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Survivorship, tunneling and feeding behaviors of Formosan subterranean termite (Isoptera: Rhinotermitidae) in response to trans-caryophyllene

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SURVIVORSHIP, TUNNELING AND FEEDING BEHAVIORS OF FORMOSAN SUBTERRANEAN TERMITE (ISOPTERA: RHINOTERMITIDAE) IN RESPONSE TO TRANS-CARYOPHYLLENE

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ABSTRACT

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, an invasive species from southern China, is one of the most destructive wood pests in the United States. Presently, termites are managed through chemical control. However, some synthetic chemicals cause serious environmental problems due to their persistence and toxicity. There is growing interest in using natural compounds with low toxicity to mammals to control termites.

Trans-caryophyllene is a component of the essential oils from many flowering plants. It is especially abundant in clove oil. It has also been found to be released from the roots of maize damaged by insects. I investigated caryophyllene as a potential control agent for Formosan subterranean termites. I evaluated caryophyllene topical toxicity, consumption of caryophyllene treated filter paper in choice and no choice tests, and effect of caryophyllene on Formosan termite tunneling behavior. Caryophyllene was moderately toxic to Formosan subterranean termites (LD_{50} =40.19 ug/insect; 95% CL 28.36-53.74 ug/insect). In no choice consumption tests, termite feeding was negatively correlated with dose of caryophyllene. In multiple choice bioassays, greater consumption occurred on untreated filter paper, suggesting caryophyllene is not a feeding stimulant, but may in fact act as a repellent. Equal tunnel volumes were recorded in chambers with or without caryophyllene but location of tunneling suggested an effect on tunnel orientation behavior and attraction to caryophyllene. However, tunnel volume increased significantly toward the center of areas where caryophyllene droplets were placed, but in untreated chambers, tunnel volume increased mainly along the edges of the chamber. These results indicate that while caryophyllene shows moderate acute

toxicity to Formosan subterranean termites and paper treated with this sesquiterpene is a feeding deterrent, termites will tunnel toward caryophyllene droplets. Further studies are needed to evaluate caryophyllene's effectiveness when used against termites.

CHAPTER 1

INTRODUCTION

Termites play important roles in ecological systems. There are more than 2300 termite species in the world (Edwards and Mill 1986). One hundred and forty seven of these species belong to the group of subterranean termites and about 28 species are of economic importance (Su and Schefferahn 1998). The genus *Coptotermes* includes the two most destructive species *Coptotermes formosanus* Shiraki and *Coptotermes havilandi* Holmgren (Edwards and Mill 1986). The destructive nature of *C. formosanus* makes it imperative that we discover a method to limit its negative influence. *C. formosanus* is native to southern China (Su and Tamashiro 1987) and it has been found in 14 southern provinces in China (Gao *et al.* 1982; He & Chen 1981; Lin 1986). Presently *C. formosanus* is found in Japan, Sri Lanka, the Phillipines, Guam, Hawaii, South Africa and the United States. *C. formosanus* is believed to have been introduced into the mainland of the U.S. through infested material from Asia after World War II (Su and Tamashiro 1987). *C. formosanus* was probably introduced to Hawaii in the late 1800s (Su and Tamishiro 1987), but expanded its distribution rapidly after its introduction (Su 2003). Formosan subterranean termites have been reported in Alabama, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee and Texas (Atkinson *et al*. 1993; Haagsma *et al*. 1995).

Formosan subterranean termites are one of the most aggressive and destructive structural pests in the United States. It is estimated that the more than $1.5 billion was used for control of this termite based on the sales of pesticides in the U.S. (Su 1994). However, the damage caused by Formosan subterranean termites is hard to estimate due to the lack of knowledge concerning their tunneling habits (Su and Scheffrahn 1998).

Termite management strategies can be divided into several groups. The first group falls under the heading of barrier treatment which includes soil termiticide barriers and physical barriers (Su and Scheffrahn 1998). Insecticides currently used in pest control as soil termiticide barriers belong to the pyrethroid and nicotinoid classes of insecticdes. Stainless steel screens are used as physical barriers to block the access of termites (Su and Scheffrahn 1998).

Soil barrier treatments and wood treatments are currently the dominant strategies employed to control Formosan subterranean termites. Population size and habit make it more difficult to apply termiticides directly to Formosan subterranean termites than to drywood termites. Therefore, toxic bait is a more practical control for *C. formosanus*. Bait systems commonly involve slow acting, non-repellent chemicals. As new pesticides are developed, new toxicants are used in bait systems, such as the chitin synthesis inhibitor hexaflumuron (Su and Scheffrahn 1991). Su and Scheffrahn (1996) developed the first registered termite bating system in United States with the chitin synthesis inhibitor, hexaflumuron and lufenuron in 1996. The second registered bait system employs sulfluramid as the toxicant (Myles 1996). A new strategy under investigation by Myles (1996) involves coating termites to transmit toxicants such as growth regulators, and microbial pathogens by trophallaxis and grooming.

Essential oils are also being examined as green alternatives to commonly used synthetic pesticides. Essential oils are a mixture of many chemical compounds which are extracted from odoriferous plants. Essential oils are commonly used in perfume fragrance, food enhancers, pharmaceuticals and insecticides (Zhu *et al*. 2001). *Trans*-Caryophyllene

($C_{15}H_{24}$) (caryophyllene hereafter) is a natural bicyclic sesquiterpene occurring in some essential oils from *Radix bupleuri*, *Syzygium aromaticum* (Myrtaceae family) *Seuvium portulacastrum* (Michael *et al*. 2006), *Leucas milanjiana* Guerke (Moody 2005), *Pinus canariensis*, *P. pinaster* (Hmamouchi *et al*. 2001), *Pinus pinea*, *Bulpleurum frutescescens*, and *Tithonia diversifolia* (Hemsl) (Moronkola *et al*. 2006). Concentrations of caryophyllene are especially high in clove *Syzygium aromaticum* (Martin *et al*. 1993). Clove oil has local anaesthetic activity and is often used to relieve toothache. Caryophyllene is one of the main components of clove oil (Tyler 1994). The anti-inflammatory activity of caryophyllene in *Bupleurum fruticescens* was tested on several animals (Martin *et al*. 1999).

The interaction between plants and herbivores involves volatile and other chemical compounds. Plants respond to herbivore damage by releasing chemicals (Tallamy and Raupp 1991; Baldwin 1994). Some of these released chemicals are volatile and attractive to natural enemies. Previous studies showed that corn (*Zea mays*) and cotton (*Gossypium hirsutum*) when stimulated by insect feeding damage can release certain types of chemical compounds including caryophyllene (Loughrin *et al*. 1994).

The objective of this research was to evaluate the usefulness of caryophyllene as component of a termite control program. The effect of caryophyllene on termite behavior and its toxicity was also investigated.

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CHAPTER 2

EVALUATION OF CARYOPHYLLENE TOXICITY ON *COPTOTERMES FORMOSANUS*

INTRODUCTION

Chemical control, at present, is the dominant termite management method. Most management strategies use synthetic insecticides in a soil treatment (Mao and Henderson 2007). However, some of the synthetic insecticides cause environmental problems (Coats 1994).

Caryophyllene is found in many plants, especially in clove *Syzygium aromaticum* (Knudsen *et al*. 1993; Borg-Karlson *et al*. 1994). Clove is known for its analgesic properties and can temporarily relieve a toothache (Ghelardini *et al*. 2001). A main component of clove oil, caryophyllene, has anti- inflammatory properties (Ghelardini *et al*. 2001).

Caryophyllene is a component of the essential oil extracted from *Calocedrus formosan* Florin, which is a species noted for its resistance to rotting. The essential oil from *C. formosan* leaves was examined for its antitermitic and antifungal effects (Cheng *et al.* 2004; Wang *et al.* 2006). *C. formosan* leaf extract with caryophyllene (19.3%) at a dose of 1mg/g was tested on Formosan termites and the toxicity of caryophyllene at a dose of 5mg/g after 14d was 44% (Cheng *et al* 2004). Caryophyllene was evaluated separately and also showed antitermitic properties (Wang *et al.* 2006). Caryophyllene was studied for its potential application in pest control since it was attractive to other insects, such as lacewings, boll weevil and corn root worms (Ghelardini *et al*. 2001; Hammack 2001). The objective of this study was to test the toxicity of caryophyllene on *C. formosanus*.

MATERIALS AND METHODS

Caryophyllene was purchased from Sigma Company (C9653-10ML, St. Louis, MO). The susceptibility of *C. formosanus* workers to caryophyllene was evaluated via a topical application assay using a Hamilton PB 600 Repeating Dispenser equipped with a 10ul syringe (Hamilton, Reno, NV.). Seven doses (0ul, 0.002ul, 0.02ul, 0.06ul, 0.08ul, 0.1ul, 0.2ul and 0.4ul per insect) were used in this experiment. HPLC grade pentane was added to caryophyllene to bring the total volume of the caryophyllene solution to 0.4ul. Termites used in this experiment were collected from two colonies in New Orleans, LA during February 2007 and May 2007 for the acute toxicity test. Termites were maintained in 250-liter trash cans with pine (*Pinus* spp.) at 24-26˚C in a laboratory after collection. Termite workers were held in a Petri dish (60 X15mm) which was chilled on an ice surface to slow down their movement. Caryophyllene solution was applied to the dorsum of termites and volume of caryophyllene solution was held constant at 0.4ul on each termite. After treatment, termites were kept in Petri dishes with moistened filter paper (Whatman$^{@}$ no.1; 42.5mm). The Petri dishes were sealed with Parafilm$^{@}$ (Pechiney, Plastic packaging, Menasha, WI) and placed in a 28˚C incubator in total darkness for three days. Caryophyllene was tested with five replicates for each dose and colony combination with 10 workers per replicate. The number of dead termites was recorded after 24h, 48h and 96h of the experiment. Termites were considered dead if they were not able to move.

DATA ANALYSIS

LD_{50} values and 95% confidence interval were estimated using SAS PROC PROBIT for acute toxicity data. Caryophyllene volume was converted into weight for LD_{50} units (0.902 g/ml at 20 °C). Acute toxicity was evaluated and the LD_{50} was obtained. The effect of colonies was tested by using two way analysis of variance (two way ANOVA) PROC GLM.

RESULTS

Tab2.1: LD_{50} of caryophyllene at 24h, 48h and 96h after application

	24h	48h	96h
LD_{50} (95%CL)	40.19 (28.36-53.74)	10.45 (6.54-15.01)	6.99 (4.12-10.44)

LD_{50} was measured as ug/insect

No colony effect was detected (F=1.47, P value=0.2342) therefore mortality data from different colonies were combined. Termite mortality increased rapidly with the increase of caryophyllene within the range of 1.81 ug to 362.08 ug. After 48h and 96h, most of the termites were dead except for those termites at the low doses of caryophyllene (Fig2.1).

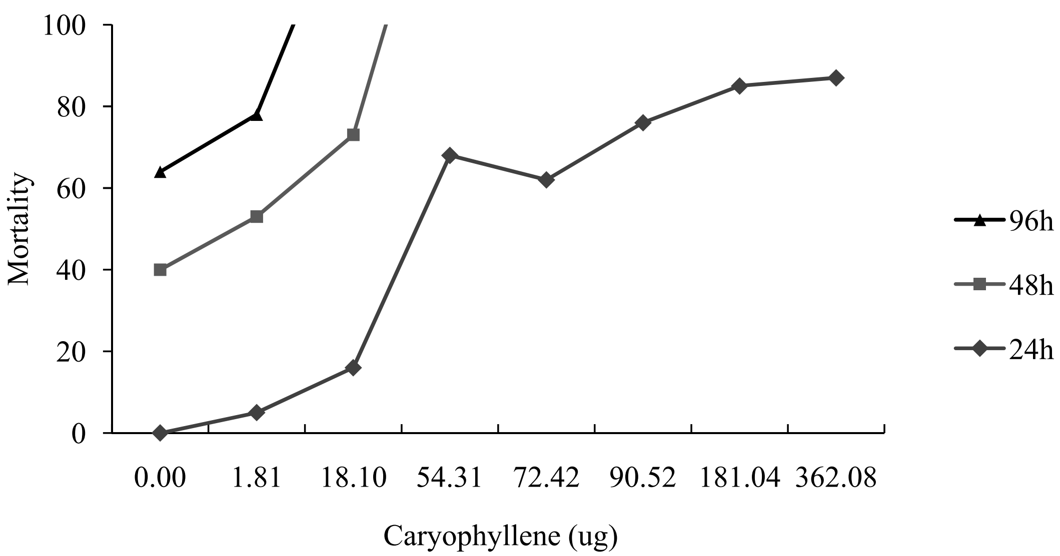

Fig2.1: Termite mortality at 24h, 48h and 96h in caryophyllene topical application

DISCUSSION

Caryophyllene was found to be moderately toxic to *C. formosanus*. It is valuable to compare the LD_{50} of caryophyllene to the LD_{50} value of nootkatone and matrine because nootkatone and matrine were studied previously for their potential application in *C.*

formosanus management. Nootkatone is an isomer of an insect-active sesquiterpene ketone in vetiver oil which is extracted from vetiver grass and considered a termite repellent (Ibrahim *et al*. 2005). Matrine is an alkaoid from Ku Shen which is an ancient Chinese herb (Mao and Henderson 2007). Previous studies showed that matrine had antifeeding activity on Formosan termites (Mao and Henderson 2007). The toxicity of nootkatone and matrine on *C. formosanus* was evaluated in our laboratory in the same way as caryophyllene was evaluated. From Table2.1 we found the LD_{50} of caryophyllene was close to nootkatone 32.8 ug/insect (with 95% confidence interval 24.4-40.5 ug/insect), but less than matrine 8.6 ug/insect (with 95% confidence interval 4.3-12.5 ug/insect).

One of the most effective termite control methods is soil treatment with termiticides and two thirds of all termite treatments involve application of termiticides to soil (Curl 2004). Some of the termiticides which were used in the past have been withdrawn from the market due to environmental concerns (Su and Scheffrahn 1998). At present, chlorphrifos, permethrin, cypermethrin, bifenthrin, fenvalerate, fipronil and imidacloprid are widely used by the pest control industry (Su and Scheffrahn 1998). Fipronil is a slow-acting termiticide; its LD_{50} for *C. formosanus* Shiraki at 72h is 1.33-1.39ng per termite (Ibrahim *et al*. 2003) and 0.16ng per worker for *Reticulitermes hesperus* Banks (Saran and Rust 2007). Caryophyllene toxicity is moderate compared to fipronil, however, caryophyllene may qualify as a termiticide in a baiting system.

Catnip is another plant extract which was evaluated for its potential application in termite control (Cornelius *et al.* 1997). The 24h LD_{50} for *Reticulitermes flavipes* is approximately 8200ug/g termite (Peterson and Wilson 2003). However, the bioassay used for catnip essential oil toxicity has not been used for previous study which makes it is impossible to compare the toxicity of catnip oil with previous research and different subterranean species was used in the catnip study.

The volatile nature of caryophyllene will also require a fumigation assay to compare with other monoterpenoids which were evaluated for their fumigation toxicity. Citral, geraniol and eugenol showed high toxicity to *C. formosanus* with LC_{50} of 2.2, 2.1 and 0.27ug/ml respectively (Cornelius *et al.* 1997).

Further study is needed to understand the horizontal transmission of caryophyllene between the colony members and the behavior change of *C. formosanus* with previous exposure to caryophyllene to a subsequent exposure. The toxicity of caryophyllene to other subterranean species needs to be examined. To reveal the possibilities of caryophyllene application as a novel biocide in termite control, short term and long term field studies are necessary.

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CHAPTER 3

EFFECT OF CARYOPHYLLENE CONSUMPTION ON *COPTOTERMES FORMOSANUS* SURVIVORSHIP

INTRODUCTION

Terpenoids, quinines, phenolics, flavonoids, lignans, lipids and alkaloids have been found in heartwood (Scheffrahn 1991). The antitermitic effects of those wood extracts were categorized into toxicity to termites either directly or to the gut symbionts. If feeding is reduced in a no choice test compared to the control it is defined as a feeding deterrent (Scheffrahn 1991).

An alternative strategy to prevent wood consumption by Formosan subterranean termites is using a soil barrier. Some natural monoterpenoid compounds (citral, geraniol, and eugenol) are potential termite bait additives which can be used to enhance the efficiency of termite bait (Cornelius *et al*. 1997). Nootkatone, a sesquiterpenoid, displayed toxic and repellent activities toward Formosan subterranean termites as well (Zhu *et al*. 2001). Catnip, which contains nepetalactone (monoterpenoid), was evaluated as a deterrent to the German cockroach *Blattella germanica* (Peterson *et al*. 2002) and two subterranean termites species, *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks) (Isoptera: Rhinotermitidae) (Peterson and Wilson 2003). Caryophyllene is a sesquiterpene and is attractive to insects at a low dose (Rasmann *et al*. 2005).

Coptotermes formosanus is more aggressive than native termite species with a higher soldier percentage and consumption rate in colonies. A feeding deterrent treatment on wood is an effective way to prevent termite damage. Chemicals with low mammalian toxicity and a high level of feeding deterrence to termites are of interest. The objective of this study was to investigate the effect of caryophyllene on termite consumption and survivorship.

MATERIALS AND METHODS

Caryophyllene was purchased from Sigma Company (C9653-10ML, St. Louis, MO). Pentane solutions with different amounts of caryophyllene were prepared and filter paper (Whatman@, radius=1.0cm) was dipped in the pentane solution. Filter paper with 2ul, 10ul, 50ul and 100u of caryophyllene were used in this experiment. In the control, filter papers were treated with pentane solution without caryophyllene (0.0ul) and in the starvation control no filter papers were provided. Treated filter paper was dried on aluminum foil in a hood for 2-3 minutes. The testing arena was a 100 x 15 mm Petri dish containing 50 g sand (Louisiana Cement Products, Greenwell Springs, LA) moistened with10 ml DDH_2O. Treated and control filter paper was placed on top of the sand. Two hundred Formosan subterranean termites (180 workers and 20 soldiers) were introduced into each dish. Filter paper in each Petri dish was the only food source and the weights of filter paper were obtained. All dishes were sealed with Parafilm® (Pechiney Plastic Packaging, Menasha, WI) to retain moisture and were weighed and placed in a 28˚C incubator in constant darkness. After fourteen days, the numbers of dead termites and consumption were determined by measuring the weight difference of filter paper before and after the experiment. Formosan subterranean termites used in this experiment were collected from New Orleans, Louisiana in February 2007 (colony 1), May 2007 (colony 2), June 2007 (colony 3) and July 2007 (colony 4). Five replicates were prepared for each colony.

DATA ANALYSIS

Colony effect on consumption and survivorship was tested by using PROC GLM. If there was no colony effect on consumption and survivorship, data from different colonies were combined. Consumption and mortality differences of termites were analyzed using an analysis of variance (ANOVA) in SAS by using PROC GLM. Significant means were separated using Tukey's studentized range test.

RESULTS

Consumption did not differ among colonies in this no-choice test (F=0.77, P value= 0.3849). Most of termites were dead by 7 days in the 10ul, 50ul and 100ul caryophyllene treatments. In the starvation control, most of termites were still alive after 14 days, which indicates the mortality of termites was not caused by starvation. A significant difference in termite survivorship was found between the filter papers treated with caryophyllene (10ul, 50ul and 100ul) compared to the control (Fig3.1). There was no significant difference between the 10ul and 50ul caryophyllene treatment and 50ul and 100ul caryophyllene treatment (Fig3.1). Termite survivorship was not significantly reduced at the low dose (2ul) compared to the control and starvation control. Survivorship in the 10ul caryophyllene treatment was significantly different from the control, but not significantly different from the starvation control. The survivorship in the starvation control was not significantly lower than the control with the food source.

Consumption of the treated filter paper was reduced in all treatments when compared to the control (Fig3.2), especially in treatments with 50ul and 100ul caryophyllene. There was no measureable consumption in the 50ul and 100ul treatments. Termites were also found avoiding direct contact with the treated filter paper in those treatments by direct observation and the 50ul and 100ul caryophyllene treated filter paper etched the Petri dish cover.

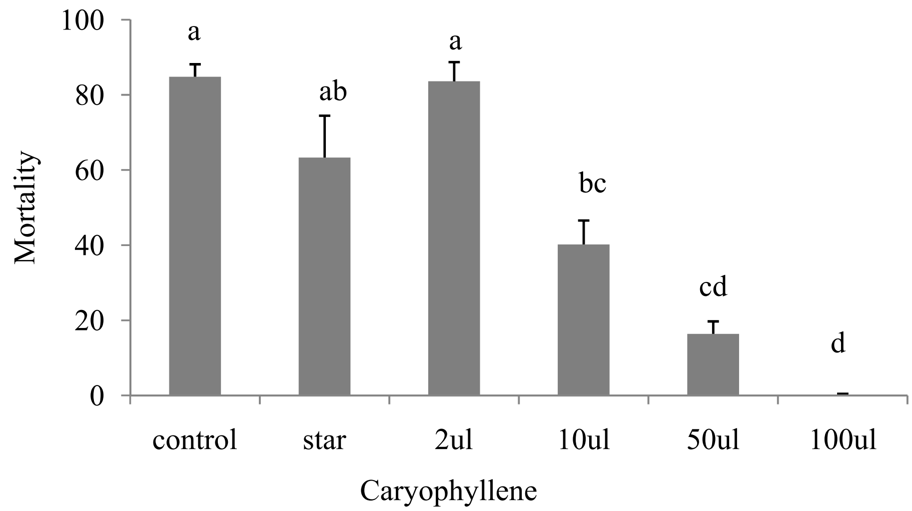

Fig3.1: Termite survivorship after consumption of caryophyllene treated filter paper

*Bars accompanied with different letters are significantly different (significant level= 0.05), bars that share the same letters are different but not significantly different.

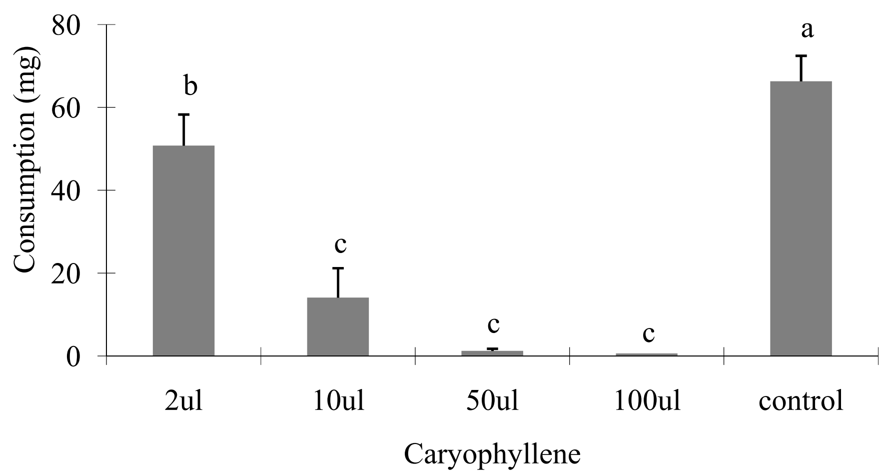

Fig3.2: No choice consumption of caryophyllene treated filter paper

* Bars accompanied with different letters are significantly different (significant level= 0.05)

DISCUSSION

Since consumption of caryophyllene treated filter paper was lower than non-treated filter paper we can assert that caryophyllene is not a feeding stimulant to Formosan subterranean termites. To the contrary, caryophyllene may have a deterrent effect on termite consumption. Survivorship in the 2ul caryophyllene treatment was not significantly different from the control, but consumption in the 2ul caryophyllene treatment was significantly reduced. This indicates that low dose of caryophyllene (2ul) decreased termite consumption. In the 10ul caryophyllene treatment, consumption was significantly lower than the control with the food and survivorship was also significantly reduced compared to the control. This may be the result of a combination effect of contact toxicity and consumption toxicity of caryophyllene. At a higher level of caryophyllene treatment (50ul and 100ul), both the survivorship and consumption were significantly reduced by the contact toxicity but not subsequent starvation. Therefore the consumption toxicity of the caryophyllene could not be judged in the 50ul and 100ul. Termites avoided direct contact with filter paper treated with caryophyllene in the 50ul and 100ul treatments. Volatile caryophyllene by indirect exposeure seems to be the main reason.

Previous studies showed that caryophyllene was attractive to other insects, such as green lacewings and boll weevils (Ghelardini *et al*. 2001; Hammack 2001). Caryophyllene is also known to be a repellent to leafcutter ant which is a part of the self defense system of some plants (Howard 1989). Caryophyllene was considered as a repellent not a deterrent to leaf cutting ants because repellent orient the movement away from the source while the deterrent has the anti-feeding effect (Howard 1989; Henderson 2008).

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CHAPTER 4

EVALUATION OF CARYOPHYLLENE ATTRACTIVENESS TO *COPTOTERMES FORMOSANUS* IN CHOICE TEST

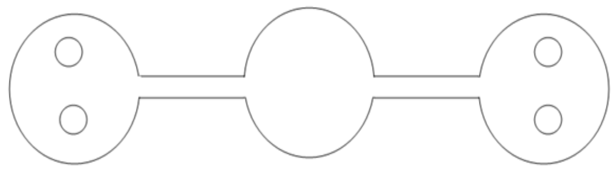
INTRODUCTION

Formosan subterranean termites are destructive pests which consume timber, trees, and buildings. Large colony size and a high feeding rate make Formosan subterranean termites more destructive than their native counterparts (Su and Tamashiro 1987; Su and Scheffrahn 1988). Preference among commercial wood types by Formosan subterranean termites was studied by Morales-Ramos and Rojas (2001). Birch (*Betula alleghaniensis* Britton), red gum (*Liquidambar styraciflua* L.), Parana pine [*Araucaria angustifolia* (Bert.)], sugar maple (*Acer saccharum* Marsh.), pecan (*Carya illinoensis* Wangenh.), and northern red oak (*Quercus rubra* L.) were the most preferred species among 28 different commercial species studied. Resistance and toxicity of wood to termites is associated with chemical constituents contained in the wood, such as terpenoids, quinones, phenolics, and flavonoids (Scheffrahn 1991).

Caryophyllene is found in essential oils of many plants, especially in clove (*Syzygium aromaticum*) (Martin *et al*. 1993). In an earlier study, specific levels of caryophyllene were attractive to Formosan subterranean termites (Mao *et al*, unpublished). The willingness of termites to consume the bait with the presence of other cellulose food is essential for bait effectiveness (Grace *et al*. 1996; Henderson and Forschler 1996). To test the efficacy of caryophyllene as a termite bait additive, caryophyllene treated paper was used to examine the preference of *C. formosanus*.

MATERIALS AND METHODS

Caryophyllene was purchased from Sigma Company (C9653-10 ML, St. Louis, MO). A three-Petri-Dish design was used and is shown in Pic4.1. Petri dishes (100 X 15mm, American Precision Plastics, Northglenn, CO) were connected by a clear vinyl tubing (3/8 X 1/4 inch, Watts, N. Andover, MA).



Pic4.1: Three Petri dishes design

Pic4.2: Three Petri dish design with termites

Two pieces of filter paper (Whatman@, radius=1.0cm) were placed into Petri dishes on each side as shown in the Pic4.2. Caryophyllene (0ul, 0.2ul, 0.6ul, 1.0ul, 2.0ul) was placed in the middle of one filter paper (marked as 1) in one Petri dish and the other filter paper (marked as 2) was untreated in the same Petri dish. On the opposite side of this three Petri dish design, caryophyllene was not added to either filter paper (marked as 3 and 4). Fifteen grams of autoclaved fine construction sand (Louisiana Cement Products, Greenwell Springs, LA) were washed with acetone and dried in an oven (80 ˚C) overnight and placed in each Petri dish. Fifteen ml of deionized water was added to the sand before sand was added into the Petri dishes on right and left side dishes. Each filter paper was weighed then placed on top of the sand in the Petri dish. One hundred termites (90 workers and 10 soldiers) were released in the middle Petri dish. The termites were able to gain access to either side through tubes which were filled with sand. Each treatment was replicated five times and termites from three colonies were used. Formosan subterranean termites were collected from New Orleans, Louisiana in February 2007 (colony 1), May 2007 (colony 2), June 2007 (colony 3). On day 7 of the experiment, the Petri dishes were opened and living termite numbers were recorded. The filter paper was placed under a fume hood to allow the sample material to dry. The weight difference before and after the experiment was considered as termite consumption. Consumption data of filter paper with caryophyllene droplet and without caryophyllene droplet in the treated Petri dish were measured and compared (1 vs 2). Data of total consumption of filter paper in the treated Petri dish and untreated Petri dish was also measured and compared (1+2 vs 3+4).

DATA ANALYSIS

Consumption and mortality difference of termites were analyzed using an analysis of variance (ANOVA) in SAS (PROC GLM). The variables were colony (1, 2 and 3) and caryophyllene treatment (0ul, 0.2ul, 0.6ul, 1.0ul, 2.0ul) with replicates. The consumption of filter paper between Petri dishes and within Petri dish comparison was conducted by using ANOVA [(1+2) vs (3+4) and 1 vs 2]). Tukey's studentized range test was used to test the significance among treatments.

RESULTS

Colony effect was tested and no significance for survivorship (F=1.08, P value= 0.3738), consumption (F=0.15, P value=0.8606 for Petri dishes consumption comparison and F=1.19, P value=0.3113 for within Petri dish consumption comparison) and termite location (F=0.16, P value=0.8511) among 3 colonies was found. Therefore the data from different colonies were combined for survivorship, consumption and termite location.

No significant difference was found for termite survivorship (F=0.39, P value= 0.8134) (Fig4.1). Most of the treatments and control had over 80% live termites at the end of the experiment (Fig4.1).

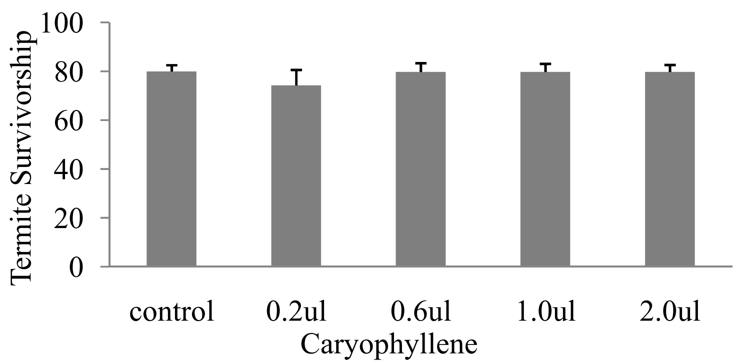


Fig4.1: Termite survivorship in choice test of caryophyllene

More consumption of filter paper was found in the Petri dish without caryophyllene in 0.6ul and 1.0ul treatments compared to the consumption with caryophyllene with statistical significance in Petri dish comparison (Fig4.2).

In the control without any caryophyllene addition, no significant consumption difference was found in control (Fig4.2).

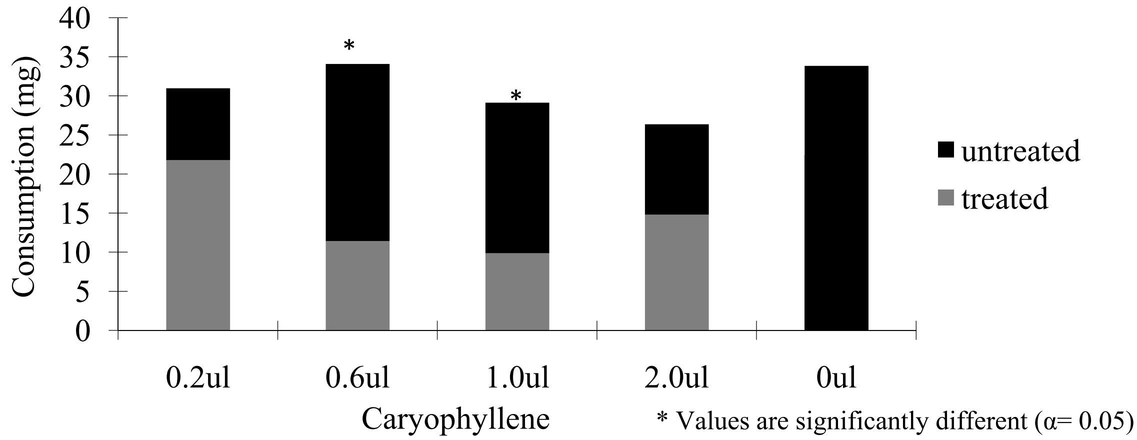


Fig4.2: Termite consumption in caryophyllene treated side and untreated side

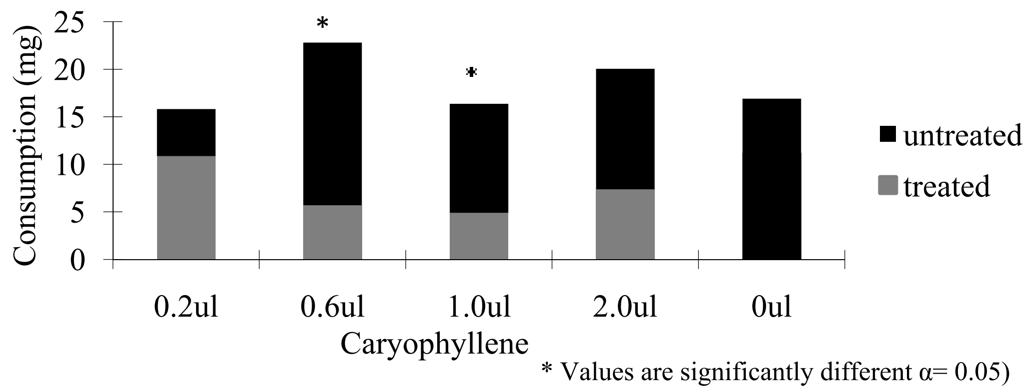


Fig4.3: Termite consumption with caryophyllene and without caryophyllene within the treated side

Consumption of filter paper with the caryophyllene droplet and filter paper without the caryophyllene droplet within the treated Petri dish were also compared (Fig4.3). More consumption was found in 0.6ul, 1.0ul and 2.0ul, however, only 0.6ul and 1.0ul treatment consumption of the filter paper with caryophyllene were significantly different from the filter paper without caryophyllene. Treated side and untreated side in control were marked as black bar and grey bar with black margin.

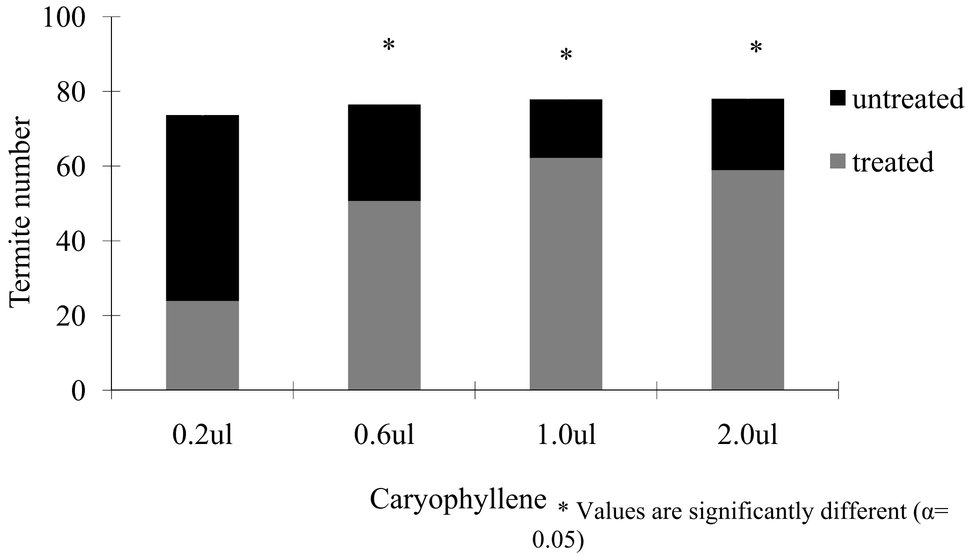


Fig 4.4: Termite distribution in caryophyllene treated side and untreated side

Number of termites in Petri dishes was tested to evaluate the attractiveness of caryohyllene. There were more termites located in the Petri dish with caryophyllene treated filter paper in all the treatments expect 0.2ul treatment and termite number in the Petri dish with caryophyllene increased as caryophyllene increased (Fig4.4).

DISCUSSION

Caryophyllene up to 2.0ul was not toxic to *C. formosanus*, as there was no significant difference in termite survivorship. More termites were found in the Petri dish with caryophyllene treated filter paper, indicating that caryophyllene is attractive to termites. However, though more termites moved to the Petri dish with treated filter paper, more consumption was found in the filter paper without caryophyllene. This suggested that termites are either more active or aggregated in the Petri dish with caryophyllene treatment; however the filter paper with caryophyllene is not preferred as a food resource. This result is consistent with the previous no choice consumption test result that caryophyllene is not a feeding stimulant. However, caryophyllene may function as an attractant to Formosan termites.

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CHAPTER 5

EVALUATION OF TUNNELING BEHAVIOR OF *COPTOTERMES FORMOSANUS* TO CARYOPHYLLENE DROPLET

INTRODUCTION

The Formosan subterranean termite, *C. formosanus* Shiraki, is a destructive world-wide pest that tunnels through soil to locate cellulose food sources underground including living trees and wood used in construction (Ibrahim *et al*. 2005). It is native to China and was accidently introduced to into the United States (Kistner 1985). One of the most effective termite control strategies is using barriers with repellent chemicals (Campora and Grace 2001). The ability of a chemical compound to repel or attract is an important factor in determining the chemical compound efficacy in termite control. A non-repellent or attractive toxic substance that kills termites or a highly repellent chemical is required in termite control (Su *et al*. 1982). An understanding of a chemical's effect on termite tunneling behavior is important to determine its candidacy for termite control.

Synthetic insecticides have been used for decades for termite control in the United States (Mao and Henderson 2007). Pyrethroids, nicotinoids, and pyrazoles and other pesticides are currently used. However, concerns about the environment have stimulated an interest in safe pesticides to replace high risk synthetic ones and have led to the studies of biochemical and botanical insecticides (Duke 1990). Essential oils, which are extracted from plant tissues, consist of terpenes, phenols, and sesquiterpenes. Some of them have been evaluated for their antimicrobial and insecticidal activity (Isman 1999). Essential oils are preferable as termiticides due to their low mammalian toxicity and minimal environmental hazard (Isman 2000).

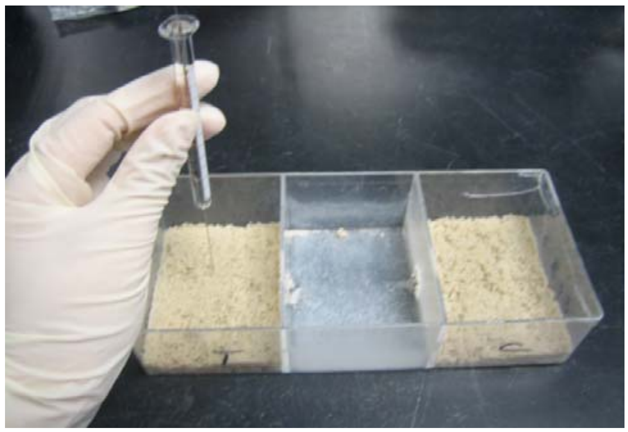
Termites tunnel in soil, foraging for wood or nesting sites. Due to the cryptic habitat of Formosan subterranean termites, it is difficult to study their tunneling behavior in the laboratory. Tunneling behavior is either studied by excavating the termite nest or using spatial-temporal and mark-release-recapture methods (LaFage *et al*. 1973; Su *et al*. 1984; Grace *et al*. 1989; Ratcliffe and Greaves 1940; Campora and Grace 2001). Two-dimensional surface tunneling methods have provided important information regarding tunneling behavior (Campora and Grace 2001). Internal and external factors influence termite tunneling behavior. Soil concentration, soil moisture, temperature, and food source all affect termite tunneling behavior (Lee *et al*. 2008). Studies of tunneling pattern include direction, distance and angle in order to understand the termite behavior (Lee *et al*. 2008).

Caryophyllene is extracted from many plants, especially clove. Caryophyllene released by maize is attractive to an entomopathogeic nematode (Rasmann *et al*. 2005). An early study of Formosan subterranean termite's response to caryophyllene was performed using a two armed Petri dish in the laboratory (Mao *et al*, unpublished). It was found that caryophyllene was attractive to Formosan subterranean termites. This led us to study the effect of caryophyllene treated sand on tunneling behavior of Formosan subterranean termites.

MATERIALS AND METHODS

Caryophyllene was purchased from Sigma Company (C9653-10 ML, St. Louis, MO). The bioassay apparatus was a three-chambered plastic container (each chamber 8.0x5.75x4.0 cm, Pioneer Packaging Co., Dixon, KY. Pic5.1). All chambers were accessible to termites through an opening (0.3 cm diameter) at the bottom of the two inner walls. Autoclaved construction sand used for the experiment was washed with acetone and dried (80°C incubator) overnight to eliminate any chemical contaminants.

Thirty grams of sand was placed in the treatment chamber and control chamber. The sand in each chamber was moistened with 8 ml of distilled deionized water (DDH_2O). Caryophyllene droplets of one of these dosages (0.0ul, 0.2ul, 0.6ul, 0.8ul and 1.0ul) were placed on the sand surface in the middle of the treatment chamber by using 10-ul syringe (Hamilton, Reno, NV.).



Pic5.1: Three chamber container with caryophyllene droplet in the middle of the sand

The center chamber was left empty for introducing the termites (release chamber). Ninety workers and 10 soldiers were placed into the release chamber of each unit. After twenty four hours in a 28˚C incubator in constant darkness, the underside of each unit was scanned to generate an image that equals the actual size of the containers for tunneling area measurement. The bottom of each 3 chamber container was scanned to measure the tunneling. The tunneling area was measured by cutting the tunneling image to gain the weight. All weights were transformed (1mg filter paper=86888 cm^2) into tunneling area data. The overall tunneling and tunneling within the circle (r=1.0cm) were analyzed.

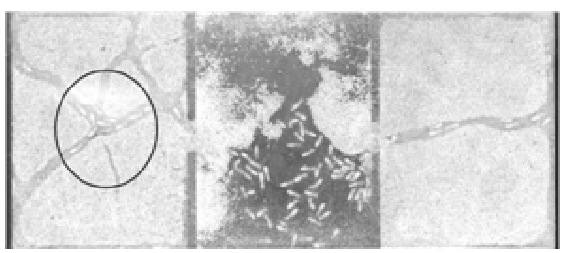
The unit was disassembled and the numbers of live termites in each chamber were counted. Formosan subterranean termites were collected from New Orleans, Louisiana in

February 2007 (colony1), May 2007 (colony2), June 2007 (colony3). Termites were maintained in 250-liter trash cans with pine (*Pinus* spp.) 24-26˚C in the laboratory after collection. Five replications of each dose were used in this test.

DATA ANALYSIS

Colony differences among treatment were analyzed using an analysis of variance (ANOVA) in SAS. The data were analyzed using the PROC GLM procedure. The variables were tunneling and caryophyllene treatments (0ul, 0.2ul, 0.6ul, 1.0ul, 2.0ul) with replicates. The tunneling in treatment was analyzed over different caryophyllene dose range to see the effect of caryophyllene dose effect (Partial factorial ANOVA). Tukey's studentized range test was used to test the significance among treatments.

RESULTS



Pic5.3: Tunneling scan of three chamber container

There was no significant variation among the colonies therefore the data from different colonies were combined for mortality and tunneling (F=0.77, P value=0.4660).

Termite survivorship was not affected by caryophyllene after 24h since there was no significant difference between the treatments and control (F=1.08, P value=0.3738) (Fig5.1).

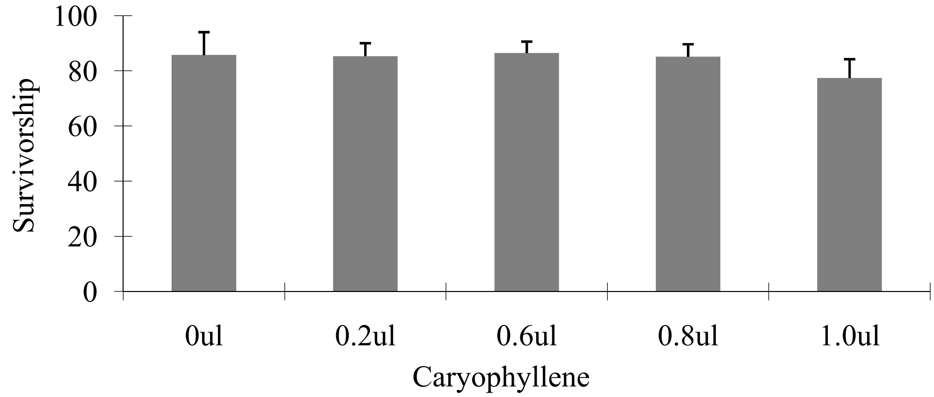


Fig5.1: Termite survivorship after 24hours with caryophyllene treatment in three chamber container

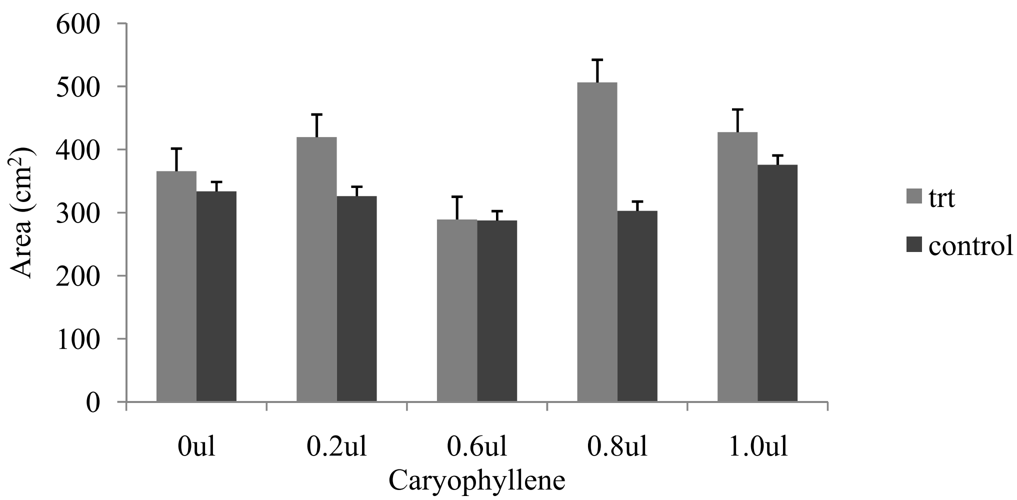


Fig5.2: Termite tunneling in three chamber container with caryophyllene droplets

There was no significant total tunneling difference between the chambers with caryophyllene droplet and chamber without caryophyllene (Fig5.2,). However, more tunneled area was observed around the caryophyllene droplets by direct observation in the treated chamber within caryophyllene treated sand therefore tunneling was analyzed within the circle (r=1.0cm) with caryophyllene droplet. Termite tunneling within the circle in 0.6ul, 0.8ul and 1.0ul caryophyllene treatments was significantly higher than the other side without caryophyllene droplet and the tunneling was increased as caryophyllene amount increased.

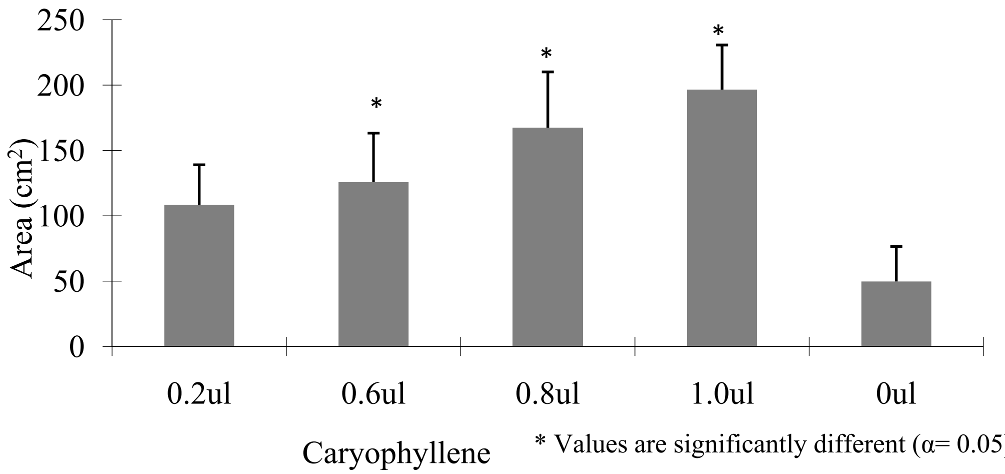


Fig5.3: Termite tunneling within two cm circles with caryophyllene droplets

DISCUSSION

No significant difference was found in any the treatments when total tunneling in the chamber was analyzed, but a significant difference was found by analyzing the tunneling within the circles (F=5.25, P value= 0.0011). Furthermore, more tunneling was found when a higher amount of caryophyllene was applied to the sand. The volatile nature of caryophyllene caused it to affect the entire space of the three chamber containers, therefore termites had the same chance to go to either side of chamber with or without caryophyllene droplets. This explained that there was no significant tunneling difference between the chamber with caryophyllene droplet and without caryophyllene droplet. However, when termites went into the chamber with caryophyllene droplets, termite tunneling was oriented to the droplet therefore more tunneling activities were found closer to caryophyllene droplets. This result showed that caryophyllene is attractive to termites.

The response of different insect species to caryophyllene varies. Caryophyllene was found in potato plants (Agelopoulos *et al*. 2000), tobacco (Heiden *et al*. 1999), sunflower (Schuh *et al*. 1997), maize (Turlings *et al*. 1998) and cotton (Rodriguez-Saona *et al*. 2001). The release of caryophyllene from cotton attracts many insects including green lacewing (*Chrysopa carnea*), rove beetle (*Collops vittatus*), and pink bollworm (*Pectinophora gossypiella*) (Takchev 1986). Caryophyllene released by the rootworm wounded maize is attractive to an entomopathogenic nematode (Rasmann *et al* 2005). However, the attractiveness of caryophyllene on entomopathogenic nematode on termite was not evaluated.

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CHAPTER 6

SUMMARY AND CONCLUSIONS

Caryophyllene is a compound found in many flowering plants, including Brassicaceae (Rohloff and Bones 2005), Solanaceae (Farag and Paré 2002), and Poaceae (Dean and De Moraes 2006). Previous studies identified caryophyllene from the headspace of *Harmonia axyridis* (Brown *et al*. 2006). The *cis* isomer of caryophyllene is usually not found with the presence of *trans*-caryophyllene (Tkachev 1988).

Early studies of caryophyllene were started as synthetic derivatives in aromatic additives for tobacco and food industry (Tkachev 1987). It has been reported that caryophyllene caused aggregation of female Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) (Verheggen *et al*. 2007). Attractiveness of caryophyllene was also found in green lacewing *Chrysopa carnea*.

One of the important synthetic derivatives which has biological importance is caryophyllene epoxide (III). Caryophyllene epoxide (III) was attractive to rove beetle *Collops vittatus* (Flint et al. 1979). Both caryophyllene and caryophyllene epoxide (III) attract male pink bollworm *Pectinophora gossypiella* (Tkachev 1987). However, the attractiveness of caryophyllene and caryophyllene epoxide (III) is not always consistent. *Campoletis sonorensis* is attracted by caryophyllene epoxide (III) but is not attracted by caryophyllene (Elzen et al. 1984). The anti-termite activities were reported from the extract of *Calocedrus formosana*, a tree with decay resistance properties. Caryophyllene and caryophyllene oxide are the two major components of the essential oil of *Calocedrus formosana* and both of them were toxic to *C. formosana* (Cheng et al. 2004). Caryophyllene repellent activities were found in leaf cutting ants *Atta cephalotes* Linnaean and *Acromyrmex octospinosus* Reich (Howard et al. 1989).

In our study, caryophyllene showed its attractiveness to the Formosan subterranean termite by stimulating its tunneling behavior; however, the consumption of Formosan subterranean termites was not increased. The tunneling of Formosan subterranean termites was significantly increased even when low amounts of caryophyllene were applied in the sand. The tunneling was increased in all the treatments including the control chambers compared to the control container without addition of caryophyllene due to the volatility of caryophyllene. The volatility of caryophyllene caused an increase in consumption in all of the test chambers. The rate of volatility was not tested in this study.

Genetic difference and previous exposure to chemical compounds influence insect behavior. However, colony variation in the all the experiments was not statistically significant. Possible prior exposure to caryophyllene was minimized by maintaining colonies under the same laboratory conditions and by feeding all colonies the same food.

The first bait product for subterranean termites is the Sentricon® colony Elimination System (DowElanco, Indianapolis, IN) using hexaflumuron, a slow-acting insecticide (Su, and Scheffrahn 1998). Several metabolic inhibitor bait products are also available using sulfluramid (FirstLine®) and hydramethylnon (Subterfuge®), however, FirstLine® and Subterfuge® require additional pesticide (Su and Scheffrahn 1998). Caryophyllene showed its attractiveness with low dose, however, the low acute toxicity was moderate based on our study. Therefore addition of other termiticides may be needed when using caryophyllene as a potential bait product. However, due to its volatility, the efficacy of caryophyllene in termite bait need further study.

Essential oils are mixtures of monoterpenes, phenols, and sesquiterpenes. The recent interests in essential oils focus on their application in pest control from their traditional use. The action model of essential oil is not clear, however, it has been suggested that some essential oils affect the GABA-gated chloride channel (Priestley *et al* 2003).

Further studies are needed to develop a methodology for utilizing caryophyllene as termite bait. More research will also be needed to determine the efficacy of caryophyllene as a termiticide to analyze the marketability of using caryophyllene as a termiticide. The longevity of its effects in particular will need to be examined. Previous studies showed that caryophyllene in moist sand had a rapid diffusion rate and high stability. These properties make caryophyllene an exceptionally suitable below ground attractant. Further experiments in the field are also needed to evaluate the effectiveness of caryophyllene under ground.

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