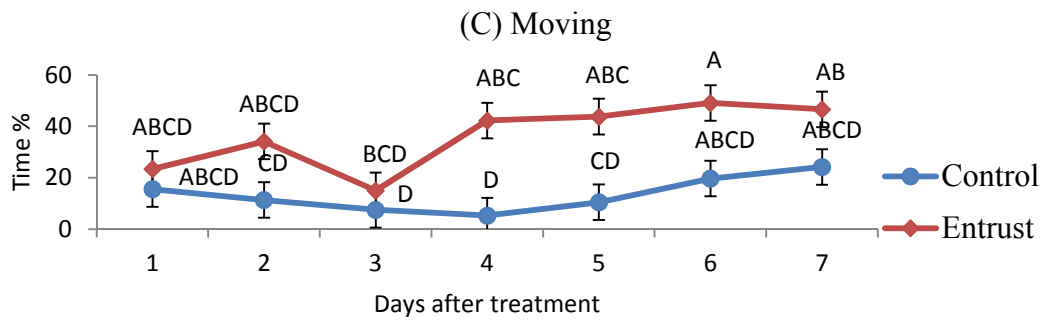
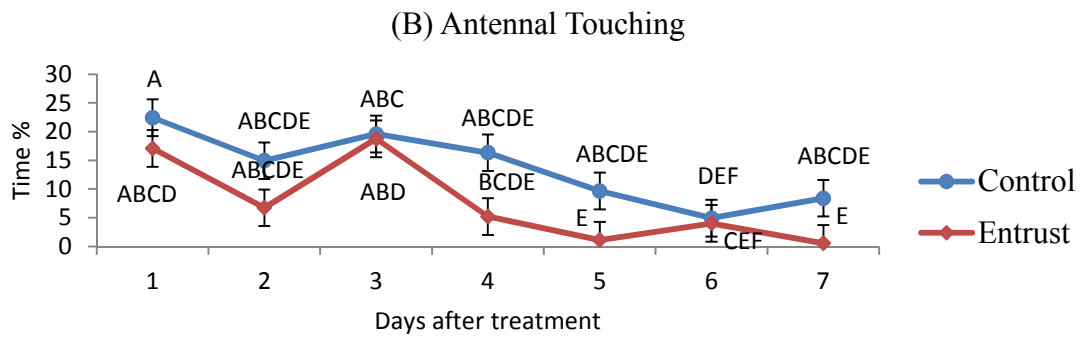
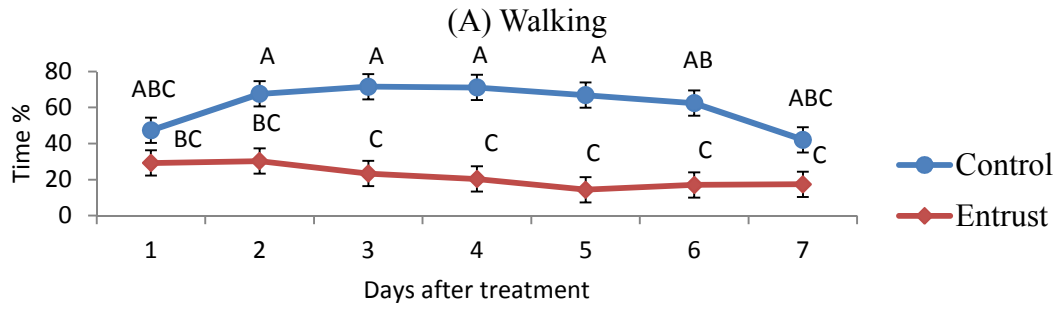
Colonies were considered as a random factor. Behaviors were analyzed in SAS 9.4 by using Proc Mixed repeated ANOVA (SAS Institute, Cary, NC, 2013). Means were compared by Tukey's HSD at $\alpha = 0.05$.

3.3 Results

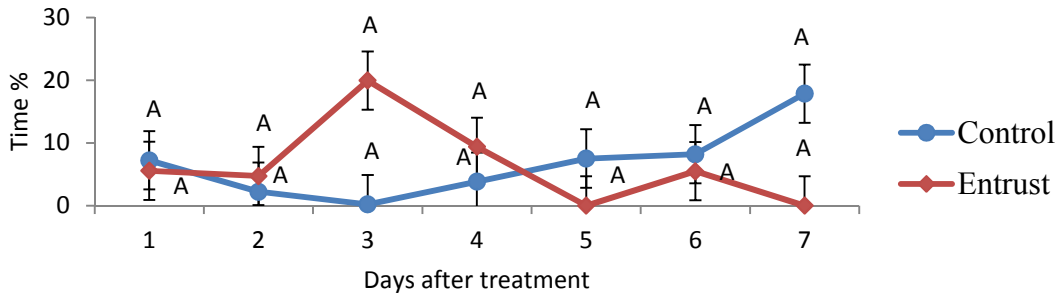
Termites in the controls budgeted the highest percent of their time in walking over the 7 days. From 2-6 days after exposure, the allocation of time in walking was significantly higher in controls than treated dishes (Figure 3.03, A). Chemical ($F_{1,2} = 47.19$; $P \leq 0.0205$), days ($F_{6,60} = 0.0475$; $P \leq 0.0475$) and chemical X days ($F_{6,60} = 2.39$; $P \leq 0.0392$) was significantly different for walking between controls and treated dishes. Likewise, for antennal touching, termites in controls budgeted more time than those in spinosad; however, a significant difference was not found (Figure 3.03, B). There was a decreasing trend of walking and antennal touching in both treatments over time. As the behaviors are mutually exclusive, the increase in one behavior would necessarily affect the other behaviors. After the onset of mortality from day 3 (Figure 3.04), most of the termites in spinosad treatment were rarely walking and remained in one place with their antennae moving. This resulted in the increase in moving behavior from 15% at day 3 to 46% at day 7 (Figure 3.03, C). After 5 days, most of the treated termites laid on their dorsum with occasional movements of appendages. Similarly, for grooming, spinosad treated termites spent more time at this than did controls (Figure 3.03, D). However, termites in both treatments showed an increasing trend for movement and grooming over time.

Gnawing, shaking, digging, and sand deposition behaviors between controls and treated termites were not significantly; however, the trend of those behaviors decreased in treated termites over time (Figure 3.03, E-H).

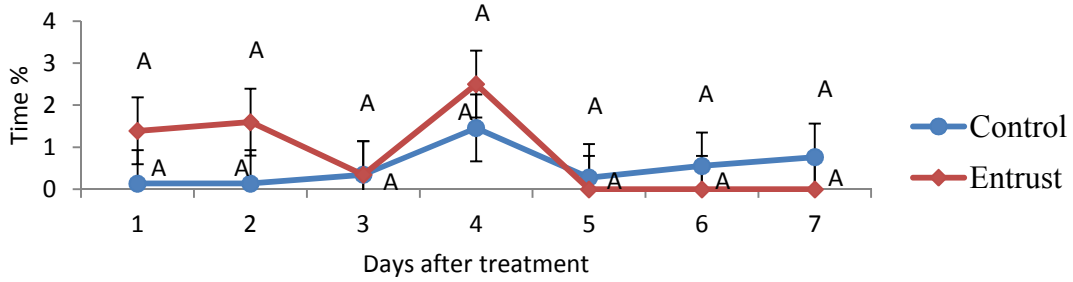
Figure 3.03. Mean percentage (\pm SE) of time budgeted by control and treated termites in: (A) Walking, (B) Antennal touching, (C) Moving, (D) Grooming, (E) Gnawing, (F) Shaking, (G) Digging, and (H) Sand depositing. Different letters indicate significant differences ($P < 0.05$).



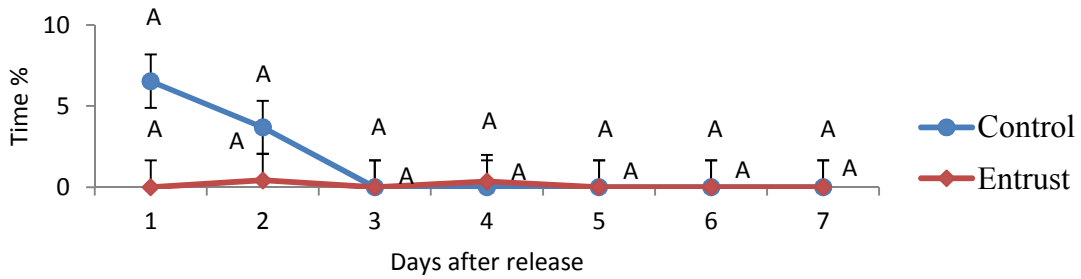
(E) Gnawing



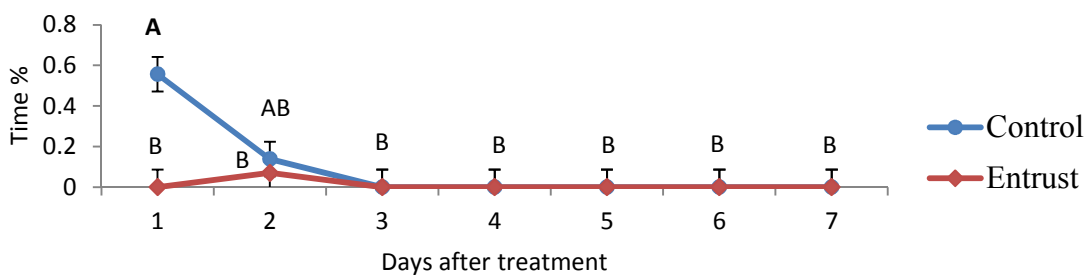
(F) Shaking



(G) Digging



(H) Sand Depositing



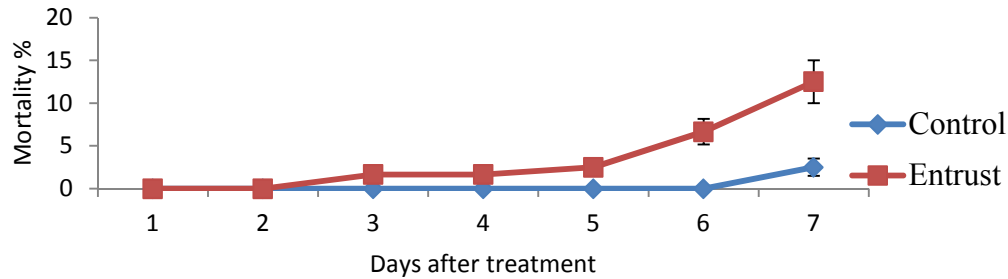


Figure 3.04. Mean percent mortality of control and Entrust 1ppm treated termites over 7 days.

3.4 Discussion

Our study showed that walking was less frequent in treated termites than in controls as the exposure time increased. Although the treated termites were affected by the toxin, immediate immobility was not observed. For colony mortality, 24h is enough for intoxicated termites to walk back to their colony and interact with healthy termites (Neoh et al. 2012). In harvester ant colonies, before the foragers leave for foraging, the patrollers explore the foraging areas (Gordon 1991). The rate of patrollers coming back into the colony determines the level and range of foraging (Greene and Gordon 2007). In termites, if there is mass mortality in a treated area, healthy termites modify their foraging away from the corpses (Su et al. 1982). Meanwhile, if the intoxicated foragers could get back to their colony, they would recruit healthy termites into the treated areas.

Antennal touching is an essential behavior that not only induces grooming but also helps in recognition of nestmates (Dhanarajan 1980; Yanagawa et al. 2009). The higher amount of antennal touching in controls might be due to the termites effort to maintain sanitation.

The difference in budgeted time in moving behavior between treated and control termites at 1 day was very low which allow treated termites to walk or carry out other social behaviors that could help in horizontal transfer of toxin. However, after the onset of mortality at 3 days in treated dishes, moving behavior was significantly higher. Thus, the increase might be due to the

effect of the toxin. Similar to the results of a behavioral study on subterranean termites with a sublethal dose of imidacloprid (Thorne and Breisch 2001) and indoxacarb (Quarcoo et al. 2010), we also recorded that intoxicated termites were laying on their dorsum with occasional moving of appendages after a longer exposure of 5 days, ultimately increasing the moving behavior.

The study of Thorne and Breisch (2001) noted that sublethal dose of imidacloprid affects mobility and reduces walking, grooming and tunneling behavior. In our study, a sublethal dose of Entrust reduced walking, digging and sand depositing behavior, however the trend of grooming behavior increased. Grooming behavior can be induced by dust particles (El-Awami and Dent 1995) or chemical irritation (Reingold and Camhi 1977). Mutual grooming is common in social insects to maintain the hygiene of nestmates (Hefetz et al. 2001; Hughes et al. 2002; Fussnecker et al. 2006). During grooming, termites use their glossae first to remove the foreign material present on the body of nestmates which is often consumed (Yanagawa et al. 2009). Grooming could lead to the possibility that the uncontaminated termites will acquire a toxin either by trophallaxis or by body contact with contaminated nestmates (Neoh et al. 2012; Neoh et al. 2013).

Gnawing behavior is induced by the labial gland secretion on the food sources which results in aggregated gnawing for the exploitation of food by foragers (Reinhard and Kaib 1995). The decreasing trend of gnawing behavior in treated termites might be due to less secretion of labial gland compared to the control which has increasing trend. However, our study cannot adequately address the effect of a sublethal dose of spinosad on labial gland secretion. Termites showing less gnawing behavior are likely to be more starved, resulting in a greater need to be cared and fed from untreated nestmates which could be the possible way for toxicant transfer (Valles and Woodson 2002; Hu et al. 2005; Huang et al. 2006).

Termites shake or vibrate their body as a signal for danger after getting direct contact with fungal spores (Rosengaus et al. 1999). It is generally considered an alarm signal (Stuart 1963). The decreasing trend of shaking behavior in treated termites might be due to the spinosad. Suppression of alarm behavior could increase the interaction of treated and untreated termites in the field but moribundity may counteract this effect.

Behavioral studies are important to determine the efficacy of termiticides as intoxicated termites show social behavior like grooming, care giving and body contact in the pre-moribund phase (Quarcoo et al. 2010). Hence, our behavioral study reflects the potentiality of spinosad as an effective termiticide that could be transferred horizontally from intoxicated to healthy nestmates for colony mortality.

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CHAPTER 4. HORIZONTAL TRANSFER OF SPINOSAD IN *COPTOTERMES FORMOSANUS* (ISOPTERA: RHINOTERMITIDAE)

4.1 Introduction

Before 1990, fast acting and repellent termiticides with long term persistence were used for soil treatment around structures that would create a chemical barrier against termite penetration (Su and Scheffrahn 1990). However, a mature colony of Formosan subterranean termites, *C. formosanus*, may have 10^6 individuals and a foraging territory of 100 meters (Tamashiro et al. 1980). Due to the large number of individuals and extended foraging areas, mortality of a small unit at the treated site would not be enough to protect the structures because termites can cause damage through untreated or inadequately treated soil (Su and Scheffrahn 1990). Therefore, after 1990, non repellent and slow acting termiticides were developed with delayed effects on termite mortality that allowed the interaction of intoxicated foragers with healthy foragers (Mullins 1993; Potter and Hillery 2002). Termites are social insects and the social behaviors like grooming and trophallaxis can be exploited to transfer the chemicals from treated nestmates to untreated nestmates. Slow acting and non repellent termiticides that is transferred from treated termites to untreated termites is known as horizontal transfer can cause mortality of the whole colony. The repellent pyrethroids and non repellent but fast acting chlorpyrifos are less effective than the non repellent and slow acting termiticides (Kard 2001; 2003; Osbrink et al. 2001; Thorne and Breisch 2001). The major non repellent and slow acting termiticides used today are imidacloprid (Premise), fipronil (Termidor), indoxacarb (Aperion) and chlorantraniliprole (Altriset) (Potter and Hillary 2002; Wagner et al. 2002; Ibrahim et al. 2003; Remmen and Su 2005; Mao et al. 2011). According to data of 2009, among the different measures of termites

control, liquid termiticides for soil treatment are covering about 80% of the total termite control market (Rust and Su 2012).

The efforts for colony mortality can be inadequate without the sufficient knowledge of chemically induced behavioral changes in termites, toxicity, repellency and the bioavailability of active ingredients. From the results of our 2nd and 3rd chapter we have enough information that spinosad is non repellent and effective against termites. Moreover, it does not cause any abnormal or immediate behavioral changes, but as the exposure period increases, termites show some changes in major behaviors like walking and grooming. The main objective of this study was to examine the horizontal transfer of spinosad from treated to untreated workers of *C. formosanus*.

4.2 Materials and Methods

4.2.1 Termites

For this experiment, two different colonies were collected from Brechtel Park, New Orleans, LA, USA, by using a milk crate technique as described in Gautam and Henderson (2011a). Collected termites were maintained in the lab in Roughneck trash cans (121 liters, Rubbermaid, Huntersville, NC) with high relative humidity before the onset of the experiment.

4.2.2 Termiticides

A DowAgroSciences product, Entrust® (a.i. 80%) was provided by the soybean entomology lab at LSU. Three concentrations 1ppm, 25ppm and 50 ppm of Entrust were tested in this study.

4.2.3 Recipient Termites

The weight of the filter papers (Whatman, 5.5-cm diameter) and Nile blue A (Sigma-Aldrich Co. LLC., St. Louis, MO) was measured, and Nile blue A 0.1% (1mg Nile blue A

/10000 mg of filter paper) was mixed with warm water and the solution was used to dye each filter paper. Nile blue A does not have any effect in termites mortality (Su et al. 1991). The dyed filter papers were dried under a hood for 1 day and were moistened with distilled water until saturation and placed individually in each Petri dish (Fisherbrand, 100mmX15mm, Thermo Fisher, Walthman, MA). About 500 termites of each colony were released into two Petri dishes to feed them the dyed filter papers (Figure 4.01, A). After 4 days, most termites became blue in color. Thence, the blue colored termites were referred as recipient termites.

4.2.4 Substrate

This experiment was conducted in sand and soil. Sand was purchased from Louisiana Cement Products, Baton Rouge, LA and soil was collected from pesticide free area at Benhur research station, LA, USA. Then the soil was sent for texture and pH analysis to the Coastal Wetlands Soils Characterization Lab, LSU. Both sand and soil were autoclaved before the onset of experiment.

4.2.5 Donor Termites

One ppm, 25ppm and 50ppm of Entrust in sand and soil were prepared on a wt/wt basis. Thirty grams of each treated substrate was added to two Petri dishes (Fisherbrand 100mmX15mm, Thermo Fisher, Walthman, MA) for a total of 16 Petri dishes including controls. Fifty worker termites were released onto each treated and untreated (as controls) substrate for one hour (Figure 4.01, B) then termites were shifted to an empty larger Petri dish (Fisherbrand, 150mmX15mm, Thermo Fisher, Walthman, MA) for 2 minutes so that they could discard any clinging substrate particles (Figure 4.01, C). Thence, these undyed termites were called donor termites.

4.2.6 Mixing Donor and Recipient Termites

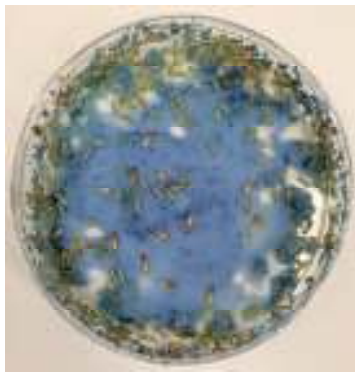
Donor and recipient termites were mixed in a Petri dish (Falcon® 60X15mm, Becton Dickinson Labware, Franklin Lakes, NJ) thence called exposure dish (Figure 4.01, D) containing 5g of untreated substrate (sand or soil). Ten donor termites and 10 recipient termites were released in each exposure dish. There were six replications for each treatment, 3 from each colony for a total of 48 exposure dishes. Mortality of donor and recipient termites was recorded after 1, 3, 7, 14 and 21 days. In addition to mortality, the interactions between donor and recipient termites were observed and recorded (Figure 4.01, E).

4.2.7 Statistical Analysis

Colonies were considered as blocks. Average percent mortality of donor and recipient termites was analyzed by PROC MIXED repeated ANOVA in SAS 9.4 (SAS Institute, Cary, NC, 2013). Means were compared at $\alpha < 0.05$ by Tukey's Honestly Significant difference.

4.3 Results

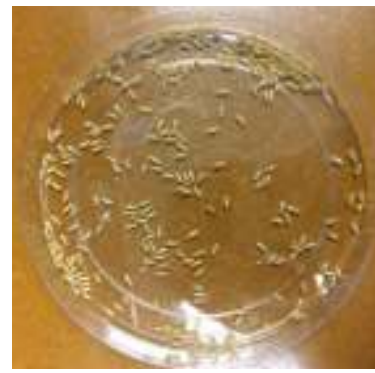
The potential of horizontal transfer indicated by the combined mortality of donor and recipient termites in soil and sand was dependent on concentration ($F_{7, 40} = 5.53$; $P \leq 0.0002$), day ($F_{4, 160} = 68.63$; $P \leq 0.0001$), and concentration X day ($F_{28, 160} = 4.13$; $P \leq 0.0001$). Donor and recipient termites began to contact and groom each other immediately after releasing them together. At 1 and 3 days exposure no significant mortality was observed in either sand or soil (data not shown in graph). The role concentration and different substrates played in toxin transfer was observed after 7 days when 50ppm in sand caused significantly higher mortality compared to control sand, 1ppm sand and all treatments in soil including 50ppm (Figure 4.02). Moreover,



(A) Termites fed 0.1% Nile blue A treated filter papers for 4 days



(D) Donor + Recipient at the ratio of 1:1



(C) Termites transferred to a clean Petri dish for 2 minutes



(B) Termites exposed for 1 hour in control or 1ppm or 25ppm or 50ppm sand or soil



(E) Interaction between donor and recipient termites after mixing them

Figure 4.01. Transfer bioassay: (A) recipient termites, (B-C) donor termites, (D-E) exposure of donor termites with recipient termites.

25ppm sand also caused higher mortality than control and 1ppm sand at 7 days. At 14 days, 25 and 50ppm sand caused higher mortality than other treatments including 1ppm sand, and 25 and 50ppm soil. Similar to 14 days, high mortality was recorded in 25 and 50ppm sand at 21 days. In soil at 21 days, unlike 7 days and 14 days, all three concentrations caused significantly higher mortality than control.

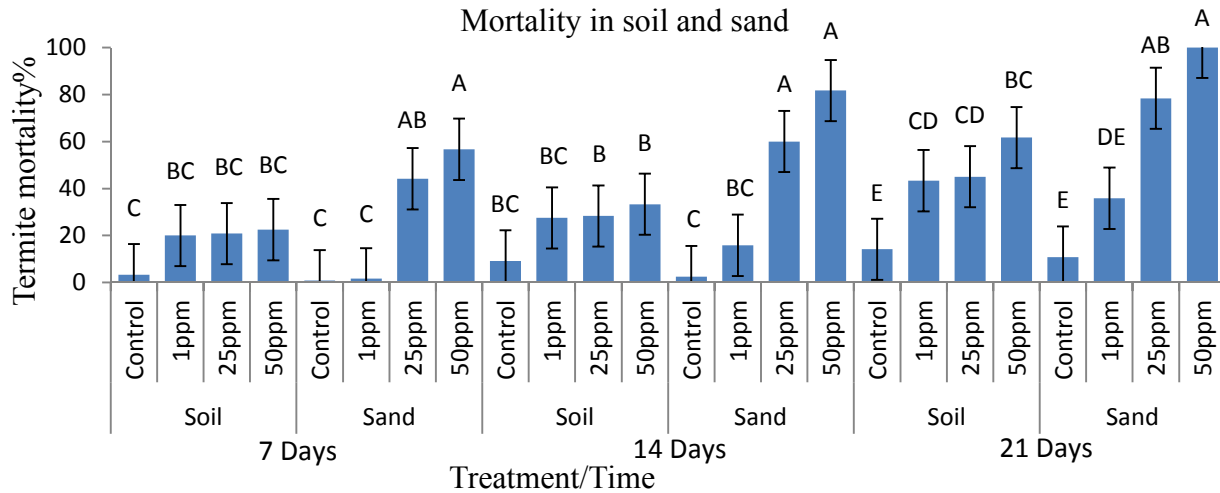


Figure 4.02. Combined mean percent mortality (\pm SEM) of donor and recipient termites in soil and sand. Different letters within each time frame indicate significant differences ($P < 0.05$).

In 1ppm sand at 14 and 21 days, donor termites caused significantly higher recipient termite mortality compared to control (Figure 4.03, A). At day 7, 25 and 50ppm sand caused higher donor and recipient mortality compared to control (Figures 4.03, B-C). More than 50% of the termites exposed to 25 and 50ppm sand were dead at day 7 which was followed by more than 50% recipient mortality at the next observation. Moreover, 50ppm in sand, after 21 days, caused 100% mortality of both donor and recipient termites.

In treated soil, mortality of donor and recipient termites was not significantly different from the controls until day 21. due to 1, 25, and 50ppm exposure with control donors and recipients, significant difference until 14 days after treatment was not observed (Figure 4.04, A,

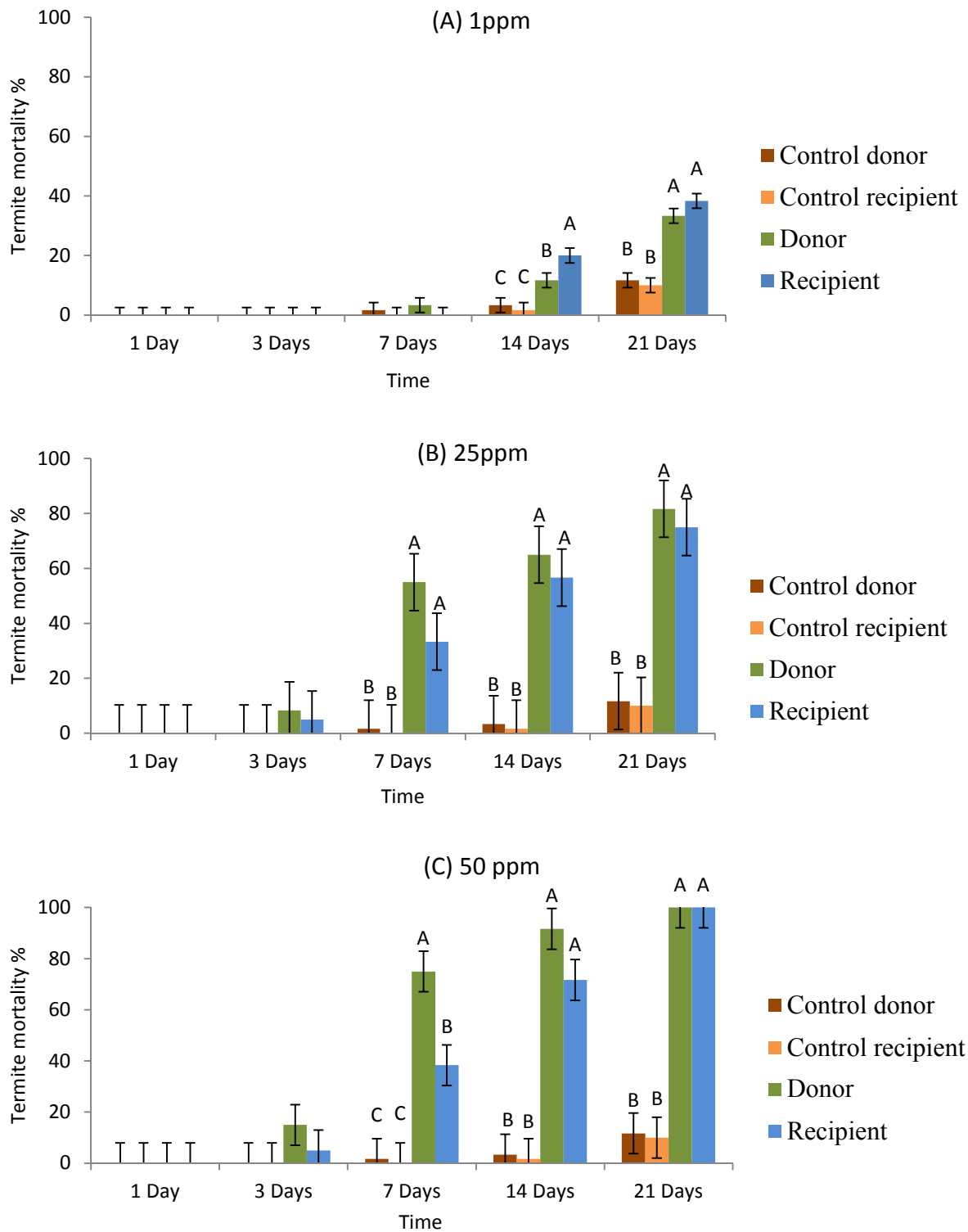


Figure 4.03. Mean percent donor and recipient mortality (\pm SEM) in sand caused by termites exposed to control and (A) 1ppm, (B) 25ppm, and (C) 50ppm. Different letters within each time frame indicate significant differences ($P < 0.05$).

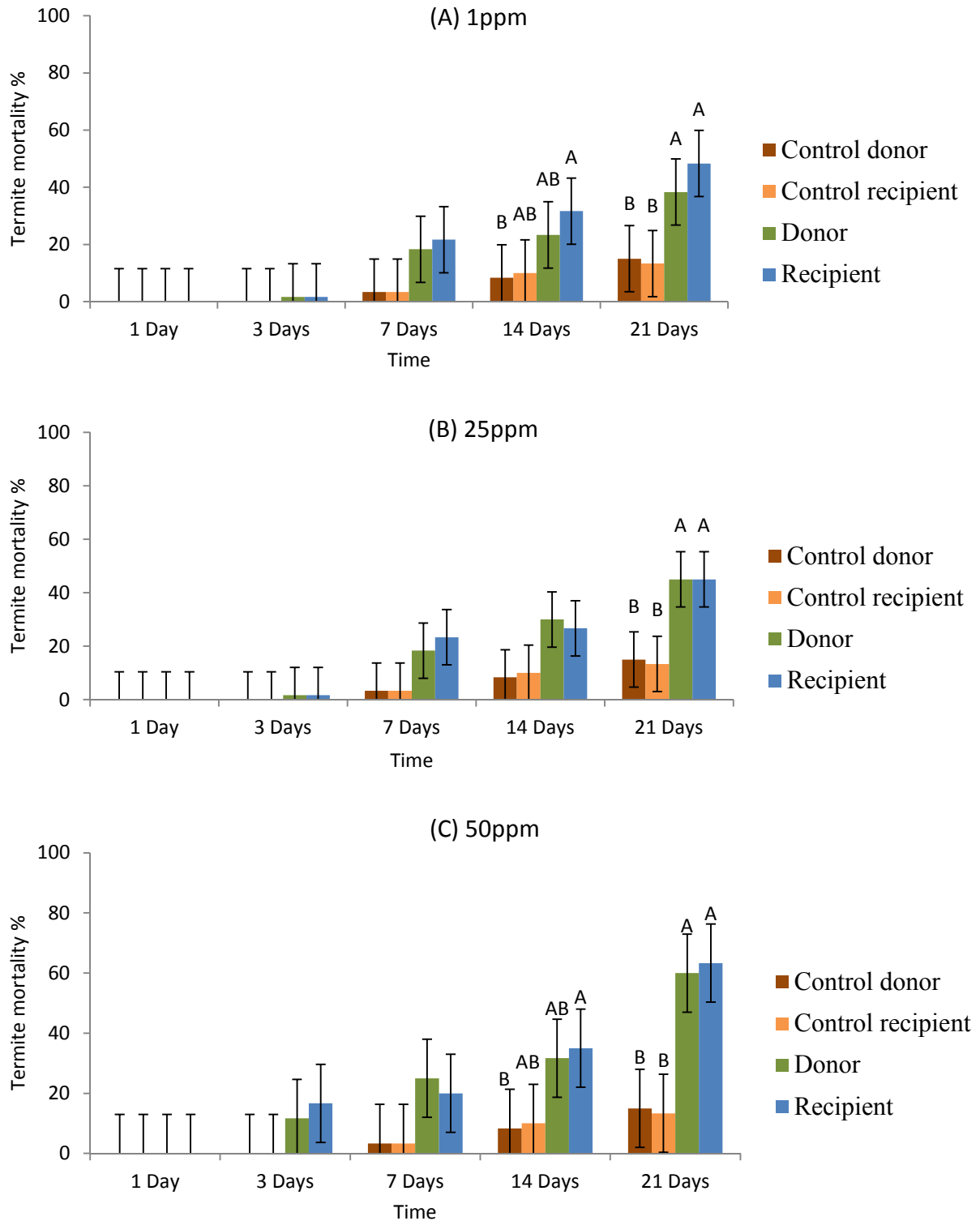


Figure 4.04. Mean percent donor and recipient mortality (\pm SEM) in soil caused by termites exposed to control and (A) 1ppm, (B) 25ppm, and (C) 50ppm. Different letters within each time frame indicate significant differences ($P < 0.05$).

B, C). Meanwhile, 50ppm caused more than 50% mortality of both donor and recipient termites at 21 days.

4.4 Discussion

Our study showed that transfer of spinosad by donor to untreated recipient termites depended on the type of substrate, concentration of the product and donor-recipient post introduction duration. The results of our 2nd chapter revealed that the toxicity of spinosad is higher in sand than in soil. In addition to higher toxicity in sand in the no choice tests, this study demonstrates that termites exposed to treated sand caused faster horizontal transfer than the termites exposed to soil. Termites exposed to 50ppm soil even after 21 days caused <65% overall mortality which was lower than the 100% mortality in sand. Bioavailability of chemicals depends on the organic matter content and physical properties of the substrate (Gold et al. 1996; Spomer and Kamble 2011). Insecticide toxicity and the organic quality of substrate are inversely related (Harris 1966). Higher organic matter reduces the bioavailability and hence retards the horizontal transfer of toxin (Gautam and Henderson 2011b). Horizontal transfer of fipronil and chlorantraniliprole in sand and soil is well documented in the study of Gautam and Henderson (2011b) and Neoh et al. (2012). They reported that termites in sand become intoxicated comparatively earlier than in soil with higher mortality of both donors and recipient termites. Likewise, in our study, termites exposed to spinosad treated sand had higher mortality than termites exposed to treated soil. The organic matter content of soil that we used in our test was 5.95% which was higher than of sand.

Intensity of the toxin transferred from donor termites to recipient termites also depends upon the concentration of termiticides (Rust and Saran 2006; Shelton et al. 2006). The concentration must be effective enough to kill both donor and recipient termites. In sand until 14

days, the combined donor and recipient mortality caused by 1ppm was not significantly different from the control. However, 25 and 50ppm caused significantly higher mortality after 7 days in sand. Higher concentrations cause comparatively early and higher mortality of donor and recipient termites. Buczkowski et al. (2012) reported that termites exposed to chlorantraniliprole 25 and 50ppm caused significantly higher mortality than 5ppm after 21 days in sand. Nonetheless, quicker mortality or abnormal behavioral changes of donor termites can repel the recipient termites (Su et al. 1982; Fei and Henderson 2005). Thus, the concentration that causes quicker mortality and behavioral changes can hinder the transfer of toxin from treated to untreated termites.

Donor and recipient ratio also plays a significant role for the effective transference of toxin to a termite population. The study of Ibrahim et al. (2003) demonstrated that for the maximum horizontal transmission of topically applied 2.5 ng fipronil, at least 40% of the termite population should be treated foragers. Similarly, Saran and Rust (2007) showed that 50% of termite individuals should have acquired toxin from treated areas before colony mortality can occur. In addition to the role of ratio of donor and recipient termites for toxin transfer and mortality, number of donor and recipient termites is also important for high mortality. In a study of fipronil against *C. formosanus*, Wang et al. (2013) reported that 2% (n=10) donor termites in 50 termites caused significantly higher mortality than 2% (n=4) donor termites in 20 termites.

Post exposure time, the time after mixing donor and recipient termites, also plays an important role for chemical transfer. In a study of spinosad transfer, Ferster et al. (2001) reported that, mortality of drywood termites, *Incisitermes snyderi*, increased as the exposure duration of donors with recipient termites increased. The interaction opportunities among donor and recipient termites can be increased with increased post exposure time. Moreover, the chances of

lethal toxin transfer or acquire can be high when social interaction high. The study of Gautam and Henderson (2011b) showed that slow acting and non repellent termiticide, chlorantraniliprole at 50ppm in sand caused 100% mortality after 21 days. Similarly, in our study, 50ppm spinosad in sand caused 100% mortality with the minimum post exposure time of 21 days.

The possible way for toxin transfer from donor to recipient termites can be grooming, direct body contact, trophallaxis and cannibalism of intoxicated termites (Myles 1996; Shelton and Grace 2003; Tomalshi and Vargo 2004; Haagsma and Rust 2005). Our ethogram analysis (chapter 3) showed that the trend for grooming among intoxicated termites steadily increased. In this study, we observed that the both donor and recipient termites were grooming each other. Termites that used their mouth parts for feeding or grabbing the treated substrate might transfer the toxin while grooming the unexposed nest mates (Tomalshi and Vargo 2004). Moreover, treated termites that acquire the toxin on their body surface can cause recipient termites to be intoxicated by grooming the treated termites. Ferster et al. (2001) reported that untreated termites got a lethal dose from several toxicants due to contact with either treated termites or carcasses of treated termites or both. The lethal dose transfer to recipients might not have occurred at one time; thus, the mechanism of transfer could have been repeated over time for the same individual (Hu et al. 2005)

Spinosad is effectively carried by the donor termites and a lethal dose is transferred to the recipient termites. This laboratory study suggests that spinosad can be used for colony mortality of Formosan subterranean termites. However, further investigations on donor recipient ratio, soldier and worker proportion, and exposure duration of donor termites in treated substrate are needed since they affect the rate of transfer under field conditions.

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CHAPTER 5. SUMMARY AND CONCLUSION

Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is an economically important structural pest that is distributed in humid tropical and subtropical climates throughout the world. In the United States, they are causing huge economic losses mainly in southern temperate regions. The range of damage extends from private properties having aesthetic and utility value like houses, trees, underground telephone cables, railway sleepers, boats and ships. Among many techniques and measures that have been developed to control them, liquid insecticides have predominated for the last six decades. However, the consequences of chemical application compromise human health and environmental safety. In mind, we tested the effect of spinosad against *C. formosanus*. Spinosad is a biopesticide derived from naturally occurring soil bacteria, *Saccharopolyspora spinosa*. The Environment Protection Agency has classified spinosad as a reduced risk compound.

In the first part of the study, we tested the toxicity of spinosyns against *C. formosanus*. Three commercially available spinosyn products Entrust®, Tracer® and Radiant® at 1, 25 and 50ppm were tested in sand, soil and filter paper for 14 days. We found that all the products were toxic against *C. formosanus*. Besides mortality, spinosyns also caused lower consumption and reduced tunnel area over a period of 14 days. After confirming that spinosyns were toxic to *C. formosanus*, we conducted two-choice and multiple-choice tests in sand with the same products and concentrations. From both tests we could confirm that spinosad and spinetoram were non repellent and toxic to termites.

Toxicity and non repellency are not the only features of a termiticide to cause colony mortality. Abnormal behaviors in intoxicated termites can disrupt the transfer of chemical from poisoned to healthy nestmates. That's why in the 2nd part of our study we conducted an ethogram

analysis to observe the behavioral differences between control and spinosad treated termites for 7 days. Termites were marked on the dorsum of their abdomens and released in untreated and Entrust 1ppm treated sand and filter paper. During our test, termites spent their maximum amount of time in four behaviors namely walking, antennal touching, moving and grooming. Walking and antennal touching behaviors were higher in untreated than treated termites over 7 days. Contrastingly, grooming and moving behaviors were higher in treated termites. Due to the absence of immediate behavioral changes in treated termites, the healthy termites could interact with the intoxicated termites and acquire the toxin through grooming, contact or trophallaxis.

In the last part of the study, we tested the horizontal transfer of spinosad from treated to untreated termites. Termites were exposed for 1 hour in Entrust 1, 25 and 50ppm and then mixed with the healthy untreated termites for 21 days. Mortality of both intoxicated and healthy termites revealed that spinosad was acquired by healthy termites from the treated termites. Meanwhile, treated sand was more effective than soil for horizontal transfer of spinosad.

Results of our laboratory study can have implications for the field. Similar to the characteristics of most chemical termiticides our study revealed that spinosad is toxic, slow acting, non repellent and horizontally transferrable and can result in whole colony mortality of *C. formosanus*.

THE VITA

Dependra Bhatta was born and raised in Silgadhi, Doti, Nepal. He received his bachelor's degree in agriculture with a major in agricultural economics in 2011 from the Institute of Agriculture and Animal Sciences, Tribhuvan University, Nepal. After getting his bachelor's degree, he was employed as an agriculture instructor for diploma level students in Far West, Nepal. In the fall of 2013, he started his master's degree in entomology at Louisiana State University (LSU), Baton Rouge, Louisiana under the supervision of Dr. Gregg Henderson. His research was focused on the effects of sublethal and lethal doses of spinosad on the behaviors and survival of Formosan subterranean termites.