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VOLATILES ASSOCIATED WITH FORMOSAN SUBTERRANEAN TERMITES AND RELATED METHODS DEVELOPMENT

A Thesis

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

in

The Department of Entomology

by
Paul McLaughlin
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ABSTRACT

An investigation was conducted to identify volatiles associated with active Formosan termites. Using a combined technique of short path thermal desorption-gas chromatography/mass spectrometry (SPTD-GC/MS), qualitative comparisons were made between compounds detected in treatments containing active Formosan termites versus those detected in controls without Formosan termites. Except for dimethyl disulfide, none of the compounds were consistently detected in the treatments but not the controls for the four termite groups tested. However, in each of the three groups that dimethyl disulfide was detected, dead termites that were decomposing along with active termites were present. Therefore, none of the compounds could be classified as volatiles associated with active Formosan termites.

Qualitative and quantitative comparisons were made between compounds detected in treatments that contained carton nests with active Formosan termites and controls that contained neither Formosan termites nor carton nests. Two methods were used, one using unwashed Nalgene 550 platinum-cured silicone tubing and the other using unwashed fluorinated ethylene propylene (FEP) teflon tubing in the experimental set-up. Qualitative analysis for both methods indicated that none of the compounds could be consistently detected in treatments but not the controls. Quantitative analysis for both methods indicated that the concentrations of naphthalene, and butylated hydroxytoluene and nine unknown volatiles were not significantly different between the treatments and controls at the 0.01 level as determined by the paired t-test. Therefore, using the methods described herein, none of the analyzed compounds could be classified as volatiles associated with active Formosan termites. However, changes in the methods may enable the detection of volatiles associated with active Formosan termites.

The concentrations of three suspect reporter molecules, which include naphthalene and two unknown compounds, were significantly lower using fluorinated ethylene propylene (FEP) teflon tubing than using unwashed Nalgene 550 platinum-cured silicone tubing in the experimental set-up as determined by 95% confidence intervals. This suggested that a source of these volatiles was unwashed silicone tubing, which was relevant to this study because it aided in the determining whether the volatiles are associated with active Formosan termites.

INTRODUCTION

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is currently one of the most destructive pests in the U.S. (Lax and Osbrink, 2003). In the U.S. alone, it is estimated that Formosan termites cost homeowners approximately 1 billion dollars a year in damages (Suszkiw, 2000; Lax and Osbrink, 2003). Formosan termites are currently distributed in 11 states within the U. S. including Louisiana, Texas, Mississippi, Alabama, Florida, Georgia, South Carolina, North Carolina, Hawaii and California. Furthermore, the expansion of range of this pest shows no sign of slowing (Woodson *et al*., 2001).

To combat this pest, extensive research has been done to improve the control of Formosan termites. An area of termite control that needs improvement is that of the detection of termites in structures (Scheffrahn *et al*., 1993; Henderson *et al*., 2001; Evans, 2002). It is difficult for pest management professionals to detect termites because their cryptic behavior often results in infestations that are concealed from view. Once subterranean termite infestations are established, visual inspections that entail such things as searching for live termites, swarm ports, imaginal wings shed from alates, mud tunnels and damaged wood are relied upon (Henderson *et al*., 2001; Evans, 2002; and Brooks *et al*., 2003). In fact, visual inspections are the primary method of termite detection (Scheffrahn *et al*., 1993). This dependence on visual inspections to detect termites can result in misdiagnosis due to an inability to search inaccessible areas, which may exceed 45 percent of the total area searched (Lewis *et al*., 1997; Henderson *et al*., 2001). Therefore, the presence of termites in a structure may go undetected until substantial damage has occurred (Lewis *et al*., 1997; Henderson *et al*., 2001).

To enable inspectors to locate termite infestations before the substantial damage occurs, the pest management industry has begun to use several tools to inspect areas inaccessible by visual

inspection. These tools include electronic stethoscopes, moisture meters, acoustic emission detectors and infrared technologies (Lewis *et al*., 1997; Suszkiw, 2000; Yanase *et al*., 2000).

Another promising technique is the detection of volatiles associated with the termites (Lewis *et al*., 1997; Koestler *et al*., 2000; Stušek *et al.*, 2000; Henderson *et al*., 2001). Several studies indicate that volatiles are associated with Formosan termites (Henderson *et al.*, 1999; Henderson *et al*., 2001; and Nix *et al*., 2003). For example, several volatiles from Formosan termite carton nests with living termites were tentatively identified (Henderson *et al.*, 1999). In related work, naphthalene was detected in solvent extracts of Formosan carton nests (Chen *et al*., 1998). This suggested that naphthalene would be present as a volatile associated with Formosan termites and that it could be used to identify the presence and location of termite nests (Henderson *et al.*, 1999).

In subsequent work, volatiles from Formosan termite nests with living termites were compared to volatiles from controls (Henderson *et al*., 2001). The results from these experiments indicated that naphthalene and butylated hydroxytoluene volatiles were in higher concentrations in treatments containing carton nests with active termites than in the controls. In addition, volatiles tentatively identified as ethyl benzene, ethylmethylbenzene, and dichlorobenzene were present as volatiles from active carton nests but were absent in controls (Henderson *et al*., 2001).

In related work by researchers at the LSU AgCenter, two compounds found in Formosan termite nests, naphthalene and butylated hydroxytoluene, were presented to a dog that had been trained to detect termites by their signature odor (Nix *et al*., 2003). Although the dog alerted to naphthalene in the same way it alerted to termites, it did not alert to butylated hydroxytoluene.

The results therefore indicated that termite detection canines are likely to key in on naphthalene but not butylated hydroxytoluene to find Formosan termite nests (Nix *et al*., 2003).

In addition to work done by researchers at LSU AgCenter, studies indicate that there are volatiles associated with other species of termites (Zimmerman *et al*., 1982; Rasmussen and Khalil, 1983; Seiler *et al*., 1984; Fraser *et al.*, 1986; Khalil *et al*., 1990; French *et al.*, 1997; Koestler *et al*., 2000). For example, in a study in which the influence of termites on atmospheric gases was determined**,** several volatiles were found to be in higher concentration in termite mounds than in the surrounding ambient air (Khalil *et al*., 1990). For the species *Amitermes laurensis* Mjoberg, *Nasutitermes magnus* Froggatt, *Drepanotermes perniger* Froggatt, *Tumulitermes pastinator* Hill, *Coptotermes lacteus* Froggatt and *Nasutitermes triodae* Froggatt, those volatiles included methane, carbon dioxide, chloroform and nitrous oxide. For the species *C. lacteus* and *A. laurensis*, volatiles included light hydrocarbons such as ethene, propene, and propane. For *C. lacteus* alone, butane was found (Khalil *et al*., 1990). Furthermore, in a similar study, dimethyl disulfide (DMDS) was shown to be emitted by *Reticulitermes tibialis* Banks (Zimmerman *et al*., 1982).

To develop a method of detection of termites based on volatiles associated with Formosan termites, a multiple-phased research project was designed (Henderson *et al*., 1999). The first phase involved the identification of volatiles from active Formosan termite nests in order to create a profile of “reporter molecules” (Henderson *et al*., 1999). Reporter molecules are volatiles that are present in Formosan termite nests with active termites but not in the ambient environment or volatiles that are in higher or lower concentrations in Formosan termite nests with active termites than the ambient environment. The second phase involves taking air samples from houses, identifying their constituent compounds, and determining if Formosan termites are

present based on whether the identified compounds match those of the profile of reporter molecules (Henderson *et al*., 1999). The last phase involves developing an odor detection device for both pest control operators and homeowners (Henderson *et al*., 1999).

The objective of this thesis was to make significant progress in the first phase of research project. Specifically, the objectives were: 1) to determine qualitative differences between volatiles detected in treatments containing active Formosan termites versus those detected in controls without termites and 2) to determine qualitative and quantitative differences between volatiles detected in treatments containing carton nests with active Formosan termites versus those detected in controls with neither carton nests nor Formosan termites.

CHAPTER 1

QUALITATIVE ANALYSIS OF VOLATILES DETECTED IN VESSELS CONTAINING FORMOSAN SUBTERRANEAN TERMITES

INTRODUCTION

Several studies conducted in the laboratory indicate that volatiles are associated with active termites without their nests (Zimmerman *et al*., 1982; Rasmussen and Khalil, 1983; Fraser *et al.*, 1986; and Koestler *et al*., 2000). For example, by using between 119 and 2000 termites, methane, carbon dioxide, carbon monoxide, and molecular hydrogen were shown to be produced by the species *Reticulitermes tibialis* Banks and *Gnathamitermes perplexus* Banks (Zimmerman *et al*., 1982). In addition, dimethyl disulfide (DMDS) was shown to be emitted by *R. tibialis* (Zimmerman *et al*., 1982). Similarly, in another study using 20 worker termites, carbon dioxide was shown to be produced by the *Reticulitermes* sp. (Koestler *et al*., 2000). In addition, using 25 workers and a queen, methane was shown to be produced by the species *Zootermopsis angusticollis* Hagen (Rasmussen and Khalil, 1983). Furthermore, using groups of 200 individuals, methane was shown to be produced by the species *Coptotermes lacteus* Frogatt, *Coptotermes acinaciformis* Frogatt, *Nasititermes exitiosus* Hill and *Coptotermes formosanus* Shiraki (Fraser *et al.*, 1986).

Relative to the other species, however, *C. formosanus* produces practically no methane (Fraser *et al.*, 1986; Khalil and Rasmussen, 1983). This indicated that the amount of methane produced by different species of termites varies significantly, even for termite species within the same genus (Khalil and Rasmussen, 1983). Furthermore, it also suggested that *C. formosanus* may not produce or produce relatively less of volatiles that are produced by other species.

Although this may be true, the above studies suggested that active Formosan termites without their carton nest produce volatiles. To determine if this is the case, the following

hypothesis was tested: Volatiles will be consistently present in treatments containing active Formosan termites that are not present in controls without termites. A volatile is classified as being consistently present in the treatments but not the controls if it is present in treatments but not the controls in groups of active Formosan termites from different source colonies.

MATERIALS AND METHODS

Termites

Four groups of termites were used and the number of termites in each group was determined by dividing the total weight of the termites within each group by the average weight of three sets of 50 termites for each group. The first group was collected in Lake Charles, LA on January 15, 2003 and contained approximately 4,838 termites. The second group was collected on March 9, 2003 from a carton nest in New Orleans, LA and contained approximately 4,480 termites. The third group was collected in Lake Charles, LA on March 7, 2003 and contained approximately 8,020 termites. The fourth group was collected on February 25, 2003 from Memorial Brenthel Park in New Orleans, LA and contained approximately 2,650 termites.

Sampling Protocol

1,200 grams of sand were rinsed three times with acetone and then dried in an oven at 121°C for approximately 1 hour, 30 minutes. 600 grams of the sand, 2 pieces of filter paper (each weighing 1.6 grams), a piece of pine wood (*Pinus* sp.) weighing 5 grams and 61 milliters of deionized water (10% by weight of the total weight of the other materials) were added to each of two Wheaton purge and trap vessels (Scientific Instrument Services, Ringoes, NJ), each having a capacity of 1.8 liters. To prevent air leaks at the connection between the vessels and the vessels' adapters and between the vessels and vessels' caps, T. F. E. tape was wrapped around the orifices to form a tight seal. To prevent contamination by fungal spores and bacteria, the vessels were

autoclaved using the following parameters: sterilization temperature = 250ºF and sterilization time = 15 minutes. Formosan termites were then added to a vessel that served as the treatment while no termites were added to the other vessel that served as the control. An adsorption tube (Scientific Instrument Services, Ringoes, NJ) packed with a multiple layered resin (100 mg of carboxen 569, 100 mg of Tenax TA and 100 mg of glass beads) was attached to the vessel. Nalgene 550 platinum-cured silicone tubing (Fisher Scientific, Springfield, NJ) measuring 60 cm in length was used to connect the adsorption tube to a Welch® vacuum pump (Model 2545B-01, Thomas Compressor and Vacuum Corp, Skokie, IL). Note: The Nalgene 550 platinum-cured silicone tubing was used as is from the manufacturer and was not washed with solvents. To reduce contamination of the vessels by volatile compounds from the ambient air, an adsorption tube packed with multiple layered resins was attached to the inlet adapter on the Wheaton vessel. The experimental set-up is shown on the following page (Figure 1).

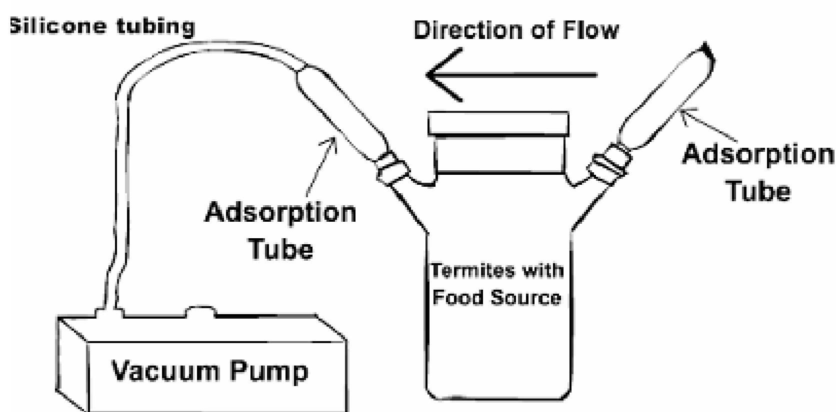

Figure 1. Set-up for the collection of volatiles facilitated by a vacuum pump and using Wheaton vessels to contain the Formosan termites.

The apparatuses were placed in an incubator set at a temperature of 29ºC. The volatiles from within the vessels were then vacuumed onto the adsorbent resins. The flow rate was determined

by connecting the inlet adsorption tube to a Gilmont® flow meter with unwashed silicone tubing (30 cm). The flow rate before and after sampling was recorded and averaged. The average readings were converted to units of ml/min and the total volume sampled was calculated by multiplying total sampling time in minutes by the flow rate. The sampling times for each treatment and corresponding control were equal. However, there was variation in the sampling times and thus the sampling volumes between the pairs of treatments and corresponding controls (Appendix A, Table 9, Page 50). Each treatment (group of termites) with its corresponding control was considered a repetition, so there were four repetitions. New pieces of unwashed silicone tubing were used each time samples were taken. For the fourth group, the percent relative humidity was recorded five times at the completion of sampling the treatment and corresponding control with a Traceable® Hygrometer (Control Company, Friendswood, TX). All of the readings were 100 percent.

Instrumental Methods

The samples were then analyzed using the Model TD-4 Short Path Thermal Desorption (SPTD) system (Scientific Instrument Services, Ringoes, NJ) accessory connected to the Finnigan GCQ system (Thermo Electron Corporation, Austin, TX). After analysis, the adsorption tubes were cleaned using a Thermal Desorption Conditioning System (Scientific Instrument Services, Ringoes, NJ). The parameters set for the Short Path Thermal Desorption System (SPTD) system were as follows: gas purge before injection = 2 minutes; injection time = 15 seconds; desorption time = 5 minutes at 250°C; and delay time = 5 seconds. The gas chromatograph (GC) and mass spectrometer (MS) parameters were as follows: The GC injection port was set to split less mode at a temperature of 250°C; the helium carrier gas was set to 1.5 ml/min; the MS ion source temperature was set at 180°C; and the GC-MS interface was set at

260°C. For the first group of termites that were tested, the GC oven temperature program was as follows: 40°C (5 min); 10°C / min, 80°C, 0.0 min; 4°C/min, 200°C, 0.0 min; 10°C / min, 260°C, 0.0 min. The MS was set to full scan mode from 41-400 daltons and data acquisition began when the oven temperature had reached 60°C. For the remaining three groups of termites tested, the scan mode was set to 35 to 550 atomic mass units, the data acquisition began when the oven temperature was 35°C, and the GC oven temperature program was changed to the following: 35°C, 7 min; 10°C / min, 80°C, 0.0 min; 4°C/min, 200°C, 0.0 min; 10°C / min, 260°C, 2 min. In addition, to detect light hydrocarbons, a Micro Cryo-Trap™ (Scientific Instrument Services, Ringoes, NJ) with Liquid Nitrogen cooling (230 L Nitrogen Dewar, BOC Group, Inc.) was set at a temperature of -175°C during the desorption process after which it was ballistically heated to 250°C.

Data Analysis

For the first group of termites sampled, mass chromatograms were run on each consecutive base peak from 41 to 300 daltons on the chromatographs of the treatment and the control. For the remaining three groups of termites in which the full scan mode was set to 35 to 550 daltons, mass chromatograms were run on each consecutive base peak from 35 to 300 daltons on the chromatographs of the treatments and the controls. For all four of the groups of termites tested, each of the peaks of the mass chromatograms were integrated using the default values in the Xcaliber® software, which are the following: baseline window = 40; area noise factor = 5 and peak noise factor = 10. For each peak of each mass chromatogram, the corresponding retention time and mass spectrum were recorded. A profile of compounds in the chromatographs of the treatments and controls was then created.

Identification of Dimethyl Disulfide

The identification of a compound detected only in the treatments, dimethyl disulfide was confirmed using the following procedure. As a standard, a solution of dimethyl disulfide (Sigma Aldrich, St. Louis, MO) in methanol was prepared at a concentration of 100 nanograms/µl and then analyzed using the instrumental methodology described above. The mass spectrum and GC retention times of peaks in the sample were then compared with those of the dimethyl disulfide standard.

RESULTS

Dimethyl disulfide was detected in treatments but not the controls in three of the four groups of termites (Table 1). However, in each of the three groups of termites that it was detected, decomposing termites along with active termites were observed in the vessels. The identification of dimethyl disulfide was confirmed since its GC retention time and mass spectrum in the samples matched those in a standard (Table 2).

Table 1. Comparison of compounds detected in the treatments and controls. m/z =mass to charge ratio; G = group; + =detection in treatment; ○ =detection in control; ● = detection in both treatment and control; - = not detected in treatment or control.

Compound Name or Unknown #	Retention time	m/z	Detection symbol – G1	Detection symbol – G2	Detection symbol – G3	Detection symbol – G4
1	0.79	40,42	-	●	●	●
2	1.35	44, 45	-	●	●	●
3	1.61	101,103	-	●	+	●
4	2.16	47	-	●	●	●
5	2.16	59, 43	-	●	●	●
6	2.59	107, 109, 75, 89, 181	-	●	●	●
Dimethyl disulfide	4.32	94, 45, 79, 61	-	+	+	+
7	5.06	91, 92, 65	●	●	●	●
8	6.22	83, 55, 99	-	○	-	●
9	6.63	229, 227, 151	●	●	●	●
10	7.85	91, 106, 77	●	●	●	●
11	8.20	57, 86, 115	-	-	+	-
12	10.35	281, 299, 267	●	●	●	●
13	8.99	105, 120, 77	●	●	+	●
14	11.06	146, 148, 111, 75	●	-	-	●

Table 1 Continued

Compound Name or Unknown #	Retention time	m/z	Detection symbol – G1	Detection symbol – G2	Detection symbol – G3	Detection symbol – G4
15	9.49	93,91,79,67, 121,136	●	●	●	●
16	11.26	117,118,115,91	●	●	●	●
17	11.36	119,134, 91	●	●	●	●
18	12.53	105,77,51,50	●	●	+	●
19	13.81	57,71,85,99	●	●	+	●
20	13.47	67, 57, 81, 143	●	○	●	●
21	14.14	355, 286, 267	●	●	●	●
22	12.94	132, 117, 115, 91	●	●	●	●
23	13.94	142, 99, 127	-	-	+	-
Naphthalene	15.69	128,102	●	●	●	●
26	16.14	148, 147, 117, 77	●	●	+	●
28	19.38	429, 73, 430, 445, 325, 341, 359, 149	●	●	●	●
31	20.31	121, 166, 91, 77	-	●	+	●
32	20.45	161, 91, 105, 133, 189,204	●	●	●	●
33	22.84	180, 166, 193		-	+	-
34	22.65	156, 141, 115, 76	+	-	+	○
35	24.13	503, 519, 73, 281, 299, 149, 345	●	●	●	●
Butylated Hydroxytoluene	25.14	205,220,177	●	●	+	●
37	25.78	159, 131, 202	●	●	+	-
38	26.55	219, 191, 234	+	+	○	-
40	27.68	173, 243, 71	●	-	-	●
41	27.80	149, 177, 105	●	-	-	○
42	30.02	183,198,168	●	●	-	●
43	30.00	181, 210, 165	●	○	-	○
44	36.69	257,272,161	●	○	+	○
45	27.80	149, 150	●	-	+	-
48	39.29	255, 159, 173, 270	-	○	+	-
49	42.79	322,81,121, 136,217	-	-	-	●
50	44.66	207, 91, 117, 129	●	●	●	●
51	46.13	262, 263, 261	●	-	-	●

Based strictly on qualitative analysis, none of the remaining compounds could be considered reporter molecules since the compounds could be classified into one of the following categories. 1) The compound was detected in both the treatments and controls (Table 1). 2) The compound

Table 2. Comparison of the GC retention time and mass spectra of the peak in a representative sample with those of a standard for dimethyl disulfide. RT = Retention time, RS = Representative Sample, STD = stock solution of standard.

Volatile	RT in RS	RT in STD		m/z in RS	m/z in STD
Dimethyl disulfide	4.92	4.77		45, 94, 79, 61, 64	45, 94, 79, 64, 61

was detected only in a treatment using this methodology but was detected in both the treatments and controls using other methodologies (Table 1; Appendix B, Table 10, Pages 51-52). 3) The compound was detected in a treatment but not the controls using this methodology but was not detected in the treatments or controls in any other methodologies (Table 1; Appendix B, Table 10, Pages 51-52).

DISCUSSION

Except for the presence of termites in the treatments, all of the factors for each treatment and corresponding control were the same. Furthermore, as indicated above, all of the materials in the experimental vessels such as the sand, wood, deionized water, and filter paper were autoclaved prior to analysis. Therefore, it is unlikely that contaminating volatiles from bacteria or fungi were present. Thus, it is likely that dimethyl disulfide is associated with the presence of decomposing termites only or both decomposing and living termites. Furthermore, dimethyl disulfide was detected in treatments containing termites from at least two different source colonies. Therefore, the presence of dimethyl disulfide cannot be attributed to a characteristic particular to a colony.

Although results of this methodology suggest that dimethyl disulfide is associated with decomposing Formosan termites rather than active Formosan termites, dimethyl disulfide has been associated with the active termites of another species, *Reticulitermes tibialis* Banks (Zimmerman *et al.*, 1982). When determining the effect of gases produced by termites on the atmosphere, it was reported that *R. tibialis* emit dimethyl disulfide at a rate of 0.005 percent in

units of grams of dimethyl disulfide emitted per gram of carbon ingested (Zimmerman *et al.*, 1982).

Two explanations are proposed to account for the apparent inconsistency that active termites of *R. tibialis* emit dimethyl disulfide but that only decomposing termites of *C. formosanus* produce dimethyl disulfide. First, it is possible that living *R. tibialis* do not emit dimethyl disulfide and dimethyl disulfide is only produced by decomposing termites of *R. tibialis* and *C. formosanus*. This is plausible explanation because although it was indicated that dead termites were present in the study on *R. tibialis* (Zimmerman *et al*., 1982), the dead termites were not considered as a possible source of the dimethyl disulfide. A second explanation is that dimethyl disulfide is associated with both living and decomposing termites of both species. This is also a plausible explanation since another possible source of dimethyl disulfide in a termite colony is the decomposition of fecal material and/or secretions.

Assuming that the second explanation is true, however, an explanation is necessary for the fact that dimethyl disulfide was not detected in the first group used in this methodology and only in two of the eight subgroups (pieces of carton nests with active Formosan termites) tested in the methodologies that follow (See chapter two). An explanation as to why dimethyl disulfide was not detected in the first group used in this methodology is the change in the instrumental methodology. For the first group, the data acquisition began when the oven temperature had reached 60°C and the Micro-Cryo trap was not used to trap volatiles with low boiling points at the front of the GC column. However, in the remaining three groups, data acquisition began when the oven temperature was 35°C and a Micro-Cryo Trap was used. Thus, since dimethyl disulfide eluted at 35°C, it is possible that dimethyl disulfide was present in the first group of termites as well but that it was not detected because it eluted from the GC prior to data

acquisition. It should be noted that the first group was included in this analysis because other than the change in the instrumental methodology, it was sampled and analyzed as the remaining groups. Therefore, only compounds having boiling points less than 60°C would have been affected by the change.

A possible explanation for the fact that dimethyl disulfide was only detected in two of the eight subgroups tested in the following chapter is that the concentration of dimethyl disulfide produced from a decomposing termites may be at a much higher concentration than that produced from decomposing fecal material or secretions from the termites. Therefore, it is possible that the concentration of dimethyl disulfide theoretically produced by fecal material or secretions was below the detection limit of the instrument using the methodologies described herein.

In summary, although the results suggest that dimethyl disulfide is associated with decomposing Formosan termites, the results do not negate the possibility that it is also associated with active Formosan termites. Moreover, the fact that active termites of *R. tibialis* reportedly emit dimethyl disulfide raises a reasonable concern that dimethyl disulfide may also be associated with active termites of *C. formosanus*.

CHAPTER 2

QUALITATIVE AND QUANTITATIVE ANALYSIS OF VOLATILES DETECTED IN VESSELS CONTAINING ACTIVE FORMOSAN SUBTERRANEAN TERMITES WITHIN CARTON NESTS

INTRODUCTION

Previous research in the laboratory indicated that some volatiles were detected in treatments with active Formosan termites in carton nests but were absent in controls with neither carton nests nor Formosan termites (Henderson *et al.*, 2001). For example, volatiles tentatively identified as ethyl benzene, ethylmethylbenzene, and dichlorobenzene were present in treatments containing carton nests with active termites but were absent in controls (Henderson *et al*., 2001). In addition, volatiles from air within a Formosan termite nest were tentatively identified (Henderson *et al*., 1999). To verify these results and to determine if additional volatiles can be detected, the following hypothesis was tested: volatiles will be consistently present in treatments containing carton nests with active Formosan termites that are not present in controls that contain neither carton nests nor Formosan termites. A volatile is considered consistently present in the treatments but not the controls if it is present in the treatments but not the controls for different source colonies.

Several studies also indicate that there are volatiles in higher concentration in termite nests than in the ambient air (Seiler *et al*., 1984; Fraser *et al.*, 1986; Khalil *et al*., 1990; French *et al.*, 1997; Henderson *et al.*, 2001). For example, the concentration of methane was shown to be higher in the termite nests of *Coptotermes acinaciformis* Frogatt and *Coptotermes lacteus* Frogatt than in the ambient air (Fraser *et al.*, 1986). Methane and carbon dioxide were shown to be emitted from termite nests of species from genera including *Hodotermes*, *Macrotermes*, *Odontotermes*, *Trinervitermes*, *Cubitermes*, and *Amitermes* (Seiler *et al*., 1984). In addition, the

concentrations of carbon monoxide, chloroform, nitrous oxide were shown to be in higher concentrations in termite mounds than in the ambient air for several species of termites (Khalil *et al*., 1990). Furthermore, for the Formosan termite, the concentrations of naphthalene and butylated hydroxytoluene were reported to be in higher concentrations in treatments containing carton nests with active termites than in controls without carton nests or termites (Henderson *et al.*, 2001).

Studies also indicate that some volatiles are in lower concentrations in association with termites than in ambient air (Khalil *et al*., 1990; Stušek *et al*. 2000). For example, molecular hydrogen was shown to be in lower concentration within the termite mounds of *C. lacteus* than the ambient air (Khalil *et al*., 1990). Moreover, *Reticulitermes lucifugus* Rossi consume oxygen and the presence of the termites in wood was shown to be detectable respirometrically (Stušek *et al*. 2000).

Besides consumption by termites themselves, volatiles may be removed from the nest through the decomposition by microorganisms in the nests (Seiler *et al*., 1984). For example, it has been hypothesized that methane volatiles were decomposed by microorganisms present within the nests of the genera *Trinervitermes* (Seiler *et al*., 1984). In addition, bacteria are also known to biodegrade aromatic compounds (Bielefeldt and Stensel, 1999). Since microorganisms are known to be present in Formosan termite nests (Osbrink *et al*., 2001), it is possible that those microorganisms biodegrade volatiles and the nests act as an overall sink for those volatiles.

Taking into consideration the fact that volatiles may be in higher or lower concentrations within Formosan termite nests than in the ambient air, the following hypothesis was tested: The concentrations of volatiles will be significantly different in the treatments containing carton nests

with active Formosan termites than in controls that contain neither carton nests nor Formosan termites. The two methodologies that are described below were used for this purpose.

METHODOLOGY #2

Materials and Methods

Termites Two carton nests with active Formosan termites were collected, one in Westlake, LA on June 12, 2003 and the other in New Orleans, LA on March 8, 2003.

Sampling Protocol Except for the following changes, the sampling technique was the same as that used in chapter one. Two pieces of the carton nest with active termites were gently separated from the first colony from Westlake, LA and placed into each of two previously autoclaved Wheaton vessels (Figure 2).

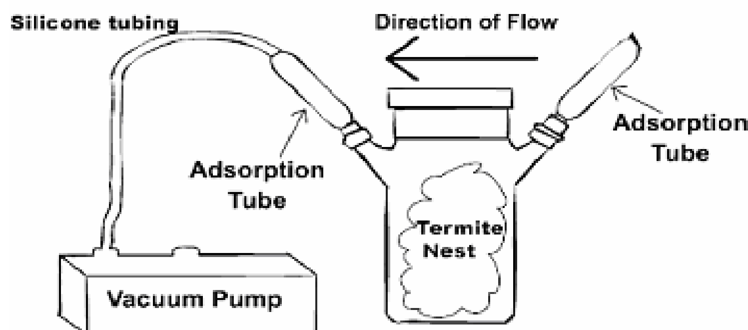

Figure 2. Set-up for the collection of volatiles facilitated by a vacuum pump and using Wheaton vessels to contain a carton nest with active Formosan termites.

The dimensions of the first piece of carton nest with active termites were 8.5 by 6.5 by 5.0 centimeters while the dimensions of the second piece were 16 by 7.5 by 8 centimeters. A third empty, autoclaved Wheaton vessel served as the control. The procedure was repeated for the second group from New Orleans, LA. Although the dimensions of each of the two pieces of carton nest for the second were not recorded, they were approximately the same size as that of

the first group. Each time the treatments and corresponding controls were sampled was considered a repetition and there were thirteen repetitions. The sampling times and thus the total volumes of air sampled for each repetition varied. However, this variation was corrected for by using the concentrations of the volatiles in units of nanograms per liter of air sampled (Appendix A, Table 9, Page 50). For each treatment and corresponding control, the same type of adsorbent resin, either multiple layered or only Tenax TA resins, was used. However, there were variations between the pairs (Appendix A, Table 9, Page 50). For the first group only, the percent relative humidity for the treatments and corresponding control was recorded three times at the completion of sampling with a Traceable® Hygrometer (Control Company, Friendswood, TX). The average readings for the treatments and control were respectively 100 and 46 percent. In addition, for qualitative analysis only and for five repetitions, the control for the first group collected from Westlake, LA was changed so that it contained three pieces of cypress tree wood (*Toxodium distichum* (L.) Rich.) from the original food source that had the following dimensions: 20.5 by 25 by 2 cm, 18 by 2.3 by 1 cm, and 15 by 21 by 0.6 cm.

Instrumental Methods After sampling, an internal standard consisting of a 1µl solution of a one hundred nanograms 2,6-diisopropylnaphthalene per one microliter of methanol was injected onto the resin in the adsorption tube. The SPTD/GC-MS parameters were identical to those used in the chapter one except for the following changes to the oven temperature program: 35°C, 5 min; 10°C / min, 80°C, 0.0 min; 4°C/min, 188°C, 0.0 min; 10°C / min, 260°C, 18 sec. The parameters for Micro Cryo-Trap™ were also identical to those used in chapter one.

Creation of a Calibration Curve A 250 ml azulene/methanol solution was prepared at a concentration of 100 ng/ul. A 10.0 mg sample of the solid naphthalene was transferred into a 10 ml volumetric flask. The 100 ng/ul azulene solution was added to the flask so that the total

volume was 10 ml. Starting with this solution mixture of 1000 ng/ul naphthalene and 100 ng/ul of azulene, a series of dilutions will be prepared down to a final concentration of 1.0 ng/ul of naphthalene using the 100 ng/ul azulene solution as the diluent. Stock solutions with the following concentrations of naphthalene in methanol were thus obtained: 1000 ng/ul, 500 ng/ul, 100 ng/ul, 50 ng/ul, 10 ng/ul, 5 ng/ul and 1 ng/ul. One microliter of the first solution was injected into a clean adsorption tube and was analyzed by SPTD-GC/MS. The procedure was repeated for each of the remaining solutions. Using X-caliber software, the following calibration curve was generated (Figure 3).

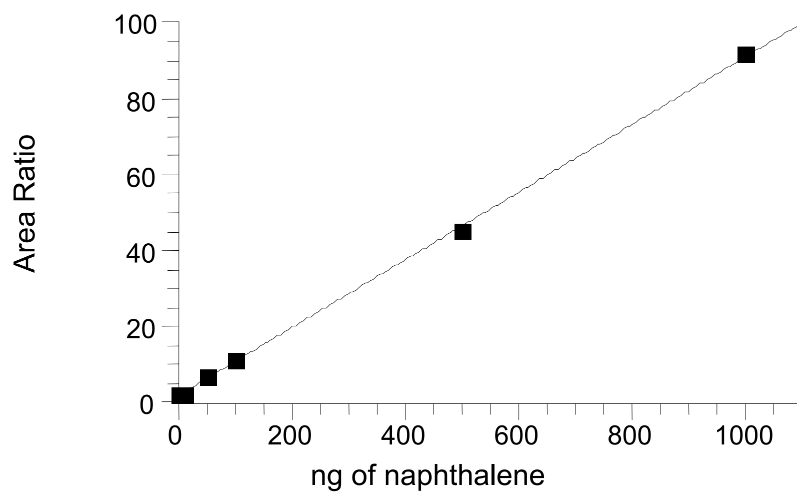

Figure 3. Calibration curve for naphthalene.

The concentrations of 5 ng/ul and 1 ng/ul were not detected. Therefore, only the concentrations from 10 to 1000 ng were used creating the calibration curve. The calibration curve was linear for the range of concentrations from 10 to 1000 ng (R^2 = 0.9997 where R^2 = correlation coefficient).

Data Analysis For qualitative analysis, mass chromatograms were run on each consecutive base peak from 35 to 300 daltons on the chromatographs of the treatments and the controls. For each peak of each mass chromatogram, the corresponding retention time and mass spectrum

were recorded. A profile of compounds in the chromatographs of the treatments and in the controls was then created.

For quantitative analysis, the procedure was the following. The peak area of the internal standard 2,6-diisopropylnaphthalene was determined by obtaining a mass chromatogram of peak 197, which is the base peak for 2,6-diisopropylnaphthalene, and then integrating that peak. The peak area for base peak of naphthalene, 128, was determined in the same way. A ratio was then obtained by dividing the peak area of naphthalene by the peak area of 2,6-diisopropyl-naphthalene. To convert units into nanograms per liter, the peak area ratio was then multiplied by the slope of the calibration curve and then divided by the total volume in liters of air sampled. To compare the concentrations of volatiles in treatments with the concentration of volatiles in the controls, a paired t-test was performed (SAS Version 8.2). The data were judged at $\alpha = 0.01$.

Results

Qualitative analysis indicated none of the compounds could be considered reporter molecules because the compounds could be classified into one of the following categories: 1) the compound was detected in both the treatments and controls; or 2) the compound was not consistently detected in the treatments but not the controls (Table 3; Appendix B, Table 10, Pages 51-52). It was noted that dimethyl disulfide was detected in a treatment but not the control in one of the subgroups collected on June 12, 2003 from Westlake, LA. However, its appearance in the chromatographs coincided with the death of the termites in that subgroup.

The mass spectrums of unknowns 47 and 48 were similar to the mass spectrums of manool (Table 4), which are known to be present in cypress tree wood (Scheffrahn *et al*., 1988). Furthermore, these compounds are only detected in treatments or controls in other methodologies

Table 3. Comparison of compounds detected in the treatments and controls. m/z =mass to charge ratio; G = group; C1 = control was an empty vessel; C2 = control was a vessel with wood; + =detection in treatment; ○ =detection in control; ● = detection in both treatment and control; - = not detected in treatment or control.

Compound Name or Unknown #	Retention time	m/z	Detection symbol – G5/C1	Detection symbol – G5/C2	Detection symbol – G6
1	0.63	40,42	●	●	●
2	1.30	44, 45	●	●	●
3	1.63	101,103	●	●	●
4	1.71	47	●	●	●
5	1.93	59, 43	●	●	●
6	2.51	107, 109, 75, 89, 181	●	●	●
7	5.03	91, 92, 65	●	●	●
8	6.95	83, 55, 99	-	○	-
9	6.97	229, 227, 151	●	●	●
10	7.82	91, 106, 77	●	●	●
12	10.80	281, 299, 267	●	●	●
13	10.59	120, 105, 77	●	●	●
14	11.06	146, 148, 111, 75	●	○	●
15	11.98	93,91,79,67, 121,136	●	○	●
16	11.52	117, 118, 115	+	●	●
17	14.34	119,134, 91	●	●	●
18	12.64	105,77,51,50	●	○	●
19	18.51	57,71,85,99	●	-	●
20	13.47	67, 57, 81, 143	●	●	●
21	14.13	355, 286, 267	●	●	●
22	13.48	132, 117, 115, 91	+	●	●
24	15.47	122, 107, 167, 182	+	+	-
Naphthalene	15.67	128,102	●	●	●
26	16.10	148, 117, 77	○	-	-
28	19.51	429, 73, 430, 445, 325, 341, 359, 149	●	●	●
29	26.23	142, 141, 115	○	-	○
30	21.63	123,95,109, 165,208	-	○	-
32	23.03	119, 133, 161, 204	○	●	●
34	23.80	156, 141, 115, 76	-	-	●
35	24.55	503, 519, 73, 281, 299, 149, 345	●	●	●
36	25.67	159, 131, 202	●	●	●
Butylated hydroxytoluene	26.12	205,220,177	-	●	+
40	27.54	173, 143, 71	●	+	●
41	34.80	149, 176	-	-	●
42	30.12	183,198,168	●	+	●
44	35.87	257,272,161	●	○	●
45	27.80	149, 150	-	-	●
47	41.73	255,270, 275	-	●	●
48	39.27	255, 159, 173, 270	+	●	●
51	41.35	262, 262	●	●	●

Table 4. Comparison of the mass spectrums of unknown 47 and 48 with that of manool, a compound to be present in cypress tree wood.

m/z of Unknown 47	m/z of Unknown 48	m/z of Manool
81, 95, 67, 275, 257	255, 159, 173, 270	137, 95, 257, 272

in which cypress tree or pine wood (*Pinus* sp.) was present (Table 3; Appendix B, Table 10, Pages 51-52). This indicated that the source of these volatiles was the wood used as the food source.

Quantitative analysis indicated that there were no significant differences in the concentrations of unknowns 2 through 5, unknown 10, unknown 13, unknown 28, unknown 29, unknown 35, naphthalene and butylated hydroxytoluene between the treatments and controls at the 0.01 level as determined by the paired t-test (Table 5; Figures 4-6).

Discussion

The results reported here indicate that there were no significant differences in concentrations of the eleven analyzed volatiles between the treatments and controls. Although

Table 5. Comparison of the concentrations between treatments and controls for unknowns 2 through 5, unknown 10, unknown 13, unknown 28, unknown 29, unknown 35, naphthalene, and butylated hydroxytoluene. * m/z = mass to charge ratio; T = t value; d.f. = degrees of freedom; P = p value.

Compound Name or Unknown #	m/z*	T	d.f.	P
Unknown #2	44, 45, 46	1.73	12	0.1090
Unknown #3	101, 103	-0.52	12	0.6157
Unknown #4	47, 48	0.37	12	0.7210
Unknown #5	59, 43	0.63	12	0.5403
Unknown #10	91, 106, 65, 77, 51	-2.12	12	0.0552
Unknown #13	105, 120, 77, 91	-1.76	12	0.1034
Unknown #28	429, 73, 430, 445, 325, 341, 359, 149	-2.04	12	0.0635
Unknown #29	142, 141, 115, 63	-1.38	12	0.1954
Unknown #35	503, 519, 73, 281, 299, 149	-1.28	12	0.2242
Naphthalene	128,102	-0.90	12	0.3874
Butylated hydroxytoluene	205, 220, 177	1.29	12	0.2228

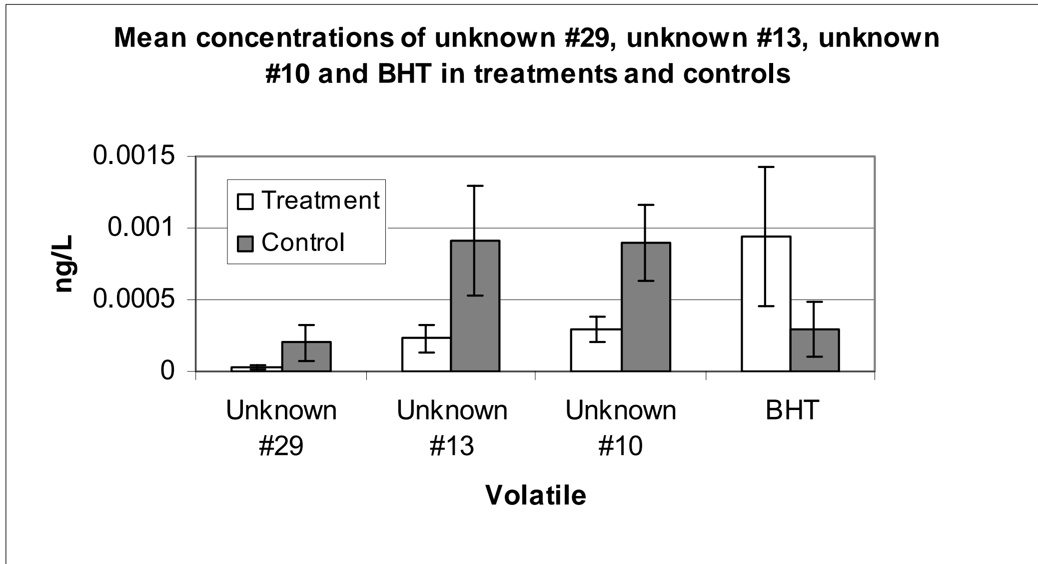

Figure 4. Mean ± SE concentrations of unknown #29, unknown #13, unknown #10 and butylated hydroxytoluene (BHT) in the treatments and controls.

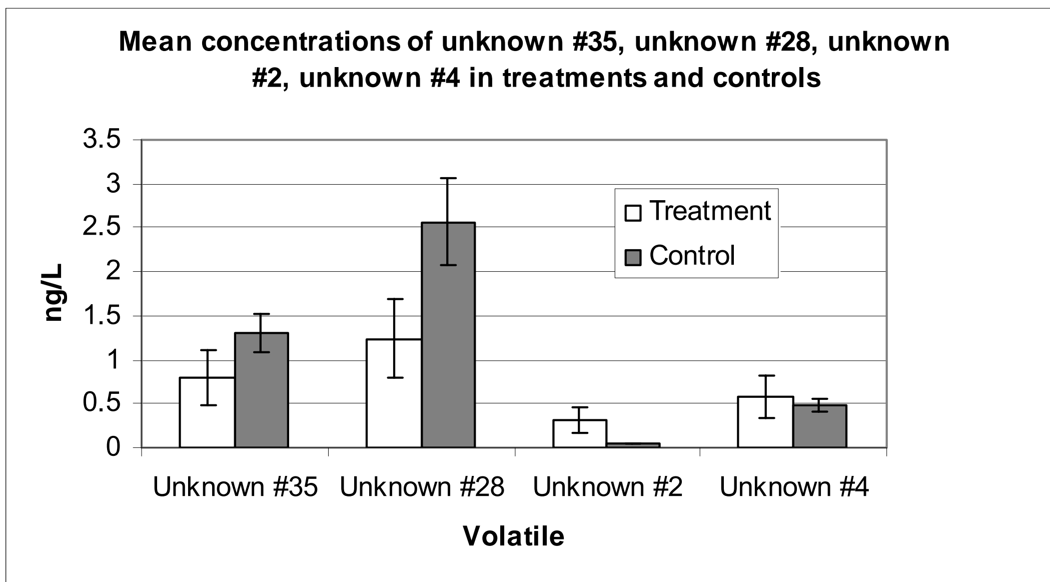

Figure 5. Mean ± SE concentrations of unknown #35, unknown #28, unknown #2 and Unknown #4 in the treatments and controls.

the differences were not significant for unknowns 10, 13, 28, and 29, there was a tendency for the concentration of these volatiles to be higher in the controls than the treatments (Table 5; Figures 4 and 5). Two possible explanations are proposed to account for this trend. First, it is

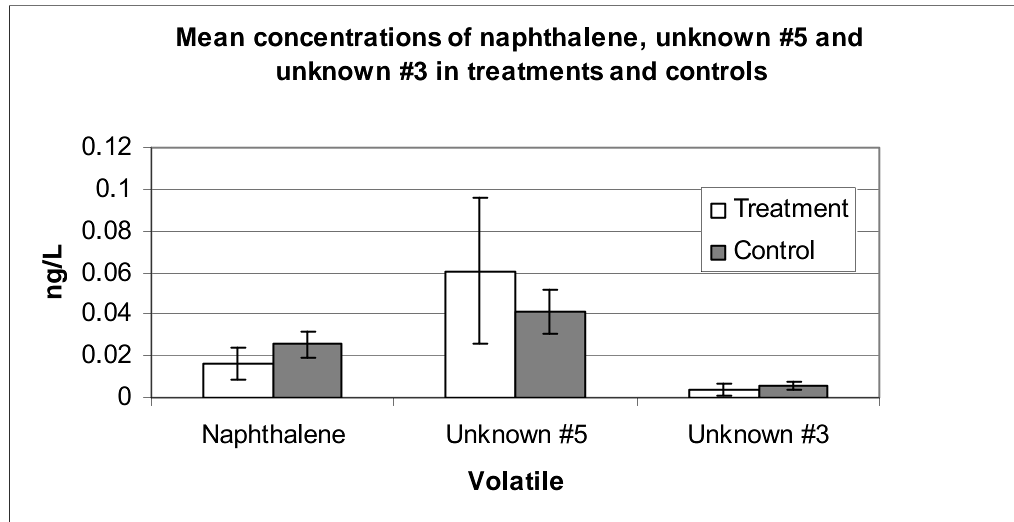

Figure 6. Mean ± SE concentrations of naphthalene, unknown #5, and unknown #3 in the treatments and controls.

possible that the carton nests were adsorbing the volatiles and thus decreasing the amount available for collection by the adsorbent resins. Second, it may be due to the differences in the relative humidity within the treatments and the controls. As indicated above, average relative humidity for the treatment of the carton nest with active termites from Westlake, LA was greater than that of the control, with respective readings of 100 and 46 percent. Furthermore, although the Tenax™ TA adsorbent resin is especially useful for trapping of volatiles from samples with high moisture content due to its low affinity for water (Scientific Instrument Services, 2004a), high moisture can cause pores of the resin matrix to be blocked and thus reduce the pores available for adsorption of other volatiles (Overton and Manura, 1999). Therefore, it is possible that less volatiles were trapped in the treatments than in the controls due to the higher relative humidity in the treatments than the controls. It should be noted that difference in relative humidity is not considered an error in experimental design because the relative humidity within air of a carton nest was naturally higher than the relative humidity in the ambient air and thus the design simulated field conditions.

There are limitations of this analysis, however, which are the following. First, in comparing the concentrations of volatiles in the treatments versus that in the controls, a paired t-test was used, a test that assumes that that each repetition is independent. In this analysis, each of the four treatments was independent but the repeated measures taken from each treatment were not independent. However, since the paired t-test is robust, it may be used for the analysis but the results should be interpreted with caution (Susanne Hoeppner and Dr. James Geahgan, Louisiana State University, personal communication). Second, it was assumed that the use of a paired t-test would compensate for the fact that between the repetitions, either the multiple layer or Tenax TA resins were used. This assumption is theoretically correct since the adsorbent resin for each pair of treatment and corresponding control was the same. Therefore, the differences between the concentrations of volatiles collected in the treatment versus that collected in the control are comparable. Furthermore, while the range of volatiles the were collected could potentially increase by using the multiple layer rather than the Tenax TA resin only, there would be not be a major effect in the concentrations of the volatiles collected (Robert Frey, Scientific Instrument Services, personal communication). Third, it was assumed if there was variation in the concentration of the volatiles caused by differences in the size of the carton nests between the group collected from Westlake, LA and the group collected from New Orleans, LA, then it was negligible. This is supported by the fact that for each of the analyzed volatiles, there were not significant differences between the two groups at the 0.01 level as determined by the t-test. Fourth, the total volume of air sampled for each repetition varied. However, this variation was corrected for by using the concentrations of the volatiles in units of nanograms per liter of air sampled. In order to make this correction, however, it was assumed that there is a linear relationship between the amount of the volatile collected and the total volume of air sampled.

For reasons that will be explained in chapter three, the Nalgene 550 platinum-cured silicone tubing was replaced with fluorinated ethylene propylene (FEP) teflon tubing in the experimental set-up. The methodology using FEP teflon tubing in the experimental set-up was as follows.

METHODOLOGY #3

Materials and Methods

Termites Two carton nests with active Formosan termites were collected, one in Westlake, LA on August 12, 2003 and the other in New Orleans, LA on May 16, 2003. Each carton nest was divided into two subgroups for a total of four subgroups. The number of termites in each subgroup was determined by dividing the total weight of the termites within each subgroup by the average weight of three sets of 50 termites within each subgroup. The numbers of termites in the first and second subgroup collected from New Orleans, LA were approximately 3,460 and 5,063 termites. Since these carton nests of these subgroups appeared dry, each received along with their corresponding control 20 milliters of deionzed, autoclaved water. The numbers of termites in the first and second subgroups collected from Westlake, LA were approximately 1,228 and 2,675. So that air samples were taken from intact carton nests, the numbers of termites within piece of carton nest (subgroup) were counted after sampling was completed.

Sampling Protocol The procedure was the same as that used in the previous methodology except for the following changes. A 40 cm piece of Fluorinated Ethylene Propylene (FEP) teflon tubing was connected at one end to the Welch vacuum pump and the other end to an adsorption tube. The adsorption tube was placed at this location because contaminating volatiles were suspected to have originated from the Welch vacuum pumps. However, further analysis suggested that those volatiles originated silicone tubing (See chapter three). This adsorption tube

was in turn connected to another adsorption tube used to collect volatiles flowing out of the vessel (Figure 7).

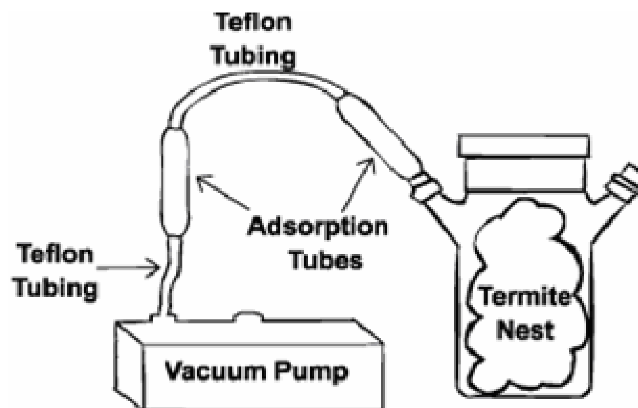

Figure 7. Set-up for the collection of volatiles from a Formosan termite nest with active termites.

For each group of termites each having two subgroups, an empty vessel served as a control. Each time the treatments and corresponding controls were sampled was considered a repetition and there were thirteen repetitions. For all but one of the repetitions, volatiles flowing out of the vessels were sampled for 18 hours. One repetition was sampled for 19 hours, which was corrected for since the amount of each volatile was divided by the total volume of air sampled. The teflon tubing used to connect the adsorption tube to the Welch® vacuum pump was washed with deionized water and then dried with compressed air from a cylinder (BOC Gases, Murray Hill, NJ) between each repetition. In addition, for qualitative analysis only and seven repetitions, the control from the first group collected from New Orleans, LA consisted of a vessel with three pieces of pine wood (*Pinus* sp.) having the following dimensions in centimeters: 10.5 by 3.5 by 3.5, 10.5 by 3.5 by 3.5 and 15 by 3.5 by 3.5.

Instrumental Methods The instrumental methods were identical to those used in methodology #2.

Data analysis The qualitative and quantitative data analysis of the treatments and controls was identical to that used in methodology #2. In addition, to check if some of the light hydrocarbons that have been associated with other species of termites were detected in this study, mass chromatograms for the base peaks of propene, propane, and butane were run on the chromatographs of the treatments and controls for this methodology as well as for methodologies one and two.

Results

Qualitative analysis indicated none of the compounds could be considered reporter molecules because each of the compounds could be classified into one of the following categories: 1) the compound was detected in a treatment but not a control but not on a consistent basis as defined above; 2) the compound was detected in both the treatments and controls in this methodology; 3) the compound was detected only in the treatments using this methodology but both in the treatments and controls using other methodologies (Table 6; Appendix B, Table 10, Pages 51-52). It was noted, however, that dimethyl disulfide was detected in a treatment but not the control in one subgroup of the carton nest collected on May 16, 2003 from New Orleans, LA (Table 6). In that subgroup, it was observed that along with the healthy, active Formosan termites, some dead termites, which were killed when the termites were transferred to the vessel, were present.

The mass spectrums of propene, propane, and butane did not match any of the mass spectrums of the compounds listed in tables one, three and six. Furthermore, analysis by mass chromatograms for the base peaks of each of the three volatiles on the chromatographs of the treatments and controls in this methodology as well as methodologies one and two indicated that the volatiles were not present.

Table 6. Comparison of compounds detected in the treatment and control. m/z =mass to charge ratio; G group; C1 = control was an empty vessel; C2 = control was a vessel with wood; + =detection in treatment; ○ =detection in control; ● = detection in both treatment and control; - = not detected in treatment or control.

Compound Name or Unknown #	Retention time	m/z	Detection symbol – G7/C1	Detection symbol – G7/C2	Detection symbol – G8
1	0.63	40,42	●	●	●
2	1.30	44, 45	●	●	●
3	1.71	101, 103	●	●	●
4	1.78	47	●	●	●
5	2.15	59, 43	●	●	●
6	2.51	107, 109, 75, 89, 181	●	●	●
Dimethyl disulfide	4.93	94, 45, 79, 61	+	-	-
7	5.63	91, 92	●	●	●
9	7.00	229, 227, 151	●	●	●
10	7.82	91, 106, 77	●	●	●
12	10.78	281, 299, 267	●	●	●
13	11.19	120, 105, 77	●	●	●
14	11.74	146,148, 111, 75	●	-	●
15	11.98	93,91,79,67, 121,136	●	●	●
16	12.15	117,118,115,91	●	●	●
17	12.71	119,134, 91	●	●	●
18	12.91	105,77,51,50	●	●	●
19	13.73	57, 71, 85	●	+	-
20	14.39	67, 57, 81, 143	-	○	●
21	14.73	355, 286, 267	●	●	●
23	15.26	132, 117, 115, 91	●	●	●
24	16.22	122,107,167, 182	-	-	+
25	16.40	131,91, 115, 146	-	-	●
Naphthalene	16.49	128,102	●	●	●
26	16.99	148, 147, 117	-	○	+
27	19.50	159, 128, 174	+	+	-
28	19.72	429, 73, 430, 445, 325, 341, 359, 149	●	●	●
29	20.06	142, 141, 115	○	○	●
32	29.38	91, 107, 161, 204	-	-	+
35	24.73	503, 519, 73, 281, 299, 149, 345	●	●	●
Butylated hydroxytoluene	25.97	205,220,177	+	○	+
36	26.50	159, 131, 202	-	○	-
37	27.00	157, 200, 142	-	-	+
40	28.36	173, 243, 71	●	○	●
42	30.00	183,198,168	-	-	+
43	31.86	181, 210, 165	-	○	-
44	37.59	257,272,161	+	●	●
47	38.81	81, 95, 67, 275, 257	-	○	+

Table 6 Continued

Compound Name or Unknown #	Retention time	m/z	Detection symbol – G7/C1	Detection symbol – G7/C2	Detection symbol – G8
48	39.43	255, 159, 173, 270	-	○	+
51	43.02	262, 263, 261	+	-	+

Quantitative analysis indicated that there were no significant differences in the concentrations for unknowns 2 through 5, unknown 10, unknown 13, unknown 29, unknown 35, naphthalene and butylated hydroxytoluene between treatments and controls at the 0.01 level determined by the paired t-test (Table 7; Figures 8-11).

Discussion

Quantitative analysis indicated that there were no significant differences in concentrations of the nine unknown compounds, butylated hydroxytoluene and naphthalene between the treatments and controls at the 0.01 level as determined by the paired t-test. However, naphthalene and unknown #29, with respective p-values of 0.067 and 0.102, had general tendencies to be in higher concentrations in the controls than the treatments (Table 7; Figures 9 and 10). As elaborated in the previous method, possible causes for the tendencies are that

Table 7. Comparison of the concentrations between treatments and controls for unknowns 2 through 5, unknown 10, unknown 13, unknown 29, unknown 35, naphthalene, and butylated hydroxytoluene. * m/z = mass to charge ratio; T = t value; d.f. = degrees of freedom; P = p value.

Compound Name or Unknown #	m/z*	T	d.f.	P
Unknown #2	44, 45, 46	0.86	12	0.4049
Unknown #3	101, 103	-0.75	12	0.4660
Unknown #4	47, 48	0.08	12	0.9369
Unknown #5	59, 43	-0.90	12	0.3862
Unknown #10	91, 106, 65, 77, 51	1.13	12	0.2800
Unknown #13	105, 120, 77, 91	1.15	12	0.2732
Unknown #28	429, 73, 430, 445, 325, 341, 359, 149	0.25	12	0.8041
Unknown #29	142, 141, 115, 63	-1.77	12	0.1019
Unknown #35	503, 519, 73, 281, 299, 149	0.50	12	0.6264
Naphthalene	128,102	-2.01	12	0.0670
Butylated hydroxytoluene	205, 220, 177	2.28	12	0.0416

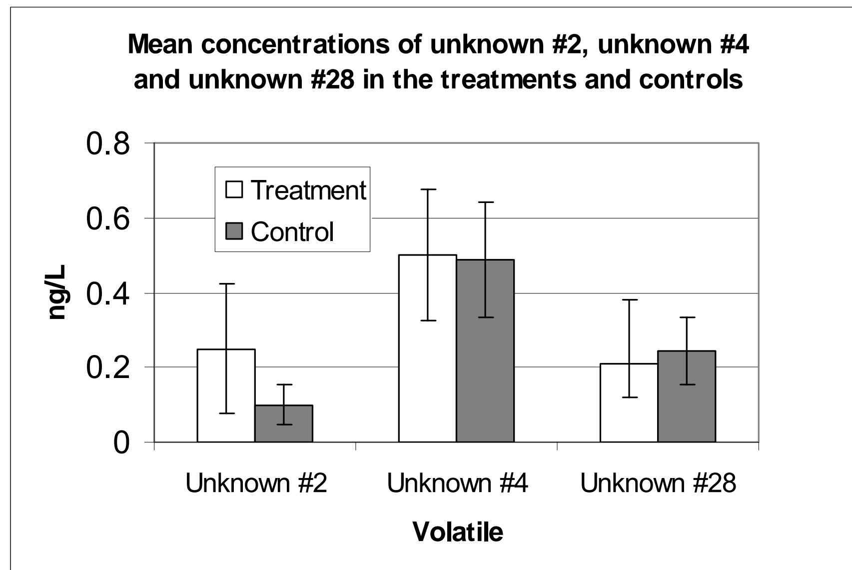

Figure 8. Mean ± SE concentrations of unknown #2, unknown #4 and unknown #28 in the treatments and controls.

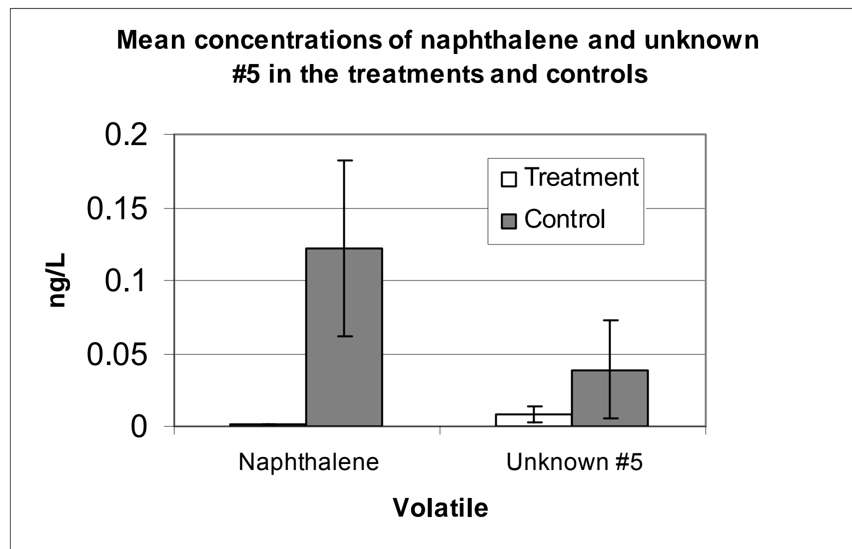

Figure 9. Mean ± SE concentrations of naphthalene, and unknown #5 in the treatments and controls.

carton nests were adsorbing the volatiles or that the air in the treatments had a higher relative humidity than that in the controls. In addition, with a p-value of 0.042, there was a tendency for butylated hydroxytoluene to be higher in the treatments than in the controls (Table 7, Figure 10).

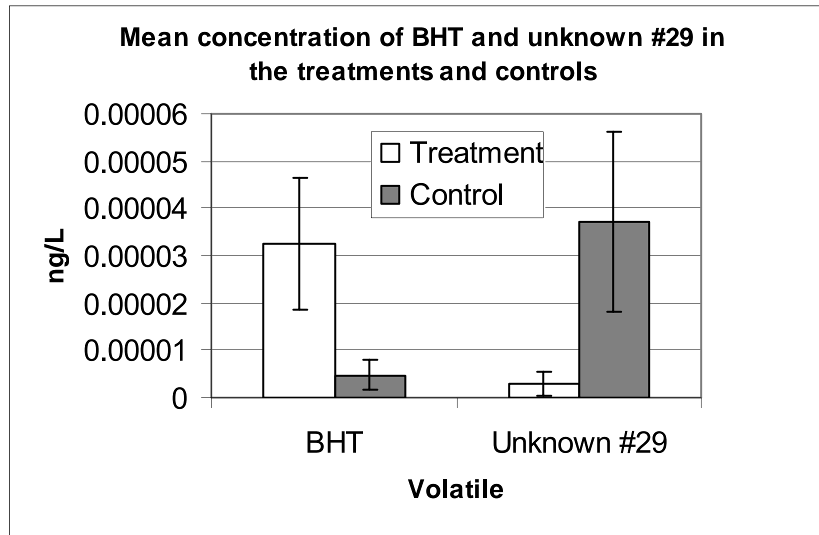

Figure 10. Mean ± SE concentrations of BHT and unknown #28 in the treatments and controls.

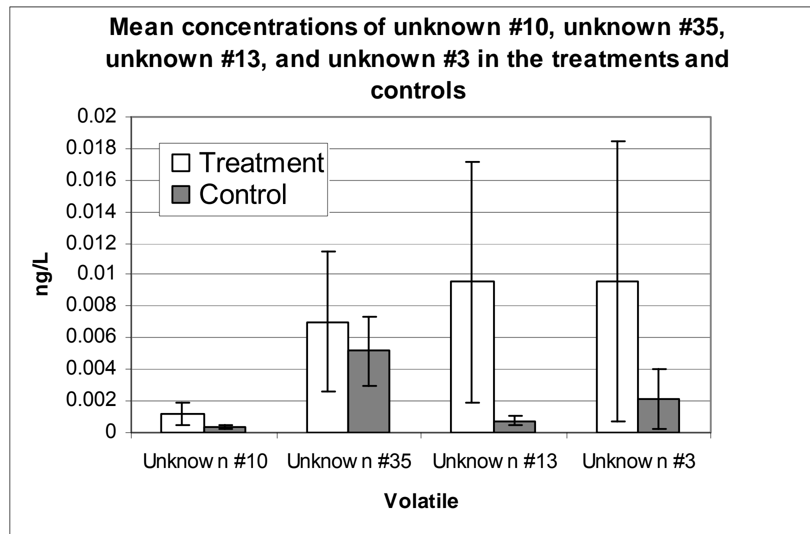

Figure 11. Mean ± SE concentrations of unknown #10, unknown #35, unknown #13 and unknown #3 in the treatments and controls.

However, butylated hydroxytoluene is a frequently occurring contaminant in GC/MS analysis because it is used as an antioxidant in plastics and as a stabilizer in technical grade mixtures of isomers (Hübschmann, 2001). Therefore, it is likely that the source of this compound in the chromatographs is due to contamination.

The results are important because in a previous report unknown #10 and unknown #13, which were tentatively identified as ethyl benzene and ethylmethylbenzene, were listed as being potential reporter molecules because they were present in treatments containing carton nests with Formosan termites but were absent in controls (Henderson *et al*., 2001). The results presented here indicated that these volatile were consistently present in both the treatments and controls (Appendix B, Table 10, Pages 51-52). Furthermore, there were no significant differences in these volatiles concentrations in the treatments and controls even after a source of these volatiles was eliminated from the experimental set-up (See chapter three).

The results are also important because it conflicts with a previous report that naphthalene volatiles were in higher concentrations in samples of air from carton nests with active termites than in controls without termites or carton nests (Henderson *et al*., 2001). It is important to note, however, that the results do not conflict with another study that indicated that naphthalene was present in the carton nests of Formosan termites (Chen *et al.*, 1998).

There are limitations of this analysis, however, which include the following. First, it was assumed that each repetition was independent. Second, it was assumed that the paired t-test would compensate for use of either multiple layered or Tenax TA resins between repetitions. Third, it was assumed that the effect of the addition of 20 milliters of water to the control of one group but not to the other was negligible. Fourth, it was assumed that the washing the teflon tubing with water and the then drying with compressed air between repetitions removed all of the volatiles that may have adhered to the tubing. Fifth, it was assumed that if the differences in the numbers of termites within the carton nest collected from Westlake, LA and the carton nest collected from New Orleans, LA caused variation in the concentration of the volatiles, then it

was negligible. This is supported by the fact that for each of the analyzed volatiles, there were not significant differences between the groups at the 0.01 level as determined by the t-test.

Although there are limitations to this analysis, the results reported here are considered more reliable for the following reasons. First, the adsorption tube resins were cleaned according to the specifications of the manufacturer prior to use. Second, the concentrations of each volatile were calculated using an internal standard. Third, the total volumes of air sampled in the treatments and the corresponding controls were included in the calculation of the concentrations. Finally, each of the treatments had a contemporaneous control.

When comparisons of this study with similar studies on termite species other than the *C. formosanus* are made, the following points were noted. First, some of the volatiles that have been associated with the activity of other species of termites would not be detected using the methods described in this study even if those volatiles are also associated with active Formosan termites. For example, carbon dioxide, carbon monoxide, nitrous oxide, molecular hydrogen and oxygen cannot be trapped by the adsorbent resins used in this study, which include carboxen 569, Tenax TA and glass beads (John Manura, Scientific Instrument Services, personal communication). Furthermore, molecular hydrogen could not be detected by the mass spectrometer since the lowest mass that can be scanned by the mass spectrometer is ten daltons.

The second important point is that three of the volatiles associated with other species of termites, namely propene, propane, and butane, can be trapped using carboxen 569 and Tenax TA and be detected by a mass spectrometer (Scientific Instrument Services, 2004a; and Scientific Instrument Services, 2004b). However, the volatiles were not detected in the chromatographs of treatments or controls in any of the methodologies of this study.

Consequently, in future analysis, the following changes to the methodology are recommended. First, for the potential collection of carbon dioxide, carbon monoxide, nitrous oxide, molecular hydrogen and oxygen, samples of air from an active Formosan termite nest should be collected either by a gas tight syringe as described by Fraser *et al*., 1986 or into stainless steel flasks as described by Khalil *et al.*, 1990. These methods of sampling are also recommended for those volatiles that can be trapped by adsorbent resins for the following reason. Since the range of volatiles associated with active Formosan termites are not known, the breakthrough volumes of the volatiles, which are the volumes of gas that will purge the volatiles off the adsorbent resins, are not known either. Thus, the optimal sampling volume is not known and it is possible that some of the volatiles associated with Formosan termites will be trapped but then subsequently purged off the resins. This possibly occurred in this study because the sampling volumes typically exceeded 15,000 liters and the breakthrough volume of n-butane on 100 mg of Tenax TA is 7.94 liters (Scientific Instrument Services, 2004a). However, by sampling with syringes or flasks, it can be certain that all of the volatiles that are present will be collected and subsequently analyzed. In addition, a second recommended change in the methodology is that in order to detect molecular hydrogen, a flame ionization detector should be used.

A third recommended change in the methodology is sampling from a larger carton nest with a greater number of active Formosan termites. In this study the number of termites ranged from 1,228 to 5,063 individuals. In the study by Khalil *et al*., which was the study that detected the greatest number of volatiles associated with termites, the samples were taken from termite mounds of *C. lacteus* in the field. In their study, the number of termites was not recorded,

however, it is known that colonies of *C. lacteus* can produce approximately one million individuals (Krishna and Weesner, 1969).

Also in terms of method development, software development may be necessary for both qualitative and quantitative analysis. For qualitative analysis, the running of mass chromatograms for each base peak was an effective method for determining the presence of all of the compounds in each chromatogram and thus for making qualitative comparisons between chromatographs of the treatments and controls. However, the process is very time consuming. Thus, development of software is needed to facilitate this analysis. Regarding quantitative analysis, the X-caliber software used in this analysis is designed to determine a concentration of a compound only when a calibration curve for that compound is created (Richard White, Thermo Electron Corporation, personal communication). To accomplish this for this study requires the following steps: First, an initial phase of sampling and instrumental analysis to determine what compounds are present; Second, the identification of those compounds by standards; Third, the selection of internal standard for each compound with the subsequent creation of calibration curves for each compound using those standards; and Finally, the inclusion the internal standards each time samples from treatments and controls are run. This process is unnecessary since the main objective is to determine if there is a difference between the concentrations of compounds detected in the treatments versus those detected in the controls rather to determine the actual concentrations. Consequently, the main objective can be met with the use of one to three internal standards and appropriate software development.

CHAPTER 3

ANALYSIS OF A SOURCE OF SUSPECT REPORTER MOLECULES

INTRODUCTION

Three suspect reporter molecules, naphthalene, unknown #10 and unknown #13 were consistently detected in the controls (Appendix B, Table 10, Pages 51-52). Therefore, there was a contaminating source of those volatiles. If this contaminating source is eliminated or reduced in the analysis, it may assist in determining whether the above volatiles are associated with Formosan termite nests with active termites. For example, it is possible carton nests with active Formosan termites contain a higher concentration of naphthalene volatiles than the ambient air (Henderson *et al*., 1999). However, it is also possible there was contamination of naphthalene volatiles in the experimental set-up that is not normally present in the ambient air. If this is true, the naphthalene volatiles present due to contamination in the experimental set-up may be significantly higher than the concentration of naphthalene associated with the active Formosan termite nest thereby making the quantization of the concentration of naphthalene volatiles associated with the nest very difficult.

In this study, the source of contaminating volatiles was suspected to be unwashed Nalgene 550 platinum-cured silicone tubing. In addition, it was suspected that the contaminating volatiles were not present in fluorinated ethylene propylene (FEP) teflon tubing. Therefore, the following hypothesis was tested: The concentrations of some of volatiles that are collected when unwashed Nalgene 550 platinum-cured silicone tubing is used in the experimental set-up will be significantly greater than when fluorinated ethylene propylene (FEP) teflon tubing is used in the experimental set-up.

MATERIALS AND METHODS

Termites

The two treatments and the control for the carton nest collected in New Orleans, LA on March 8, 2003 that were used in methodology #2 were used again.

Sampling Protocol

The concentrations of three volatiles were compared using three different procedures. The sampling apparatus was set-up so that air from outside was used to replace air vacuumed out of the experimental vessels. This was accomplished by attaching unwashed silicone tubing measuring 80 cm in length to the inlet adsorption tube of the vessels and allowing the free end of the tubing to hang outside of a window. Rather measuring the volatiles trapped in the adsorption tube that collects volatiles flowing out of the vessel, the volatiles trapped in the adsorption tube that collects volatiles flowing into the Wheaton vessels were measured (Figure 12).

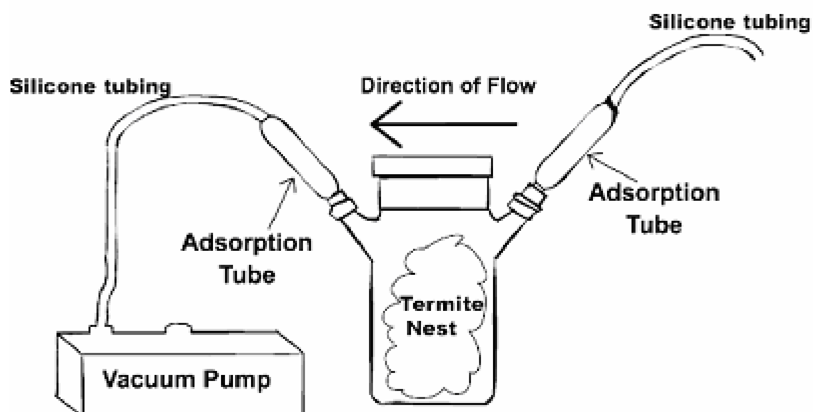

Figure 12. Set-up modified for the collection of volatiles from unwashed silicone tubing and other contaminants flowing into the vessel.

The treatments were sampled five times and the control three times. It was assumed that materials present downstream from the adsorbent resin used for this analysis, which includes the vessels with termite for the treatments and the vessel without the termite nests for the control,

had a negligible affect. Therefore, the treatments and controls were pooled making a total of eight repetitions.

The experimental set-up for the second procedure was the same at that above except for the following changes. Rather than using unwashed silicone tubing to connect the inlet adsorption tube of the vessels to the outside air and the outlet adsorption tube to the Welch® vacuum pump, fluorinated ethylene propylene (FEP) teflon tubing measuring 50 cm in length was used (Figure 13). For this procedure, there were three repetitions.

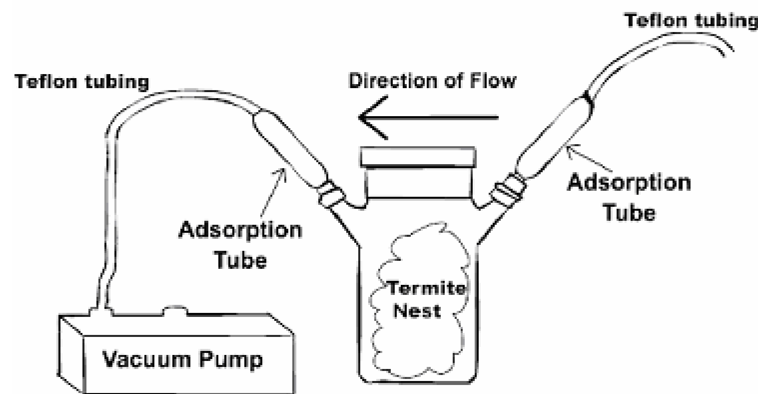

Figure 13. Set-up modified for the collection of volatiles from teflon tubing and other contaminants flowing into the vessel.

The experimental set-up for the third procedure was the following. First, a Welch vacuum pump was connected by 15 cm of fluorinated ethylene propylene FEP teflon tubing to an adsorption tube. Second, this adsorption tube was in turn connected by 15 cm of FEP teflon tubing to a second adsorption tube. Third, the second adsorption tube was connected to an additional 15 cm of FEP teflon tubing, which hung outside of a window (Figure 14). The concentration of volatiles from air outside of a window and from FEP teflon tubing approximately 15 cm in length that flowed into the second adsorption tube were measured.

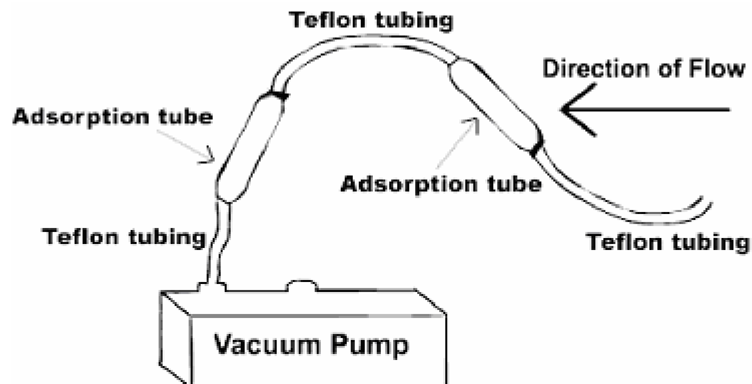

Figure 14. Set-up modified for the collection of volatiles from teflon tubing and other contaminants flowing.

For the third procedure, there were six repetitions. The teflon and silicone tubes were replaced each time a sample was taken. There was variation in the total volume of air sampled (Appendix A, Table 9, Page 50). However, this was corrected for by using the concentrations of the volatiles in units of nanograms per liter of air sampled. It was assumed that there is a linear relationship between the amount of the volatile collected and the total volume of air sampled.

Instrumental Methods

For the procedures one and two, the GC/MS parameters were as follows: 35°C, 5 min; 10°C / min, 80°C, 0.0 min; 4°C/min, 188°C, 0.0 min; 10°C / min, 260°C, 18 sec. In addition, the Micro Cryo-Trap™ was set at a temperature of -175°C during the desorption process after which it was ballistically heated to 250°C. For method three, GC/MS parameters were as follows: 35°C, 5 min; 10°C / min, 260°C, 0.0 min. In addition, the Micro Cryo-Trap™ was not used.

Data Analysis

95 % confidence intervals were used to determine if the concentrations of volatiles detected using the first procedure was significantly different from the concentrations of the same volatiles detected using the third procedure (SAS Version 8.2).

RESULTS

The concentrations of naphthalene, unknown #10, and unknown #13 were significantly higher using first procedure in which volatiles from outside air and unwashed silicone tubing were analyzed than the third procedure in which volatiles from outside air and teflon tubing were analyzed as determined by 95% confidence intervals. In fact, the differences were a approximately thousand fold for each volatile (Table 8; Figure 15).

Table 8. The was significantly higher concentrations of naphthalene, unknown 10, and unknown 13 detected using procedure one than detected using procedure three as determined by 95% confidence intervals.

Compound Name	Concentrations (ng/L) for Procedure one	Concentrations (ng/L) for Procedure three
Naphthalene	0.1591 (0.0203-0.2979)	0.0003 (0.0001 – 0.0004)
Unknown 10	0.271 (0.0586-0.4834)	0.0001 (- 2.7 $*10^{-7}$ - 0.0002)
Unknown 13	0.203 (0.0393-0.3666)	0.0002 (6.09 * 10-5 – 0.0003)

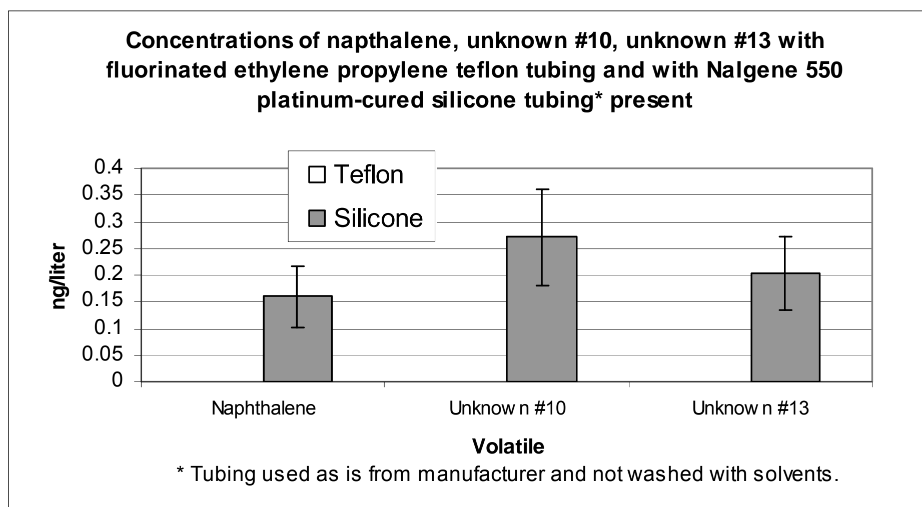

Figure 15. Mean ± SE concentrations of naphthalene, unknown #10 and unknown #13 for procedure three in which fluorinated ethylene propylene (FEP) teflon tubing was used and for procedure one in which unwashed Nalgene 550 platinum-cured silicone tubing was used.

Furthermore, naphthalene, and unknown #13 were not detected in the three repetitions using procedure two in which volatiles from outside air and FEP teflon tubing were analyzed while trace levels of unknown #10 were detected.

DISCUSSION

The concentrations of naphthalene, unknown #10, and unknown #13 were significantly lower in procedure three, in which volatiles from outside air and FEP teflon tubing were analyzed, than in procedure one, in which volatiles from outside air and Nalgene 550 platinum-cured silicone tubing were analyzed. Furthermore, naphthalene and unknown #13 were not detected while only trace levels of unknown #10 were detected using procedure two, in which volatiles from outside air and FEP teflon tubing were analyzed. These results suggest that a source of the naphthalene, unknown #10, and unknown #13 was the Nalgene 550 platinum-cured silicone tubing.

It was noted, however, that when sampling was done in the laboratory and silicone tubing was not used in the experimental set-up as in methodology #2 of chapter #2, the mean concentration of naphthalene detected in the controls was $1.22 * 10^{-1}$ ng/l, which is comparable to the mean concentration of naphthalene detected in procedure one, $1.59 * 10^{-1}$ ng/l. This was not true for unknown #10 and unknown #13 since the volatiles mean concentration in the controls in methodology #2 were respectively $2.99 * 10^{-4}$ ng/l and $7.34 * 10^{-4}$ ng/l while their mean concentrations in procedure one were respectively $2.71 * 10^{-1}$ ng/l and $2.03 * 10^{-1}$ ng/l. Therefore, another contaminating source of naphthalene volatiles is likely, possibly being air in the laboratory. (Statistical analysis was not conducted on the data sets because more than one variable had changed, namely that volatiles flowing into the vessel were analyzed in procedure #1 while volatiles flowing out of the vessel were analyzed in methodology #2).

It is possible that one or more of the volatiles originating from Nalgene 550 platinum-cured silicone tubing could be removed by washing with solvents. However, according to the manufacturer, Nalgene 550 platinum-cured silicone tubing is not marketed for volatile analysis and the company does not have a washing procedure for this application (Dan O'Nare, Nalgene®

Labware, personal communication). However, extractables have been reported from DOW CORNING® brand Pharma tubing, which is also platinum-cured silicone tubing (Malczewski and Inman, 2003). Using acetone as a solvent, a number of unidentified residues were extracted (Malczewski and Inman, 2003). Thus, it is possible that one or more of the contaminants detected in this study can be removed by washing with acetone. However, since the author was not aware of this procedure at the time of analysis, this option was not explored.

Since there is not an established protocol for the washing of Nalgene 550 platinum-cured silicone tubing to analyze volatiles, a protocol must be developed if it is to be used in experimental set-up of a future investigation of volatiles associated with Formosan termites. This procedural development can be avoided, however, if teflon tubing rather than silicone tubing is used in the experimental set-up. In addition to the results of this study, several studies show that teflon tubing can be used in volatile analysis (Zimmerman *et al.*, 1982; Fraser *et al.*, 1986; Justus and Cardé, 2002). For example, teflon tubing has been used to sample volatiles from termite mounds (Fraser *et al.*, 1986).

To make the quantitative comparisons of volatiles between the first and third procedures, it was assumed that the use of the Micro Cryo-Trap™ in first procedure but not in third procedure did not significantly affect the analysis. Two lines of evidence can be used to support this assumption. First, all of the analyzed volatiles were effectively trapped without the Micro Cryo-Trap™, as indicated by their presence in the results of the first group of the first methodology in chapter one (Table 1; Appendix B, Table 10, Page 51). Second, although the Micro Cryo-Trap™ was not used in the third procedure, it was used in both first and second procedure.
When comparing the results of first and second procedure, it was clear that the concentrations of the volatiles were greater when using the first procedure than when using the second procedure

because naphthalene and unknown #13 were not detected while only trace levels of unknown #10 were detected when using the second procedure.

SUMMARY AND CONCLUSIONS

Qualitative comparisons were made between compounds detected in treatments containing Formosan termites versus those detected in controls without termites. Except for dimethyl disulfide, none of the compounds were consistently detected in the treatments but not the controls. However, in each of the three different groups that dimethyl disulfide was detected, dead termites that were decomposing along with active termites were present. Therefore, none of the compounds could be classified as volatiles associated with active Formosan termites. However, active termites of a related termite species, *Reticulitermes tibialis* Banks, reportedly emit dimethyl disulfide. Therefore, more research is recommended to determine if dimethyl disulfide is also associated with active Formosan termites.

Qualitative and quantitative comparisons were made between compounds detected in treatments that contained carton nests with active Formosan termites and controls that contained neither Formosan termites nor carton nests. Two methods were used, one using unwashed Nalgene 550 platinum-cured silicone tubing and the other using unwashed fluorinated ethylene propylene (FEP) teflon tubing in the experimental set-up. Qualitative analysis for both methods indicated that none of the compounds could be consistently detected in treatments but not the controls and therefore could be classified as volatiles associated with active Formosan termites. It was noted, however, that dimethyl disulfide was detected in two of the eight subgroups tested but that dead termites that were decomposing were present in each of those subgroups.

Quantitative analysis for both methods indicated that the concentrations of naphthalene, and butylated hydroxytoluene and nine unknown volatiles were not significantly different between the treatments and controls at the 0.01 level as determined by the paired t-test. Therefore, none of

the quantitatively analyzed compounds could be classified as volatiles associated with active Formosan termites.

Although the differences were not significant, there was a tendency for the concentration of naphthalene and several unknown volatiles to be higher in the controls than the treatments. The tendencies may be due to higher relative humidity in the treatments than the controls because in samples with high moisture content, the pores of the Tenax™ TA adsorbent resin may be blocked by water molecules. Therefore, for future analysis, steps should be taken to minimize this effect. Since, however, the adjustment of the relative humidity so that it is equal in the treatments and controls would not simulate field conditions, another sampling technique is recommended such as sampling with syringes as described by Fraser *et al*., 1986.

The concentrations of three suspect reporter molecules, which include naphthalene and two unknown compounds, were significantly lower using fluorinated ethylene propylene (FEP) teflon tubing than using unwashed Nalgene 550 platinum-cured silicone tubing in the experimental set-up as determined by 95% confidence intervals. This suggested that a source of these volatiles was the unwashed silicone tubing, which was relevant to this study because it aided in determining whether the volatiles were associated with active Formosan termites.

Using the methods described herein, none of the volatiles could be classified as volatiles associated with active Formosan termites. However, changes in the methods may enable volatiles associated with active Formosan termites to be detected. These changes include the sampling from a larger nest size with a correspondingly greater number of active Formosan termites, the direct injection of air from nests into the gas chromatograph, and the use of a flame ionization detector in conjunction with a mass spectrometer detector.

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APPENDIX A: SAMPLING VOLUMES AND ADSORBENT RESINS

Each treatment and corresponding control was sampled for the same length of time. In addition, the same adsorbent resin was used for each treatment and corresponding control. However, sampling times and whether a multiple layered or Tenax TA only resins were changed between the pairs. The following table indicates the type of resin and the sampling volume (sampling time * flow rate) for each pair in each methodology (Table 9).

Table 9. Sampling volumes and adsorbent resins used for each pair of treatment and corresponding control. M =methodology; G =group of termites; C = control; RM = repeated measure; P = procedure.

Identification	Adsorbent resin	Sampling volume (L)		Identification	Adsorbent resin	Sampling volume (L)
M1, G1, RM1	Multiple layer	52,502		M3, G7, RM6	Multiple layer	20,758
M1, G1, RM2	Multiple layer	22,085		M3, G7, RM7	Tenax TA	24,456
M1, G2	Multiple layer	46,057		M3, G7, RM8	Multiple layer	23,885
M1, G3	Multiple layer	24,805		M3, G7, RM9	Tenax TA	26,129
M1, G4, RM1	Multiple layer	31,880		M3, G7, M10	Tenax TA	28,167
M1, G4, RM2	Multiple layer	26,962		M3, G8, RM1	Tenax TA	22,075
M1, G4, RM3	Multiple layer	63,691		M3, G8, RM2	Tenax TA	22,350
M1, G4, RM4	Multiple layer	48,344		M3, G8, RM3	Tenax TA	22,842
M1, G4, RM5	Multiple layer	51,450		M3, G7, C2, RM1	Tenax TA	23,951
M1, G4, RM6	Multiple layer	51,955		M3, G7, C2, RM2	Tenax TA	23,187
M2, G5, RM1	Multiple layer	13,199		M3, G7, C2, RM3	Tenax TA	26,184
M2, G5, RM2	Multiple layer	14,452		M3, G7, C2, RM4	Tenax TA	51,195
M2, G5, RM3	Multiple layer	20,121		M3, G7, C2, RM5	Tenax TA	51,681
M2, G5, RM4	Multiple layer	20,713		M3, G7, C2, RM6	Tenax TA	55,737
M2, G5, RM5	Tenax TA	52,224		M3, G7, C2, RM7	Tenax TA	56,175
M2, G5, RM6	Multiple layer	20,699		P1, RM1	Multiple layer	19,418
M2, G5, RM7	Tenax TA	11,477		P1, RM2	Multiple layer	17,052
M2, G5, RM8	Tenax TA	11,566		P1, RM3	Tenax TA	28,408
M2, G5, RM9	Multiple layer	22,965		P1, RM4	Tenax TA	28,408
M2, G6, RM1	Tenax TA	27,853		P1, RM5	Tenax TA	27,862
M2, G6, RM2	Multiple layer	20,487		P1, RM6	Tenax TA	78,773
M2, G6, RM3	Tenax TA	19,527		P1, RM7	Tenax TA	75,817
M2, G6, RM4	Tenax TA	19,135		P1, RM8	Tenax TA	81,702
M2, G5, C2, RM1	Tenax TA	15,289		P2, RM1	Multiple layer	38,852
M2, G5, C2, RM2	Multiple layer	23,878		P2, RM2	Multiple layer	39,982
M2, G5, C2, RM3	Multiple layer	24,446		P2, RM3	Multiple layer	41,398
M2, G5, C2, RM4	Tenax TA	20,890		P3, RM1	Tenax TA	61,889
M2, G5, C2, RM5	Tenax TA	18,939		P3, RM2	Multiple layer	49,351
M3, G7, RM1	Tenax TA	18,403		P3, RM3	Multiple layer	64,431
M3, G7, RM2	Tenax TA	23,689		P3, RM4	Tenax TA	60,861
M3, G7, RM3	Multiple layer	24,845		P3, RM5	Multiple layer	33,529
M3, G7, RM4	Tenax TA	25,229		P3, RM6	Tenax TA	63,552
M3, G7, RM5	Multiple layer	18,074				

APPENDIX B: COMPARISONS OF VOLATILES DETECTED ACROSS DIFFERENT METHDOLOGIES

The qualitative results for each methodology were consolidated into one table to reveal similarities and differences in the results obtained by using the different methodologies. This information could be used to determine if a compound is consistently detected in the treatments but not the controls across different methodologies. The following table indicates the compounds detected across the different methodologies used (Table 10). Termites that were collected on the same date and location were considered a group. For groups six through nine in which there were two subgroups, one column is provided to show the results of the main group.

Table 10. Comparison of compounds detected in treatments and corresponding controls using different methodologies. M = method; G = group of termites; m/z = mass to charge ratio; + =detection in treatment; ○ =detection in control; ● =detection in both treatment and control; - = not detected treatment or control.

Method/Group Compound (m/z)	M1 G1	M1 G2	M1 G3	M1 G4	M2 G5	M2 G6	M3 G7	M3 G8
Unknown 1 (40,42)	-	●	●	●	●	●	●	●
Unknown 2 (44, 45)	-	●	●	●	●	●	●	●
Unknown 3 (101,103)	-	●	+	●	●	●	●	●
Unknown 4 (47)	-	●	●	●	●	●	●	●
Unknown 5 (59, 43)	-	●	●	●	●	●	●	●
Unknown 6 (107, 109, 75, 89, 181)	-	●	●	●	●	●	●	●
Dimethyl disulfide (94, 45, 79, 61)	-	+	+	+	-	-	+	-
Unknown 7 (91, 92, 65)	●	●	●	●	●	●	●	●
Unknown 8 (83, 55, 99)	-	○	-	●	○	-	-	-
Unknown 9 (229, 227, 151)	●	●	●	●	●	●	●	●
Unknown 10 (91, 106, 77)	●	●	●	●	●	●	●	●
Unknown 11 (57, 86, 115)	-	-	+	-	-	-	-	-
Unknown 12 (281, 299, 267)	●	●	●	●	●	●	●	●
Unknown 13 (105, 120, 77)	●	●	+	●	●	●	●	●
Unknown 14 (146, 148, 111, 75)	●	-	-	+	●	●	●	●
Unknown 15 (93,91,79,67, 121,136)	●	●	●	●	●	●	●	●
Unknown 16 (117,118,115,91)	●	●	●	●	●	●	●	●
Unknown 17 (119,134, 91)	●	●	●	●	●	●	●	●
Unknown 18 (105,77,51,50)	●	●	+	●	●	●	●	●
Unknown 19 (57,71,85,99)	●	●	+	●	●	●	●	-
Unknown 20 (67, 57, 81, 143)	●	○	●	●	●	●	○	○
Unknown 21 (355, 286, 267)	●	●	●	●	●	●	●	●
Unknown 22 (132, 117, 115, 91)	●	●	●	●	●	●	●	●
Unknown 23 (142, 99, 127)	-	-	+	-	-	-	-	-
Unknown 24 (122, 107, 167, 182)	-	-	-	-	+	-	-	+
Unknown 25 (131, 91, 115, 146)	-	-	-	-	-	-	-	●
Naphthalene (128,102)	●	●	●	●	●	●	●	●

Table 10 Continued

Compound (m/z) \ Method/Group	M1 G1	M1 G2	M1 G3	M1 G4	M2 G5	M2 G6	M3 G7	M3 G8
Unknown 26 (148, 147, 117, 77)	-	●	+	●	○	-	○	+
Unknown 27 (159, 128, 174)	-	-	-	-	-	-	+	-
Unknown 28 (429, 73, 430, 445, 325, 341, 359, 149)	●	●	●	●	●	●	●	●
Unknown 29 (142, 141, 115)	●	-	-	-	○	●	●	●
Unknown 30 (123,95,109, 165,208)	-	-	-	-	○	○	-	-
Unknown 31 (121, 166, 91, 77)	-	●	+	●	-	-	-	-
Unknown 32 (161, 91, 105, 133, 189,204)	●	●	●	●	●	●	-	+
Unknown 33 (180, 166, 193)	-	-	+	-	-	-	-	-
Unknown 34 (156, 141, 115, 76)	+	-	+	○	-	●	-	-
Unknown 35 (503, 519, 73, 281, 299, 149, 345)	●	●	●	●	●	●	●	●
Butylated hydroxytoluene (205,220,177)	●	●	+	●	●	+	●	+
Unknown 36 (159, 131, 202)	●	●	+	-	●	●	○	-
Unknown 37 (157, 200, 142)	○	-	-	-	-	○	-	+
Unknown 38 (219, 191, 234)	+	+	○	-	-	-	-	-
Unknown 39 (168, 167, 165)	+	-	-	-	-	-	-	-
Unknown 40 (173, 243, 71)	●	-	-	●	●	●	●	●
Unknown 41 (149, 177, 105)	●	-	-	○	-	●	-	-
Unknown 42 (183,198,168)	●	●	-	●	●	○	-	+
Unknown 43 (181, 210, 165)	●	○	-	○	-	-	-	-
Unknown 44 (257,272,161)	●	○	+	○	●	●	●	●
Unknown 45 (149, 150)	●	-	+	-	-	●	-	-
Unknown 46 (134, 119, 91, 242)	●	-	-	-	-	-	-	-
Unknown 47 (81, 95, 67, 275, 257)	-	-	-	-	●	-	○	+
Unknown 48 (255, 159, 173, 270)	-	○	+	-	●	-	○	+
Unknown 49 (322,81,121, 136,217)	-	-	-	●	-	-	-	-
Unknown 50 (207, 91, 117, 129)	●	●	●	●	-	-	-	-
Unknown 51 (262, 263, 261)	●	-	-	●	●	●	+	+

APPENDIX C: CONFIRMATION OF THE IDENTITY OF NAPHTHALENE AND BUTYLATED HYDROXYTOLUENE

INTRODUCTION

The identifications of naphthalene and butylated hydroxytoluene, which are compounds detected both in treatments containing nests with active Formosan termites and controls that contained neither nests nor termites, were confirmed for the following reason. Since previous research indicated that naphthalene and butylated hydroxytoluene were suspect reporter molecules (Henderson *et al*., 1999; Henderson *et al*., 2001), it was important to compare the results presented here regarding those compounds with the previous research. To make those comparisons, it was necessary to be certain that compounds detected in previous research were identical to those presented here.

The confirmation of naphthalene and butylated hydroxytoluene was accomplished when using two different methodologies. Since there were insufficient repetitions to evaluate those methodologies for making comparisons of volatiles detected in treatments and in controls, those methodologies were not presented above. However, there were a sufficient number of repetitions for identification purposes.

MATERIALS AND METHODS

Identification of Naphthalene

The sampling procedure was the following. Formosan termites and their food source were placed in a Rubbermaid® trash can (121.1 L). Cypress tree branches (*Toxodium distichum* (L.) Rich.) without termites were removed from the trash can and placed in another Rubbermaid® trash can, which served as the control. The lids of the trash cans were modified by installing two brass connectors. One brass connector was attached by Nalgene 550 platinum-cured silicone tubing to a adsorption tube packed with 100 mg of Tenax TA, which in turn was connected to a

Welch® vacuum pump. Volatiles in the trash cans were then vacuumed onto the Tenax TA. The treatment and corresponding control were sampled twice. The instrumental procedure was the following. The Short Path Thermal Desorption system program had the same parameters as described above in methodology number one. The GC/MS parameters were as follows: 40°C (5 min); 10°C / min, 260°C, 9 min. For use as a standard, a solution of naphthalene (Sigma Aldrich, St. Louis, MO) in methanol was prepared at a concentration of 500 nanograms per microliter and then analyzed using the instrumental methodology described above. The mass spectrum and GC retention times of peaks in the sample were then compared with those of the naphthalene standard.

Identification of Butylated Hydroxytoluene

The sampling procedure was the following. Formosan termites were visually detected at two locations within apartments 1034-1036 on Royal Street in New Orleans, LA. The first location was located at a doorway on the first floor while the second was located at a doorway on a veranda of the second floor. Samples of air were collected from two infested sites as well as from a control by using the following procedure. A small plastic funnel was attached to one end of a piece of Tygon® tubing. The other end of the tubing was attached to an adsorption tube packed with Tenax TA which in turn was attached a Welch® vacuum pump. Two more sampling apparatuses was set-up in the same way. The funnel attached to first apparatus was then taped to a small hole where the termite activity was observed. This was repeated for the second site of the infestation. The funnel of the third apparatus was placed on the floor and served as a control. The three pumps were then simultaneously turned on and sampling lasted 1 hour. The adsorption tubes were then sealed with caps. The instrumental procedure was the following. The Short Path Thermal Desorption system program had the same parameters as described above in

methodology number one. The GC/MS parameters were as follows: 60°C (1 min); 10°C / min, 260°C, 10 min. For use as a standard, a solution of butylated hydroxytoluene (Sigma Aldrich, St. Louis, MO) in methanol was prepared at a concentration of 400 nanograms per microliter and then analyzed using the instrumental methodology described above. The mass spectrum and GC retention times of peaks in the sample were then compared with those of the butylated hydroxytoluene standard.

RESULTS

The identification of naphthalene was confirmed since its GC retention time and mass spectrum in the samples matched those in a standard (Table 11).

Table 11. Comparison of GC retention time and mass spectra of a peak in a representative sample with those of a standard for naphthalene. RT = retention time, m/z =mass to charge ratio, RS = representative sample, STD = solution of standard.

Volatile	RT in RS	RT in STD	m/z in RS	m/z in STD
Naphthalene	13.85	13.80	128, 102	128, 102

Similarly, the identification of butylated hydroxytoluene was confirmed since its GC retention time and mass spectrums in the samples from infested sites as well as that of the control matched those in a standard (Table 12).

Table 12. Comparison of mass spectra and GC retention times of peaks in a representative sample with those of a standard butylated hydroxytoluene. RS = Representative Sample, STD = stock solution of standard.

Volatile	RT in RS	RT in STD	m/z in RS	m/z in STD
Butylated hydroxytoluene	11.91	12.17	205, 177, 220	205, 177, 220

DISCUSSION

The identification of naphthalene and butylated hydroxytoluene were confirmed since their GC retention times and mass spectrums in the samples matched those of the standards. The mass

spectrums of the standards of naphthalene and butylated hydroxytoluene also matched those of the compounds designated as naphthalene and butylated hydroxytoluene in methodologies one, two and three (Appendix B, Table 10, Pages 51-52). Therefore, the compounds presented here were identical to those detected in previous research.

VITA

Paul Michael McLaughlin was born July 15, 1972, in Wilmington, Delaware. He attended St. Elizabeth School until receiving his high school diploma. He has received an associate in applied science degree in bioscience from Delaware Technical and Community College and bachelor of science degree in entomology from the University of Delaware. Paul is presently a candidate for the master of science degree in entomology at Louisiana State University.