Toxicity and Repellency of Essential Oils to the German Cockroach (Dictyoptera: Blattellidae)

by

Alicia Kyser Phillips

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
December 18, 2009

Keywords: *Blattella germanica*, essential oils, toxicity,
fumigation, repellency, ootheca

Approved by

Arthur G. Appel, Chair, Professor of Entomology
Steven R. Sims, Senior Research Entomologist at BASF Pest Control Solutions
Xing Ping Hu, Associate Professor of Entomology

Abstract

The topical toxicity of 12 essential oil components (carvacrol, 1,8-cineole, *trans*-cinnamaldehyde, citronellic acid, eugenol, geraniol, S-(-)-limonene, (-)-linalool, (-)-menthone, (+)-α-pinene, (-)-β-pinene, and thymol) to adult male, adult female, gravid female, and large, medium, and small nymphs of the German cockroach, *Blattella germanica* (L.) was determined. Thymol was the most toxic essential oil component to adult males, gravid females, and medium nymphs with LD_{50} values of 0.07, 0.12, and 0.06 mg/cockroach, respectively. *Trans*-cinnamaldehyde was the most toxic essential oil component to adult females, large nymphs, and small nymphs with LD_{50} values of 0.19, 0.12, and 0.04 mg/cockroach, respectively. (+)-α-Pinene was the least toxic essential oil component to all stages of the German cockroach. S-(-)-Limonene had the least effect on ootheca hatch, with 35.21 (mean) nymphs hatching per ootheca. (-)-Menthone had the greatest effect on ootheca hatch with 20.89 nymphs hatching per ootheca. The numbers of nymphs hatching from each ootheca generally declined as dose increased.

The fumigant toxicity of the 12 essential oil components to all life stages of the German cockroach, *Blattella germanica* (L.) was determined. 1,8-Cineole was the most toxic essential oil component to adult males and females, gravid females, and large nymphs with LC_{50} values of 6.84, 8.43, 5.31, and 11.44 mg/L air, respectively. (-)-Menthone and carvacrol were the most toxic essential oil components to medium and small nymphs with LC_{50} values of 8.96 and 3.63 mg/L air, respectively. Citronellic acid

was the least toxic essential oil component to all stages of the German cockroach. Citronellic acid had the least effect on ootheca hatch (100% hatch). (-)-Menthone had the greatest effect on ootheca hatch (73% hatch). The percentage hatched oothecae decreased linearly with increasing concentration.

The repellency of the 12 essential oil components to adult male German cockroaches, *Blattella germanica* (L.) was determined using Ebeling choice boxes and the harborage-choice method. Repellency ranged from a high of 45% for citronellic acid to 6% for S-(-)-limonene in the Ebeling choice box. Repellency was negatively correlated with fumigant toxicity. Repellency ranged from 76% for carvacrol to 43% for 1,8-cineole using the harborage-choice method. Repellency was negatively correlated with vapor pressure. ANOVA showed that there was a significant effect of day on repellency for both the Ebeling choice boxes and the harborage-choice method. The Ebeling choice box is the superior method for determining the repellency of essential oils to the German cockroach because it is a better approximation of normal cockroach habitat, and it is designed to measure the percentage of repelled cockroaches, rather than repellency persistence.

Acknowledgements

I would like to thank everyone who helped and inspired me during my master's study. I especially want to thank Dr. Arthur G. Appel for accepting me as a graduate student into his urban entomology program and his support and guidance in my research. I would like to thank my committee members, Dr. Steven R. Sims and Dr. Xing Ping Hu, for their review of the manuscripts and suggestions for my research. I would like to thank Dr. Nannan Liu for her review of the manuscripts and Marla Eva for assistance in the laboratory. This research was partially supported by an AAES Hatch grant and by Whitmire Micro-Gen Research Laboratories, Inc. (BASF Pest Control Solutions).

I would like to thank Dr. John M. Aho, Auburn University at Montgomery, for believing in and encouraging me to apply for graduate school and also for his outstanding teaching ability, which stimulated my interest in entomology.

My deepest gratitude goes to my family for their continuous interest in my progress and research throughout graduate school. I would like to thank my loving husband, Jonathan D. Phillips for his encouragement and escape from the stresses of graduate school. I would like to thank my mother, Linda C. Kyser for her constant support when I encountered difficulties. I would like to thank my father Charles B. Kyser, owner of Kyser Exterminating, for sparking my interest in urban entomology.

I would also like to thank him for helping fund my 5 day a week, 55 mile commute from Montgomery to Auburn.

Table of Contents

List of Tables

List of Figures

Introduction

Economic Importance

The German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae), is a ubiquitous domiciliary pest mainly associated with urban environments. Because it is a domestic pest, it is always associated with indoor environments, such as kitchens, bathrooms, and food storage areas. The German cockroach is a major pest because it has the potential to form large populations and because some people are allergic to its feces and exuviae (Schal and Hamilton 1990). German cockroach extracts contain allergens that are both intra (only found in the German cockroach) and interspecific (found in other cockroach species) (O'Connor and Gold 1999). Cockroach allergies are more prevalent in people that have frequent contact with cockroaches in their environment (O'Connor and Gold 1999). A higher rate of cockroach encounters is associated with urban areas and low socioeconomic status (O'Connor and Gold 1999). The presence of cockroaches can also induce asthmatic reactions in asthma sufferers. In a study conducted by Kang (1976), asthma patients that were allergic to cockroach extracts applied to their skin, developed asthmatic responses when they inhaled the cockroach extracts. Cockroach allergy and asthma can be managed by reducing cockroach infestations and exposure to allergens (O'Connor and Gold 1999). This could be accomplished with chemical control, IPM practices, and wearing respiratory protection for jobs that involve exposure to dust particles (O'Connor and Gold 1999).

The German cockroach is also an experimental and natural vector of numerous microorganisms that are pathogenic to humans and wildlife. These microorganisms include viruses, bacteria, protozoa, and helminthes (Roth and Willis 1960). Focusing on microorganisms that are pathogenic to humans and carried by the German cockroach in nature, the German cockroach can carry four strains of the *Poliomyelitis* virus, which is the virus that causes polio. *Poliomyelitis* is transferred to humans, from the German cockroach, by its feces (Roth and Willis 1957). *Streptococcus* spp., a genus of bacteria, can be found in the feces and alimentary canal of the German cockroach (Roth and Willis 1957). Cockroaches can also contain the bacterium *Escherichia coli* (Migula) Castellani and Chalmers, which is capable of inhabiting human intestines and causing infections of the genitourinary tract (Roth and Willis 1957). *Paracolobactrum aerogenoides* Borman, Stuart, and Wheeler, can occur in the feces and alimentary canal of the German cockroach, and it is capable of inhabiting the human intestines and causing gastroenteritis (Roth and Willis 1957). *Salmonella typhimurium* (Loeffler) Castellani and Chalmers, a species of bacteria, can cause food poisoning (Roth and Willis 1957). *Mycobacterium leprae* (Armauer-Hansen) Lehmann and Neumann, reportedly occurs in the German cockroach, and this bacterium causes leprosy (Roth and Willis 1957). *Entomoeba histolytica* Schaudinn, a protozoan that can be found in the German cockroach, can cause amoebic dysentery (Roth and Willis 1957). The eggs of *Enterobius vermicularis* (L.) Leach in Baird, a species of helminth (nematode) also known as the human pinworm, can be mechanically vectored by the German cockroach (Roth and Willis 1957). The eggs of *Trichuris trichiura* (L.) Stiles, a species of helminth (nematode) also known as the human

whipworm, can be mechanically vectored by the German cockroach (Roth and Willis 1957).

The German cockroach is a pest because most people find it disgusting and its presence embarrassing. The German cockroach is always assumed to be associated with unsanitary conditions. The fear of others knowing and observing the infestation may cause one to experience psychological problems and social anxiety (Brenner 1995). The German cockroach can also cause some people to experience delusory cleptoparasitosis, in which a person imagines an infestation in his or her home (Grace and Wood 1987).

Another reason the German cockroach is a pest is because rapid population growth, which can be attributed to the German cockroach's short generation time, makes it difficult to control. The German cockroach has a relatively short life (about 200 days). Females produce 4-10 egg cases during their life, and each egg case contains about 30-40 eggs. Its short generation time increases its chance of becoming resistant to the insecticides used to manage its population; therefore, chemical rotation and different products and strategies should be used to reduce the chance of resistance developing in the population (Barcay 2004).

Biology

Like all cockroaches, the German cockroach is hemimetabolous, and its life cycle includes egg, nymphal, and adult stages. The German cockroach is oviparous, and its eggs are contained in a case or ootheca. A German cockroach ootheca is yellowish brown and about 6 mm long. Females produce an ootheca with or without fertilization. If fertilization does not occur, the ootheca will be deformed, and the eggs will not hatch (Roth 1970b). During the production of an ootheca, the female will partially extrude her

ootheca out of her genital chamber. Then, she will rotate the ootheca 90º, and it will remain partially exposed until the eggs are ready to hatch (Roth 1970a). Water and some nutrients for the eggs are provided by the female through the permeable anterior end of the ootheca. The ootheca hatches about 30 days from the time that it is produced (Ross and Mullins 1995). When the eggs are ready to hatch, the female will often drop her egg case, and the nymphs will break open the keel of the ootheca by swallowing air (Ross and Mullins 1995).

The nymphal stage begins when the cockroaches hatch out of the ootheca. The nymphs have two longitudinal streaks on their dorsum (Barcay 2004). There can be 6-10 instars during the nymphal stage depending on food, water, temperature, and population density (Ross and Mullins 1995). German cockroach nymphs produce an aggregation pheromone that attracts intra and interspecific cockroaches (Ishii and Kuwahara 1967). This pheromone is excreted in their feces (Ishii and Kuwahara 1967). German cockroach development is maximized when the cockroaches are aggregated together (Izutsu et al. 1970). Nymphs usually develop into adults in about 60 days (Ross and Mullins 1995).

The adult stage begins after the last molt. Both male and female German cockroaches are macropterous; however, they do not fly. Instead, they use their wings to break a fall (Ross and Mullins 1995). Adults are 16 mm long and light brown in color. Two dark brown parallel longitudinal stripes are present on the pronotum. The males have long narrow abdomens and styli on their asymmetrical subgenital plates, while the females have more robust abdomens and no styli on their subgenital plates. When a male and female first encounter one another, they will touch antennae. According to Ross and Mullins (1995), this behavioral display is used by the male to determine the sex of his

cockroach counterpart (Roth 1970b). After the male has confirmed the sex of the other cockroach, he will lift his wings to expose his tergal glands and turn 180º. The female will then crawl up on the male's back and feed on the secretions from the tergal glands. The tergal secretions will provide nutrients for the eggs (Mullins et al. 1992). While the female is feeding, the male will grab her genetalia with his left phallomere and properly position her body for copulation. He will then turn so that he is facing in the opposite direction of her. The male passes his spermatophore to the female. Later, the spermatophore is dropped (Roth 1970b), and the female ingests it (Mullins et al. 1992). Nutrients may be provided to the female from the spermatophore (Mullins et al. 1992). An ootheca is produced about 10 days after mating, and it will hatch in about 30 days. If a female does not mate within 8-14 days of her last molt, she will produce an unfertilized ootheca. This ootheca will be deformed, and it will not hatch (Roth 1970b).

Food, water, and the availability of harborages effect the growth and development of cockroaches (Appel 1997). Sanitation, therefore, is an important issue to consider when managing German cockroaches (Schal and Hamilton 1990). If an alternative food source is available to domestic cockroaches, they may choose that as their source of food instead of insecticidal baits that have been placed in the same environment in attempt to control the population (Reierson and Rust 1984). Alternative food sources must be eliminated to effectively manage German cockroaches (Schal and Hamilton 1990). Examples of alternative food sources would include dirty dishes, standing grease, uncovered food on counters, and pet food. All cockroaches must have water; therefore, any standing water should be eliminated if possible (Appel 1997). Dish water should not be left in the kitchen sink, and leaky pipes should be repaired. The presence of clutter in

a home will provide harborages for the German cockroaches (Schal and Hamilton 1990). Clutter might include dirty dishes, dirty cloths, or a stack of wood. Anything that might function as a harborage should be eliminated to effectively manage the cockroaches (Schal and Hamilton 1990).

Essential Oils

Essential oils are secondary plant substances (Isman 2006) comprised of many compounds including monoterpenoids, which are responsible for the aromatic characteristics of the plant (Appel et al. 2004). Essential oils can be obtained from aromatic plants by extraction. They have been used in the past and are still used today as fragrances for perfumes and flavorings for food items (Isman 2006). Essential oils are also used for aromatherapy and as herbal medicines (Isman 2006).

Essential oils are an excellent alternative to traditional insecticides because of their low toxicity to wildlife. (Isman 2006). Since they have low persistence, essential oils could be toxic to non-target insects initially; however, after a few days, insects that come in contact with the treated environment would not be affected as they would be with traditional insecticides (Isman 2006). Essential oils would be excellent contact sprays, but they would offer poor residual protection. A microencapsulated formulation might be useful to increase the residual activity.

Essential oils also have low toxicity to humans (Isman 2006). If essential oils were used, the applicator and the homeowner would not have to be concerned with the short or long term effects that result from exposure to the essential oils on a daily basis. The consumer would not have to wonder what kind of chemicals residues he or she is

ingesting with each meal because essential oils are often used as flavorings and would, therefore, be harmless if consumed (Isman 2000).

Compared with other botanical insecticides, the active ingredients of many essential oils are reasonably priced because they are commonly used as flavors and fragrances (Isman 2006). This is another reason why essential oils are an excellent alternative to traditional insecticides.

In the United States, all pesticides must be registered under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) by the EPA; however, minimum risk pesticides, which incorporate several essential oils, are exempt from the requirements of the EPA (EPA PR Notice 2000-6). Insecticides that are exempt from registration can be placed on the market faster than traditional insecticides (Isman 2000).

Literature Review

Numerous essential oil components, such as 1,8-cineole, *trans*-cinnamaldehyde, citronellic acid, eugenol, geraniol, (-)-linalool, (-)-menthone, (-)-β-pinene, and (+)-α-pinene are used as fragrances for perfumes and flavorings for food items (Isman 2006). Others, such as eugenol and geraniol are used as insect attractants (O'Neil 2006). Additional uses include disinfectants (carvacrol), solvents for manufacturing resins ((-)-limonene), plasticizers ((+)-α-pinene), and mold eliminators (thymol) (O'Neil 2006).

The demand for botanical insecticides is growing because of the public's increase in concern for the negative effects of traditional insecticides, (Appel et al. 2001). Essential oils are a natural botanical alternative to traditional insecticides. Numerous studies have demonstrated the efficacy of essential oils as insecticides.

Constituents of marjoram oil were tested against female German cockroaches to determine if they could be used as insecticides (Jang et al. 2005). The contact and vapor phase toxicities of marjoram oil and its constituents were determined and the results were compared to that of four conventional insecticides (Jang et al. 2005). The results from the contact toxicity bioassay (using filter paper) indicated that 1,8-cineole, linalool, α-terpineol, and thymol, the major constituents of marjoram oil, were more toxic than propoxur but less toxic than deltamethrin, dichlorvos, and permethrin (Jang et al. 2005). When compared to dichlorvos, all marjoram oil constituents showed less fumigant activity; however, the results indicated that marjoram oil, 1,8-cineole, (+)-camphor, linalool, α-terpineol, (-)-α-thujone, thymol, and verbenone could be used as fumigants to control German cockroaches (Jang et al. 2005). The method of delivery was vapor action, by way of the respiratory system (Jang et al. 2005).

Essential oils and their constituents have also been tested against the turnip aphid, *Lipaphis pseudobrassicae* (Davis), (Sampson et al. 2005); the confused flour beetle, *Tribolium confusum* (du Val), (Stamopoulos et al. 2007); the granary weevil, *Sitophilus granarius* (L.), (Kordali et al. 2006); the lesser grain borer, *Rhyzopertha dominica* (Fabricius), the rice weevil, *Sitophilus oryzae* (L.), the red flour beetle, *Tribolium castaneum* (Herbst), (Rozman el al. 2007); and the human head louse, *Pediculus humanus capitis* De Geer, (Yang et al. 2004) for contact and fumigant toxicity. Results indicated that all of the above species were susceptible to several of the essential oils and their constituents.

Carvacrol can be extracted from thyme plants (Mockute and Bernotiene 1999). It is an aromatic alcohol, and it has a density of 0.98 g/ml at 25ºC and a boiling point of

236ºC (Table 1). In a study conducted by Panella et al. (2005), essential oil constituents from the heartwood of Alaska yellow cedar were tested for insecticidal and acaricidal activity. Of the monoterpenoids tested, only carvacrol was toxic to ticks, fleas, and mosquitoes (LC_{50} = 0.0068, 0.0059, 0.0051 (wt:vol), respectively) (Panella et al. 2005). The contact toxicity (LC_{50}) (filter paper bioassay) of carvacrol to the German cockroach was 0.29 mg/cm^2 (Jang et al. 2005).

1,8-Cineole can be extracted from eucalyptus trees (Yang et al. 2004). It is a bicyclic ether, and it has a density of 0.92 g/ml at 25ºC and a boiling point of 176ºC. The majority of research on cineole has been conducted using 1,8-cineole. The fumigant toxicity of 1,8-cineole was determined for the rice weevil, the red flour beetle, and the lesser grain borer, all of which are major pests of stored grains (Lee et al. 2004). The LC_{50} values were 22.8, 15.3, and 9.5 µl/L of air, respectively (Lee et al. 2004). The contact toxicity (LC_{50}) (filter paper bioassay) of 1,8-cineole to the German cockroach was 0.13 mg/cm^2 (Jang et al. 2005), whereas the fumigant toxicity (LC_{50}) was 92.97 mg/L of air (Jang et al. 2005).

Trans-cinnamaldehyde can be extracted from the bark of cinnamon trees (Senanayake et al. 1978). It is an aromatic aldehyde that has a density of 1.05 g/ml at 25ºC and a boiling point of 250ºC (Table 1). Cinnamaldehyde was tested for acaricidal activity against the copra mite, *Tyrophagus putrescentiae* (Schrank), and, the results were compared with the following conventional insecticides: benzyl benzoate, *N*,*N*-diethyl-*m*-toluamide (DEET), and dibutyl phthalate (Kim et al. 2004). Cinnamaldehyde was toxic to the copra mite, with an LC_{50} (determined using treated fabric pieces) of 1.12 µg/cm^2. LC_{50} values for benzyl benzoate, *N*,*N*-diethyl-*m*-toluamide (DEET), and dibutyl phthalate

were 10.03 μg/cm^2, 13.39 μg/cm^2, and 12.87 μg/cm^2, respectively (Kim et al. 2004). The results indicated that cinnamaldehyde would be considered as an alternative direct contact spray for the control of mites in stored products (Kim et al. 2004). A study by Hertel et al. (2006) confirmed that cinnamaldehyde inhibits the development and affects the circulatory system and antenna-heart of the American cockroach, *Periplaneta americana* (L.). Cinnamaldehyde can also be used to protect stored products from the red four beetle and the maize weevil, *Sitophilus zeamais* Motsch (Huang and Ho 1998). The contact and fumigant toxicity (LC_{50}) of cinnamaldehyde to the adult red flour beetle and the maize weevil was 0.70 and 0.66 mg/cm^2 and 0.28 and 0.54 mg/cm^2, respectively (Huang and Ho 1998). Cinnamaldehyde also exhibits antifeedant effects against the red flour beetle and the maize weevil (Huang and Ho 1998). The toxicity (LC_{50}) (filter paper bioassay) of (E)-cinnamaldehyde was 0.23 mg/cm^2 to the German cockroach (Jang et al. 2005).

Citronellic acid, a component of citronella oil, can be extracted from the stems and leaves of citronella grass (Nakahara et al. 2003). It is an aliphatic compound that contains a carboxyl functional group, and it has a density of 0.92 g/ml at 25ºC and a boiling point of 121ºC (Table 1). Citronella oil has repellent properties, and it has been used in the production of citronella candles to repel mosquitoes. Few studies have been conducted using citronellic acid. Citronellic acid had insignificant insecticidal activity against adult German cockroaches in contact toxicity studies (Jang et al. 2005).

Eugenol can be extracted from the dried flower buds of clove trees (Park and Shin 2005). It is an aromatic ether and its structure also contains a hydroxyl functional group. Eugenol has a density of 1.07 g/ml at 25ºC and a boiling point of 254ºC (Table 1). It was

toxic to the Japanese termite, *Reticulitermes speratus* Kolbe (Park and Shin 2005). When fumigated with 1 µl/L of air of eugenol there was 100% mortality of the Japanese termite at 24 h (Park and Shin 2005). The mortality of the rice weevil, the lesser grain borer, and the red flour beetle when fumigated with 0.1 µl/720 ml volume of eugenol was 85.0, 80.0, and 12.5% at 24 h, respectively (Rozman et al. 2007).

Geraniol can be extracted from the petals of various roses (Antonelli et al. 1997), geraniums (Timmer et al. 1971), and lemongrass (Dudai et al. 2001). It is an aliphatic alcohol, and it has a density of 0.88 g/ml at 25ºC and a boiling point of 229ºC (Table 1). When geraniol was tested against the confused flour beetle in fumigant toxicity studies, LC_{50} values were insignificant to all stages; however, geraniol produced more adultoids than any other monoterpenoid tested (Stamopoulos 2007). These results indicate that geraniol has IGR-like properties and, upon further testing, could be used along with other insecticides to control the confused flour beetle (Stamopoulos 2007). There was no significant mortality of German cockroaches treated with geraniol in either contact or fumigation studies (Jang et al. 2005).

S-(-)-Limonene is an essential oil constituent that can be extracted from the rind of citrus fruits (Usai et al. 1992). It is a cyclic hydrocarbon that has a density of 0.84 g/ml at 25ºC and a boiling point of 175ºC (Table 1). Few essential oil studies have been conducted using S-(-)-limonene; however, a study done by Hink and Fee (1986) on the toxicity of D-limonene to all stages of the cat flea, *Ctenocephalides felis* Bouche, showed that adults, larvae, and eggs are about equal in susceptibility to D-limonene, and pupae are the least susceptible to D-limonene. Citrus oil, which contains d-limonene, was toxic to red imported fire ants, *Solenopsis invicta* Buren, in a study performed by Vogt et al.

(2002). The toxicity (LC_{50}) (filter paper bioassay) of (-)-limonene to the German cockroach was 2.58 mg/cm^2 (Jang et al. 2005).

(-)-Linalool can be extracted from sweet basil (Yousif et al. 1999) and several plants in the Lauraceae family (Kilic et al. 2005). It is an aliphatic alcohol, and it has a density of 0.86 g/ml at 25ºC and a boiling point of 198ºC (Table 1). Stamopoulos et al. (2007) found that linalool was toxic to 10-day-old adult male red flour beetles; the fumigant toxicity (LC_{50}) was 34.8 µl/L of air. Oils containing linalool were also toxic to small turnip aphids (Sampson et al. 2005). The LC_{50} value for coriander, another oil that contains linalool, was 2.9 mg/ml after 60 min (Sampson et al. 2005). Strong contact insecticidal activity (LC_{50} value of 0.12 mg/cm^2) was observed when linalool was tested against adult German cockroaches for contact toxicity (filter paper bioassay) (Jang et al. 2005). Strong fumigant toxicity (LC_{50} value of 26.20 mg/L of air) of linalool to the German cockroach was also observed (Jang et al. 2005).

(-)-Menthone can be extracted from peppermint plants (Baldinger 1942). It is a cyclic ketone, and it has a density of 0.89 g/ml at 25ºC and a boiling point of 207ºC (Table 1). Results from an experiment conducted by Sampson et al. (2005) indicated that oils containing menthone were minimally toxic to turnip aphids. The LC_{50} value for peppermint, an oil that contains menthone, was 8.8 mg/ml after 60 min (Sampson et al. 2005). When menthone was tested against adult German cockroaches for contact toxicity (filter paper bioassay), moderate insecticidal activity was observed (Jang et al. 2005). Contact toxicity (LC_{50}) of menthone to the German cockroach was 0.25 mg/cm^2 (Jang et al. 2005). Mint oil, which consists of menthone as a main component, was repellent and toxic to red imported fire ants (Appel et al. 2004), American cockroaches, and German

cockroaches (Appel et al. 2001). LD_{50} values for American and German cockroaches were 10 µl of 2.57% and 2 µl of 3.83% mint oil, respectively (Appel et al. 2001).

Both (+)-α-pinene and (-)-β-pinene can be extracted from pine trees (Palmer 1942). They are isomers, and both are bicyclic compounds. (+)-α-Pinene has a density of 0.86 g/ml at 25ºC and a boiling point of 155ºC. (-)-β-Pinene has a density of 0.87 g/ml at 25ºC and a boiling point of 165ºC (Table 1). When α-pinene and β-pinene were tested against adult German cockroaches for contact toxicity, poor insecticidal activity was observed (Jang et al. 2005). The contact toxicity (LC_{50}) (filter paper bioassay) of α-pinene and β-pinene to the German cockroach was 2.77 and 1.23 mg/cm^2, respectively (Jang et al. 2005). Poor insecticidal activity of these essential oils was also observed in fumigant toxicity tests (Jang et al. 2005). LC_{50} values of α-pinene and β-pinene to the German cockroach were 218.17 and 143.76 mg/L of air, respectively (Jang et al. 2005). These LC_{50} values are approximately 25-55 times greater than the pyrethroid insecticide, permethrin (Jang et al. 2005).

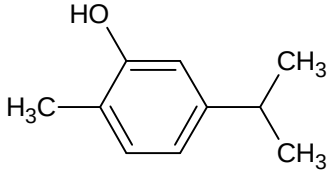
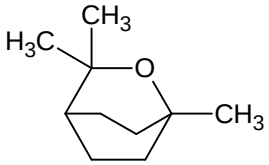
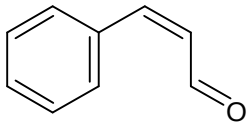
Thymol can be extracted from thyme plants (Sotomayor et al. 2004). It is an aromatic alcohol, and it has a density of 0.97 g/ml at 25ºC and a boiling point of 233ºC (Table 1). Along with other monoterpenoids and rosemary oil, thymol was tested against the wireworm (larval form of a click beetle), *Agriotes obscurus* L. (Waliwitiya et al. 2005). Wireworms are major economic pests of cereal, corn, and potatoes (Waliwitiya et al. 2005). Of the oils tested, thymol had the greatest contact toxicity (LD_{50}=196.0 µg/larva); however, thymol did not have the greatest fumigant toxicity (LC_{50}=17.1 µg/cm^3) of the four compounds tested (Waliwitiya et al. 2005). The contact toxicity (LC_{50}) (filter paper bioassay) of thymol to the German cockroach, determined by Jang et

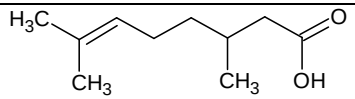
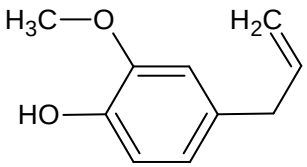
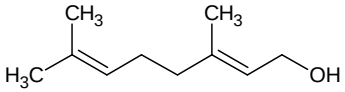
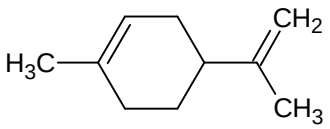
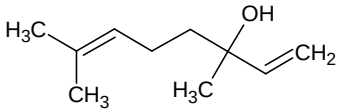
al. (2005), was 0.09 mg/cm^2. The fumigant toxicity (LC_{50}) of thymol to the German cockroach was 18.76 mg/L of air at 24h (Jang et al. 2005).

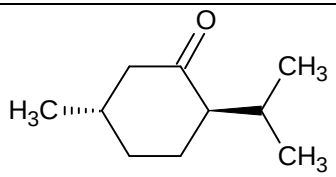
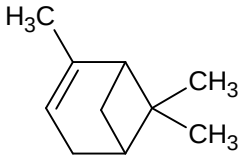
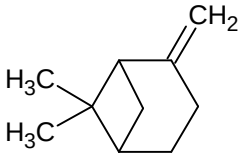
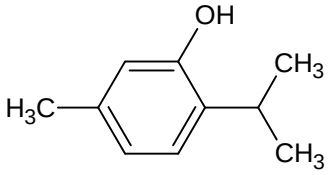
Objectives

The toxicity of essential oils in their purest form will be determined for several stages of the German cockroach. Topical applications will be used to obtain LD_{50} values for each essential oil component. The effect of the components on ootheca hatch will also be determined. Fumigation bioassays will be used to obtain LC_{50} values for each essential oil component. The effect of fumigant activity on ootheca hatch will also be determined. The repellency of essential oils to the German cockroach will be determined using Ebeling choice boxes (Ebeling et al. 1966) and the harborage-choice method (Steltenkamp et al. 1992).

Table 1. Essential oil components

Oil component	Structure[a]	Derivation[b]	Physical and Chemical Properties						
			Log P[a]	Density (g/ml)[c]	Assay[c]	Boiling Point[c]	Vapor Pressure (mmHg at 25ºC)[a]	Solubility (g/l water)[a]	Molecular Weight[a]
Carvacrol		-Thyme plant	3.16	0.98	98%	236°C	0.030	0.96	150.22
1,8-Cineole		-Eucalyptus trees	2.8	0.92	99%	176°C	1.648	0.91	154.25
Trans-Cinnamaldehyde		-Bark of cinnamon trees	1.9	1.05	99%	250°C	0.027	2.98	132.16

Citronellic acid		-Stems and leaves of citronella grass	3.16	0.92	98%	121°C	0.005	200.41	170.25
Eugenol		-Dried flower buds of clove trees	2.4	1.07	99%	254°C	0.010	1.79	164.20
Geraniol		-Petals of various roses -Geraniums -Lemongrass	2.94	0.88	98%	229°C	0.013	0.9	154.25
S-(-)-Limonene		-Rind of citrus fruits	4.55	0.84	≥95%	175°C	1.541	0.0034	136.23
(-)-Linalool		-Sweet basil -Plants in Lauraceae	2.79	0.86	≥95%	198°C	0.091	1.03	154.25

(-)-Menthone		-Peppermint plant	2.75	0.89	90%	207°C	0.256	0.85	154.25
(+)-α-Pinene		-Pine trees	4.32	0.86	98%	155°C	3.489	0.0089	136.23
(-)-β-Pinene		-Pine trees	4.24	0.87	99%	165°C	2.399	0.0106	136.23
Thymol		-Thyme plants	3.25	0.97	99%	233°C	0.038	0.87	150.22

[a]ACD/Labs 11.0 2008. Log P = log of the octanol/water partition coefficient.

[b]Compounds were described by Mockute and Bernotiene 1999; Yang et al. 2004; Senanayake et al. 1978; Nakahara et al. 2003; Park and Shin 2005; Timmer et al. 1971, Antonelli et al. 1997, and Dudai et al. 2001; Usai et al. 1992; Caredda et al. 2002 and Yousif et al. 1999; Baldinger 1942; Palmer 1942; Palmer 1942; and Sotomayor 2004, respectively.

[c]Sigma-Aldrich (St. Louis, MO).

Topical Toxicity of Essential Oils to the German Cockroach (Dictyoptera: Blattellidae)

The German cockroach, *Blattella germanica* (L.), is an important economic pest because its feces and exuviae can cause allergic reactions in sensitive people (Schal and Hamilton 1990). It can also induce asthmatic reactions in asthma sufferers (Kang 1976). German cockroaches can vector numerous microorganisms that are pathogenic to humans and wildlife, including viruses, bacteria, protozoa, and helminthes (Roth and Willis 1957, 1960). The German cockroach can also cause psychological problems. Some people experience delusory cleptoparasitosis, imagining a home cockroach infestation that does not exist (Grace and Wood 1987). The German cockroach has a short generation time and high fecundity which makes it difficult to control. Its short generation time increases the chance of developing resistance to insecticides used to manage populations (Barcay 2004). Populations of German cockroaches have become resistant to the organochlorine, organophosphate, carbamate, and pyrethroid classes of insecticide (Scott et al. 1990).

The public's increasing concern about potentially negative effects of traditional insecticides and the restricted use of traditional insecticides in commercial food preparation areas, storage buildings, apartments, and homes (Barcay 2004) has stimulated the investigation of botanical alternatives. Essential oils are safer alternatives to traditional insecticides that could be used in areas where traditional insecticides are prohibited. They are secondary plant substances (Isman 2006) comprised of many compounds, including monoterpenoids, which are responsible for a plant's aromatic

characteristics. They have been used in the past and are still used as fragrances for perfumes and flavorings for food items (Isman 2006).

Essential oils are an excellent alternative to traditional insecticides because of their low toxicity to humans and wildlife and short residual period (Isman 2006). Compared with other botanical insecticides, such as neem and pyrethrum, the active ingredients of many essential oils are reasonably priced because they are commonly used as flavors and fragrances (Isman 2006), but they usually require application at higher rates than pyrethrum and neem-based insecticides. Minimum risk pesticides, which contain one or more essential oils, are currently exempt from United States Environmental Protection Agency registration requirements (EPA PR Notice 2000-6). Insecticides that are exempt from EPA registration requirements can reach the market faster than conventional insecticides (Isman 2000).

Constituents of marjoram oil were tested against female German cockroaches to determine if they could be used as insecticides (Jang et al. 2005). Results from the contact toxicity bioassay demonstrated that 1,8-cineole, linalool, α-terpineol, and thymol, the major constituents of marjoram oil, were more toxic than a conventional insecticide, propoxur, but less toxic than deltamethrin, dichlorvos, and permethrin (Jang et al. 2005).

The toxicity and repellency of corn mint, *Mentha arvensis* L., oil to American, *Periplaneta americana* (L.), and German cockroaches was determined by Appel et al. (2001). Corn mint oil, containing menthol and menthone as main components, was repellent and toxic to both species. LD_{50} values for corn mint oil were 10 µl of 2.57% for American cockroaches and 2 µl of 3.83% for German cockroaches (Appel et al. 2001).

Essential oils and their constituents have also been tested, for contact toxicity, against a variety of other insects including the turnip aphid, *Lipaphis pseudobrassicae* (Davis), (Sampson et al. 2005); red imported fire ant, *Solenopsis invicta* (Buren), (Appel et al. 2004); confused flour beetle, *Tribolium confusum* (du Val), (Stamopoulos et al. 2007); granary weevil, *Sitophilus granarius* (L.), (Kordali et al. 2006); lesser grain borer, *Rhyzopertha dominica* (F.), rice weevil, *Sitophilus oryzae* (L.), red flour beetle, *Tribolium castaneum* (Herbst), (Rozman el al. 2007); and the human head louse, *Pediculus humanus capitis* De Geer, (Yang et al. 2004). Results demonstrated that all of the above species were susceptible to several of the essential oils and their constituents.

Because essential oils have a relatively short period of residual activity (Isman 2006) the potential efficacy of these materials as active ingredients in contact spray formulations for control of the German cockroach was investigated. The purpose of this study was to determine and compare the toxicity of several pure essential oils to several life stages of the German cockroach.

Materials and Methods

Chemicals. Essential oil components (Table 1) were obtained from Sigma-Aldrich (St. Louis, MO). Some of the essential oil components were chosen because they are present in the essential oil extracts of numerous plant species, while others were chosen because they occur at high concentrations in the essential oils of selected plants. Both aromatic and aliphatic hydrocarbons were tested; the functional groups represented included acids, alcohols, aldehydes, ketones, and ethers. Physical and chemical properties of essential oil components were either obtained from Sigma-Aldrich or

estimated using Advanced Chemistry Development software version 12.0 (ACD/Labs 2008).

Insects. An insecticide susceptible strain of the German cockroach was used in all experiments. This strain (American Cyanamid, Clifton, NJ) has been in continuous laboratory culture for >35 years. The stages used were adult males, adult females, gravid females, large nymphs (5^{th}-7^{th} instar, ≥8.5 mm long), medium nymphs (3^{rd}-4^{th} instar, 5-8 mm long), and small nymphs (1^{st}-2^{nd} instar, ≤4.5 mm long). Laboratory cultures were maintained at 28 ± 2ºC, 40-55% RH, and a photoperiod of 12:12 (L:D) h. Colonies were provided water and dog chow (Purina) as needed. Cockroaches were briefly (<5 min.) anesthetized with CO_2 to facilitate handling during topical applications. Each stage of the German cockroach was weighed to determine if the difference in toxicity of each essential oil component among the stages was due to significant differences in the mass of each stage.

Topical applications. Serial dilutions of essential oil components were made in Fisher Scientific Certified ACS acetone (99.7% purity; Fisher Scientific, Fair Lawn, NJ) to obtain the desired concentrations of 0.05-0.5 mg/cockroach. A Burkard Manufacturing Co. hand microapplicator (Hertfordshire, United Kingdom) was used to topically apply 1 µl doses of essential oil solutions in acetone between the metathoracic legs of each cockroach. Control cockroaches were treated with 1 µl of acetone. Three replicates containing six cockroaches each (total n = 18) were used for each concentration. After treatment, the cockroaches were placed in 162.65 ml (5.5 oz) plastic cups (Georgia-Pacific, Atlanta, GA) and covered with a lid. Mortality was assessed at 24 h.

Effects of essential oils on ootheca hatch. After mortality was recorded for the topical application tests, the live and dead gravid females and the dropped oothecae were held individually in 50 x 9 mm transparent plastic Petri dishes (Becton Dickinson Labware, Franklin Lakes, NJ) and observed every 7 d for 30 d. Mortality, ootheca drop, ootheca hatch, and the number of nymphs present in each Petri dish were recorded. Cockroaches were supplied with carrot slices *ad libitum* and maintained in an incubator at ≈80% RH and ≈28ºC. The carrot slice provided both food and moisture.

Data analysis. One-way ANOVA and Tukey's multiple comparison tests were used to determine the significance of differences in body mass among stages (Proc GLM, SAS 9.1, SAS Institute 2003). Probit analysis for independent data was used to estimate toxicity in the topical application tests (LD_{50}) (Proc Probit, SAS 9.1, SAS Institute 2003). Non-overlap of the 95% confidence intervals (CI) was used to estimate significant differences among LD_{50} values. The insecticidal activity was classified as follows: highly toxic, LD_{50} <0.20 mg/cockroach; moderately toxic, LD_{50} 0.20-0.50 mg/cockroach; and slightly toxic, LD_{50} >0.50 mg/cockroach. A t-test was used to test for significant differences in the mean number of hatched nymphs for control and treated females (Proc Ttest, SAS 9.1, SAS Institute 2003). One-way ANOVA and Tukey's multiple comparison tests were used to study differences in the number of nymphs responding to doses of individual essential oils (Proc GLM, SAS 9.1, SAS Institute 2003). Regression analysis was used to examine the linear relationship between doses applied to gravid females and the mean number of hatched nymphs, percentage of dropped oothecae, and percentage of hatched oothecae (SigmaPlot 11.0; SPSS 2008). Correlation analysis was

used to relate essential oil toxicity with physical and chemical properties (SigmaPlot 11.0; SPSS 2008).

Results

Body mass. Differences in body mass among stages were significant ($P < 0.0001$) with the exception between adult males and large nymphs and adult and gravid females (Table 2).

Topical applications. No control mortality was observed for any stage during the 24 h test period. Carvacrol was highly toxic to all stages of the German cockroach with LD_{50} values ranging from 0.06 to 0.19 mg/cockroach for small nymphs (Table 8) and adult females (Table 4), respectively. Homogeneity of response (slope of the log-dose probit relationship) was similar among adult stages (6.42, 6.94, and 6.69 for adult males (Table 3), adult females (Table 4), and gravid females (Table 5), respectively, but as a group, about twice that of immature stages (Tables 6, 7, and 8).

LD_{50} values of 1,8-cineole ranged from 0.13 mg/cockroach for small nymphs (Table 8) to 0.51 mg/cockroach for gravid females (Table 5). Homogeneity of response was greatest for adult males (7.87) (Table 3) and least for small nymphs (2.49) (Table 8).

Trans-cinnamaldehyde was highly toxic to all stages of the German cockroach; LD_{50} values ranged from 0.04 to 0.19 mg/cockroach for small nymphs (Table 8) and adult females (Table 4), respectively. Homogeneity of response was greatest for adult males (12.62) (Table 3) and least for small nymphs (4.93) (Table 8).

LD_{50} values of citronellic acid ranged from 0.13 to 0.64 mg/cockroach for small (Table 8) and large (Table 6) nymphs, respectively. Homogeneity of response was similar among all stages (3.62, 2.14, 3.83, 3.03, 4.05, and 2.27 for adult males, adult

females, gravid females, large nymphs, medium nymphs, and small nymphs, respectively) (Tables 3, 4, 5, 6, 7, and 8).

LD_{50} values for eugenol ranged from 0.07 mg/cockroach for small nymphs (Table 8) to 0.29 mg/cockroach for adult females (Table 4). Homogeneity of response was similar for adult females (Table 4), medium nymphs (Table 7), and small nymphs (Table 8) (4.19, 4.20, and 4.42 for adult females, medium nymphs, and small nymphs, respectively).

LD_{50} values of geraniol ranged from 0.05 to 0.83 mg/cockroach for small nymphs (Table 8) and adult females (Table 4), respectively. Homogeneity of response was greatest for gravid females (5.13) (Table 5) and least for large nymphs (1.79) (Table 6).

LD_{50} values for S-(-)-limonene ranged from 0.06 mg/cockroach for small nymphs (Table 8) to 0.60 mg/cockroach for large nymphs (Table 6), but S-(-)-limonene was not significantly toxic to adult females (Table 4) or gravid females (Table 5), $P > 0.05$. Homogeneity of response was greatest for adult males (5.45) (Table 3) and least for adult females (0.85) (Table 4).

LD_{50} values for (-)-linalool ranged 0.10 mg/cockroach for small nymphs (Table 8) to 0.32 mg/cockroach for adult males (Table 3); however, (-)-linalool was not toxic to adult females, gravid females, and large nymphs at doses up to 0.45 mg/cockroach. Homogeneity of response for adult males (Table 3), medium nymphs (Table 7), and small nymphs (Table 8) was 6.86, 3.61, and 3.64, respectively.

LD_{50} values of (-)-menthone ranged from 0.06 to 0.77 mg/cockroach for small nymphs (Table 8) and adult females (Table 4), respectively. Homogeneity of response was similar among all stages (3.71, 4.19, 3.04, 4.51, 4.48, and 2.28 for adult males, adult

females, gravid females, large nymphs, medium nymphs, and small nymphs, respectively) (Tables 3, 4, 5, 6, 7, and 8).

Both (-)-β-pinene and (+)-α-pinene were slightly toxic to adult males, but neither were toxic, to adult females, gravid females, large nymphs, medium nymphs, or small nymphs at doses up to 0.44 mg/cockroach. LD_{50} values for adult males were 0.48 and 0.64 mg/cockroach, respectively (Table 3). Homogeneity of response for adult males for (-)-β-pinene and (+)-α-pinene was 3.57 and 4.49, respectively (Table 3).

Thymol was highly toxic to most cockroach stages. LD_{50} values ranged from 0.05 mg/cockroach for small nymphs (Table 8) to 0.22 mg/cockroach for large nymphs (Table 6). Homogeneity of response was greatest for adult males (23.10) (Table 3) and least for medium nymphs (1.85) (Table 7).

Ootheca hatch. Combining all doses for each essential oil component, there were significant differences in the numbers of nymphs hatching from oothecae attached to gravid females treated with essential oil. The percentage of oothecae that dropped before hatch for the control treatment, the percentage of oothecae that hatched for the control treatment, and the mean number of nymphs that emerged from oothecae attached to control females was 93.79 ± 0.02, 93.79 ± 0.02, and 31.65 ± 0.83, respectively. The percentage of oothecae that dropped before hatch for essential oil components ranged from 32.71 ± 0.05 for thymol to 100 for S-(-)-limonene (Fig. 2b). The percentage of oothecae that hatched for the essential oil components ranged from 72.22 ± 0.04 for (-)-menthone to 95.33 ± 0.02 for S-(-)-limonene (Fig. 3b). The mean number of nymphs that emerged from oothecae attached to females treated with essential oil components ranged from 20.89 ± 1.38 to 35.21 ± 1.08 for (-)-menthone and S-(-)-limonene, respectively (Fig

1b). Oothecae that were attached to females treated with S-(-)-limonene had a significantly greater mean number of nymphs (35.21 ± 1.08) hatch than oothecae attached to females treated with other essential oil components; therefore, S-(-)-limonene treatment had the least effect on ootheca hatch (Figs. 1a and 1b). Oothecae that were attached to females treated with (-)-menthone had a significantly lower mean number of nymphs (20.89 ± 1.38) hatch than oothecae attached to females treated with the other essential oil components; therefore, the (-)-menthone treatment had the greatest effect on ootheca hatch (Figs. 1a and 1b). The essential oil components having the greatest to least effect on ootheca hatch were (-)-menthone>geraniol>thymol>1,8-cineole> citronellic acid>(-)-linalool>eugenol>(-)-β-pinene>carvacrol>*trans*-cinnamaldehyde=(+)-α-pinene>S-(-)-limonene (Figs. 1a and 1b).

The mean number of nymphs that emerged from oothecae attached to carvacrol-treated females ranged from 32.06 ± 2.51 to 13.94 ± 3.77 for 0 and 0.50 mg/cockroach, respectively (Fig. 1a). The number of hatched nymphs was not significantly different between 0 and 0.40 mg/cockroach; however, significantly fewer nymphs hatched for 0.50 mg/cockroach (13.94 nymphs) than for all other doses (Fig. 1a).

The mean number of nymphs that emerged from *trans*-cinnamaldehyde-treated females ranged from 35.06 ± 1.47 to 24.11 ± 3.16 for 0 and 0.53 mg/cockroach, respectively (Fig. 1a). The number of nymphs decreased linearly with increasing dose, and this relationship was highly significant [mean number of nymphs = 35.13 (± 0.81) – 21.46 (± 2.73) dose, $r^2 = 0.93$ ($F = 61.95$, df = 1, 5, $P = <0.001$), (Table 9)].

The mean number of nymphs that emerged from oothecae attached to citronellic acid-treated females ranged from 33.33 ± 1.08 to 18.89 ± 3.75 for 0 and 0.47

mg/cockroach, respectively (Fig. 1a). Significantly fewer nymphs hatched for 0.47 mg/cockroach (18.89 nymphs) than 0 mg/cockroach (33.33 nymphs) and for 0.47 mg/cockroach (18.89 nymphs) than 0.09 mg/cockroach (31.50 nymphs) (Fig. 1a). The number of nymphs decreased linearly with increasing dose: mean number of nymphs = 31.20 (± 2.04) – 23.58 (± 7.72) dose, $r^2 = 0.65$ ($F = 9.34$, df = 1, 5, $P = 0.028$) (Table 9).

The mean number of nymphs that emerged from oothecae attached to geraniol-treated females ranged from 29.28 ± 2.62 to 18.94 ± 3.44 for 0 and 0.44 mg/cockroach, respectively (Fig. 1a). Significantly fewer nymphs hatched for 0.36 mg/cockroach (15.61 nymphs) than 0 mg/cockroach (29.28 nymphs) (Fig. 1a). The number of nymphs decreased linearly with increasing dose: mean number of nymphs = 28.04 (± 1.94) – 23.38 (± 7.67) dose, $r^2 = 0.65$ ($F = 9.30$, df = 1, 5, $P = 0.028$) (Table 9).

The mean number of nymphs that emerged from oothecae attached to (-)-menthone-treated females ranged from 32.11 ± 2.24 to 14.61 ± 3.36 for 0 and 0.50 mg/cockroach, respectively (Fig. 1b). Significantly fewer nymphs hatched for 0.40 mg/cockroach (18.33 nymphs) than 0 mg/cockroach (32.11 nymphs), and for 0.50 mg/cockroach (14.61 nymphs) than 0 mg/cockroach (32.11 nymphs) (Fig. 1b). The number of nymphs decreased linearly with increasing dose: mean number of nymphs = 28.04 (± 2.35) – 25.07 (± 8.38) dose, $r^2 = 0.642$ ($F = 8.948$, df = 1, 5, $P = 0.030$) (Table 9). There were no significant effects ($P>0.05$) of 1,8-cineole, eugenol, S-(-)-limonene, (-)-linalool, (+)-α-pinene, (-)-β-pinene, and thymol on ootheca hatch (Figs. 1a and 1b).

Discussion

Toxicity. *Trans*-cinnamaldehyde, thymol, eugenol, and carvacrol were the most toxic essential oil components to adult and large and medium nymphs of the German

cockroach. *Trans*-cinnamaldehyde, thymol, geraniol, and carvacrol were the most toxic essential oil components to small nymphs. The topical toxicity of the four most toxic essential oil components was less than those of the conventional insecticides, such as permethrin (LD_{50} = 0.072 µg/cockroach), deltamethrin (LD_{50} = 0.006 µg/cockroach) (Pridgeon et al. 2002), and bendiocarb (LD_{50} = 0.36 µg/cockroach) (Scott et al. 1990). These results are consistent with those reported by Jang et al. (2005), who demonstrated that components of marjoram oil were less toxic than permethrin and deltamethrin to adult female German cockroaches.

The toxicity of each essential oil component differed among the stages. We used one-way ANOVA and Tukey's multiple comparison tests to verify that there were significant differences in body mass among stages ($P < 0.0001$). The differences in body mass between adult males and large nymphs and adult and gravid females, however, was not significant (Table 2). The most frequently occurring susceptibility ranking for the stages was small nymphs > medium nymphs > adult males > large nymphs > gravid females > adult females. Susceptibility differences are positively correlated to the mass of each insect stage. Our results are consistent with the studies of Gish and Chura (1970), who found that animals with a larger body mass were less susceptible to insecticides (Table 2). In addition, adult females and large nymphs have greater proportions of body lipid than gravid females and adult males, respectively (Abd-Elghafar et al. 1990). The lipid soluble oils may become trapped in the body lipid, inhibiting the oils from reaching the target site (Yu 2008). Because adult females are the most tolerant to the essential oil components, they should be the determining factor when selecting field application rates.

The Pearson product-moment correlation was used to determine the correlation between toxicity and physical properties of the essential oils. LD_{50} values of all stages were correlated negatively with the density (g/ml) of the essential oil components ($r = -0.421$, $P = 0.0011$). The density of the essential oil components may effect the penetration of the compounds through the cuticle. Essential oil components with high densities were generally more toxic than those with low densities. LD_{50} values of all stages were negatively correlated with the boiling point of the essential oil components ($r = -0.389$, $P = 0.0028$). The boiling point of a compound reflects the strength of intermolecular forces, such as dipole-dipole forces and hydrogen bonds. It represents the temperature at which the molecules of the compound hold enough energy to overcome the intermolecular forces holding the molecules together (Chang 2003). A compound with a low boiling point evaporates more rapidly than a compound with a high boiling point, which would make it less available for penetration through the insect cuticle.

Structural characteristics such as chemical class, saturation, and lipophilicity may also contribute to the toxicity of compounds. These characteristics can affect penetration through the cuticle, degradation of the essential oil component, movement of the compound to the target site (Rice and Coats 1994), and the ability of the insect to excrete the compound. The most toxic essential oil components to the majority of cockroach stages were aromatic rather than aliphatic compounds. These compounds included carvacrol, *trans*-cinnamaldehyde, eugenol, and thymol. The most toxic essential oil components to small nymphs were carvacrol, *trans*-cinnamaldehyde, geraniol (aliphatic), and thymol. Benzene is a relatively non-polar compound due to the delocalization of electrons in the ring (Morrison and Boyd 1992). Metabolism of aromatic compounds is

relatively difficult because detoxification involves a series of processes that make the compound more hydrophilic and polar so that it can be easily excreted (Yu 2008). Therefore, essential oil components containing a benzene ring are not easily metabolized and detoxified in the insect body. Because the aromatic compounds are not easily metabolized, they are more toxic than aliphatic compounds. Our results are consistent with those of Rice and Coats (1994) who found that aromatic alcohols were more toxic than aliphatic alcohols to the house fly, *Musca domestica* (L.).

We found that monocyclic aliphatic compounds tended to be more toxic than bicyclic aliphatic compounds to all stages of the German cockroach. Rice and Coats (1994) also found that monocyclic compounds, such as menthol and carvone, were more toxic than bicyclic compounds, such as verbenol and thujone. The monocyclic compounds used in our study consisted of six-membered carbon rings. Six-membered carbon rings are predicted to have bond angles of ~109 degrees, which adds to their flexibility (Morrison and Boyd 1992). Two of the bicyclic compounds used in our study, (+)-α-pinene and (-)-β-pinene, consisted of one six-membered carbon ring and one 4-membered carbon ring. Because the 4-membered carbon rings have bond angles of ~90 degrees, they are in a strained state caused by lack of flexibility (Morrison and Boyd 1992). The chemical bonds may be inclined to break more easily in response to detoxifying enzyme activity, which could lead to faster degradation in the insect body. 1,8,-Cineole is a bicyclic compound consisting of two 6-membered carbon rings, which would make it more flexible than (+)-α-pinene and (-)-β-pinene but less flexible than a six-membered monocyclic carbon ring; however, the toxicity of 1,8-cineole was not consistently lower than that of the monocyclic compounds for all stages.

Compound saturation affected toxicity to German cockroaches. The saturated essential oil components used in this study contained only single bonds, with the exception of double bonds present in the benzene ring. The unsaturated components used contained at least one double bond other than the double bonds present in the benzene ring. Saturated components, such as thymol and carvacrol, were highly, or moderately, toxic to all stages of the German cockroach. The majority of the components with low toxicity were unsaturated compounds, such as (+)-α-pinene and (-)-β-pinene. Multiple unsaturated compounds did not decrease toxicity. The degree of compound saturation may affect degradation in the insect body. It is possible that the unsaturated compounds are unable to group as closely together as the saturated compounds (Tortora et al. 2007), due to steric hindrance (Morrison and Boyd 1992). Steric hindrance may increase the solubility, allowing it to be excreted at a faster rate. In phase one of the metabolism of xenobiotics, a polar reactive group is attached to the compound to make it a more suitable substrate for enzyme attachment (Hodgson 1987). Unsaturated compounds may provide more sites for a polar group and enzyme to attach. The enzymes will attack substrates, such as sugars and amino acids, creating water soluble compound that can be more easily excreted (Hodgson 1987).

The results from a study by Rice and Coats (1994) on the insecticidal properties of several monoterpenoids to the house fly, red flour beetle, and southern corn rootworm demonstrated a positive correlation between toxicity and lipophilicity (Log P); however, the results from our study did not show such a correlation. Our results were consistent with those of Jang et al. (2005) who determined the toxicity of marjoram oil components to the adult female German cockroach. A high Log P value may be related to enhanced

cuticular penetration (Matsumura 1985); however, the essential oil components with the highest Log P value in our study were (+)-α-pinene and (-)-β-pinene, which were the least toxic to all stages of the German cockroach. Depending upon epicuticular lipid composition, it is possible that a compound can be too lipophilic to completely penetrate the cuticle (Yu 2008). A cuticle with high lipid content may act as a lipid reserve, trapping lipophilic compounds and inhibiting them from reaching the target site (Yu 2008). The injection of essential oil components under the insect cuticle would determine if the low toxicity of these compounds could be attributed to the sequestering of the oils in the cuticle; however, we injected 0.44 mg/µl of (+)-α-pinene and (-)-β-pinene under the cuticle and observed negligible mortality (unpublished data).

Ootheca hatch. Our results showed that an ootheca attached to a dead female can hatch, which is consistent with the results of Abd-Elghafar and Appel (1992). Four of the essential oil components had a significant effect on ootheca hatch (*trans*-cinnamaldehyde, (-)-menthone, geraniol, and citronellic acid). No essential oil components completely prevented ootheca hatch; therefore, from a practical standpoint, additional treatments of these oils would be required in the field to prevent reinfestation. There were also significant differences in the mean number of nymphs among the doses for carvacrol, *trans*-cinnamaldehyde, citronellic acid, geraniol, and (-)-menthone; significantly fewer nymphs hatched from the higher doses, and fewer nymphs hatched from dead females. These results are consistent with those of Abd-Elghafar and Appel (1992), who found that the numbers of nymphs hatching from oothecae declined as insecticide dose increased. We also found that significantly fewer oothecae dropped from treated than control females. These results demonstrate that *trans*-cinnamaldehyde,

citronellic acid, geraniol, and (-)-menthone reduced ootheca hatch, in part, because the large doses killed the females before they had time to deposit their oothecae, a naturally occurring process for the German cockroach before ootheca hatch (Ross and Mullins 1995). Oothecae receive nutrients and water while attached to their living mother's body (Roth 1970b.). Contamination with essential oil components or the lack of nutrients and water from dead females may also have contributed to nymph mortality. It is also possible that the body of the dead females absorbed water from the developing embryos by a passive wicking action.

Essential oil components, such as *trans*-cinnamaldehyde, thymol, carvacrol, and eugenol, that are toxic to adult females, can potentially be used as direct contact sprays against German cockroaches in areas where traditional insecticide use is restricted. However, there are several limitations that must be overcome before essential oil components can successfully be used in the field. Previous studies have shown them to be repellent (Appel et al. 2001) and to have little residual activity (Isman 2006). Because of their repellency, a pest control operator would have to apply the essential oils carefully in the field. Avoiding contact of the spray on or near insecticidal baits, bait stations, and traps would be necessary to preserve their attractiveness to German cockroaches (Appel 2004). If used cautiously, essential oils could be used as flushing agents and inspection tools for locating infested areas and reducing the availability of suitable harborages. A microencapsulated formulation might be useful to increase the residual, decrease the repellency, and eliminate the odor of the essential oils. Microencapsulated formulations contain active ingredient in microscopic polymeric capsules that rupture overtime or when stimulated by pressure, such as an insect walking over the capsule (Barcay 2004).

The essential oil components do have an effect on ootheca hatch, but they do not eliminate hatch. Follow up treatment would be necessary to prevent reinfestation by the hatched nymphs. The use of essential oil components along with other integrated pest management techniques can be an effective method for controlling the German cockroach in food preparation areas, storage buildings, apartments, and homes.

Table 2. Mean masses of German cockroach stages measured in grams

Stage	*N*	Mean ± SD[a]
Adult females	30	0.0861 ± 0.0140a
Gravid females	30	0.0853 ± 0.0110a
Large nymphs	30	0.0452 ± 0.0080b
Adult males	30	0.0443 ± 0.0042b
Medium nymphs	30	0.0105 ± 0.0037c
Small nymphs	30	0.0015 ± 0.0009d

[a]Means within a column followed by the same letter are not significantly different ($P < 0.05$)

Table 3. Toxicity of essential oils applied topically to adult male German cockroaches

Essential oil	*n*	Slope ± SE	$LD_{50,}$ mg/cockroach (95%CI)	χ^2	*P*
Carvacrol	108	6.42 ± 1.36	0.101 (0.084-0.122)	22.32	0.0001
1,8-Cineole	108	7.87 ± 1.59	0.156 (0.131-0.181)	24.57	0.0001
Trans-cinnamaldehyde	234	12.62 ± 2.70	0.078 (0.071-0.086)	21.88	0.0001
Citronellic acid	126	3.62 ± 1.08	0.252 (0.149-0.391)	11.17	0.0008
Eugenol	108	6.42 ± 1.36	0.109 (0.091-0.132)	22.31	0.0001
Geraniol	126	4.79 ± 0.95	0.262 (0.219-0.305)	25.73	0.0001
S-(-)-Limonene	126	5.45 ± 1.94	0.285 (0.183-0.406)	7.91	0.0049
(-)-Linalool	126	6.86 ± 1.41	0.316 (0.279-0.358)	23.75	0.0001
(-)-Menthone	126	3.71 ± 1.60	0.126 (0.025-0.160)	5.35	0.0207
(+)-α-Pinene	126	4.49 ± 2.16	0.644 (0.463-11293.438)	4.32	0.0377
(-)-β-Pinene	126	3.57 ± 1.45	0.481 (0.351-8.434)	5.98	0.0144
Thymol	198	23.10 ± 7.03	0.070 (0.063-0.073)	10.81	0.0010

Table 4. Toxicity of essential oils applied topically to adult female German Cockroaches

Essential oil	*n*	Slope ± SE	$LD_{50,}$ mg/cockroach (95%CI)	χ^2	*P*
Carvacrol	108	6.94 ± 1.30	0.186 (0.156-0.214)	28.60	0.0001
1,8-Cineole	108	3.30 ± 1.11	0.273 (0.164-0.531)	8.86	0.0029
Trans-cinnamaldehyde	126	7.68 ± 1.53	0.188 (0.158-0.216)	25.23	0.0001
Citronellic acid	126	2.14 ± 0.64	0.491 (0.337-1.298)	11.28	0.0008
Eugenol	108	4.19 ± 0.77	0.294 (0.244-0.349)	29.53	0.0001
Geraniol	126	1.86 ± 0.66	0.832 (0.477-11.840)	8.00	0.0047
S-(-)-Limonene	126	-	>0.50 *a*	-	0.4579
(-)-Linalool	126	-	>0.50 *a*	-	0.0747
(-)-Menthone	90	4.19 ± 1.83	0.773 (0.628-6.047)	5.23	0.0222
(+)-α-Pinene	126	-	>0.50 *a*	-	0.7931
(-)-β-Pinene	126	0	>0.50 *a*	-	-
Thymol	108	2.88 ± 0.68	0.195 (0.122-0.280)	17.84	0.0001

[a] Probit model did not work because < 20% mortality occurred

Table 5. Toxicity of essential oils applied topically to gravid female German cockroaches

Essential oil	*n*	Slope ± SE	$LD_{50,}$ mg/cockroach (95%CI)	χ^2	*P*
Carvacrol	126	6.69 ± 1.23	0.146 (0.121-0.171)	29.56	0.0001
1,8-Cineole	126	3.61 ± 1.10	0.507 (0.400-1.023)	10.75	0.0010
Trans-cinnamaldehyde	126	8.02 ± 1.79	0.133 (0.112-0.157)	20.12	0.0001
Citronellic acid	126	3.83 ± 1.25	0.518 (0.411-1.140)	9.34	0.0022
Eugenol	126	8.73 ± 2.05	0.287 (0.232-0.333)	18.17	0.0001
Geraniol	126	5.13 ± 1.61	0.452 (0.382-0.722)	10.19	0.0014
S-(-)-Limonene	126	-	>0.50 *a*	-	0.3215
(-)-Linalool	126	-	>0.50 *a*	-	0.9999
(-)-Menthone	126	3.04 ± 1.33	0.395 (0.226-23.295)	5.21	0.0224
(+)-α-Pinene	126	0	>0.50 *a*	-	-
(-)-β-Pinene	126	-	>0.50 *a*	-	0.3215
Thymol	126	4.77 ± 1.08	0.122 (0.082-0.164)	19.36	0.0001

[a] Probit model did not work because < 20% mortality occurred

Table 6. Toxicity of essential oils applied topically to large nymph German cockroaches

Essential oil	*n*	Slope ± SE	$LD_{50,}$ mg/cockroach (95%CI)	χ^2	*P*
Carvacrol	108	3.84 ± 0.59	0.129 (0.102-0.157)	42.57	0.0001
1,8-Cineole	108	6.11 ± 1.26	0.333 (0.292-0.386)	23.55	0.0001
Trans-cinnamaldehyde	198	10.43 ± 2.66	0.117 (0.110-0.131)	15.37	0.0001
Citronellic acid	108	3.03 ± 1.07	0.643 (0.458-2.968)	8.10	0.0044
Eugenol	108	3.68 ± 0.64	0.246 (0.199-0.296)	33.39	0.0001
Geraniol	126	1.79 ± 0.57	0.736 (0.441-4.700)	9.92	0.0016
S-(-)-Limonene	126	4.40 ± 1.91	0.598 (0.447-13.565)	5.28	0.0216
(-)-Linalool	126	-	>0.50 *a*	-	0.4820
(-)-Menthone	126	4.51 ± 1.04	0.370 (0.315-0.456)	18.75	0.0001
(+)-α-Pinene	126	0	>0.50 *a*	-	-
(-)-β-Pinene	126	0	>0.50 *a*	-	-
Thymol	108	2.65 ± 0.48	0.220 (0.169-0.280)	29.67	0.0001

[a] Probit model did not work because < 20% mortality occurred

Table 7. Toxicity of essential oils applied topically to medium nymph German cockroaches

Essential oil	*n*	Slope ± SE	$LD_{50,}$ mg/cockroach (95%CI)	χ^2	*P*
Carvacrol	108	3.35 ± 1.33	0.061 (0.005-0.107)	6.37	0.0116
1,8-Cineole	108	3.93 ± 1.03	0.208 (0.133-0.285)	14.58	0.0001
Trans-cinnamaldehyde	198	10.92 ± 2.37	0.082 (0.074-0.087)	21.24	0.0001
Citronellic acid	108	4.05 ± 0.76	0.248 (0.203-0.295)	28.72	0.0001
Eugenol	108	4.20 ± 0.71	0.109 (0.086-0.134)	34.86	0.0001
Geraniol	126	2.99 ± 0.49	0.145 (0.110-0.181)	36.63	0.0001
S-(-)-Limonene	126	4.49 ± 0.77	0.207 (0.170-0.243)	34.03	0.0001
(-)-Linalool	126	3.61 ± 0.72	0.195 (0.142-0.255)	24.83	0.0001
(-)-Menthone	126	4.48 ± 1.03	0.175 (0.120-0.234)	19.07	0.0001
(+)-α-Pinene	126	0	>0.50 *a*	-	-
(-)-β-Pinene	126	0	>0.50 *a*	-	-
Thymol	90	1.85 ± 0.48	0.060 (0.023-0.092)	14.95	0.0001

[a] Probit model did not work because < 20% mortality occurred

Table 8. Toxicity of essential oils applied topically to small nymph German cockroaches

Essential oil	*n*	Slope ± SE	$LD_{50,}$ mg/cockroach (95%CI)	χ^2	*P*
Carvacrol	90	3.50 ± 1.32	0.056 (0.007-0.091)	7.00	0.0081
1,8-Cineole	90	2.49 ± 0.48	0.133 (0.096-0.175)	26.68	0.0001
Trans-cinnamaldehyde	198	4.93 ± 0.98	0.036 (0.031-0.042)	25.36	0.0001
Citronellic acid	108	2.27 ± 0.51	0.131 (0.075-0.188)	19.61	0.0001
Eugenol	90	4.42 ± 1.04	0.066 (0.047-0.083)	18.19	0.0001
Geraniol	126	3.00 ± 0.70	0.049 (0.028-0.067)	18.64	0.0001
S-(-)-Limonene	126	1.68 ± 0.41	0.057 (0.022-0.088)	17.28	0.0001
(-)-Linalool	126	3.64 ± 0.59	0.096 (0.074-0.119)	38.60	0.0001
(-)-Menthone	126	2.28 ± 0.48	0.060 (0.031-0.086)	22.76	0.0001
(+)-α-Pinene	126	0	>0.50 *a*	-	-
(-)-β-Pinene	126	0	>0.50 *a*	-	-
Thymol	90	4.94 ± 1.73	0.047 (0.023-0.059)	8.08	0.0045

[a] Probit model did not work because < 20% mortality occurred

Table 9. Relationship between doses applied to gravid females and the mean number of hatched nymphs

TMT	Slope ± SE	Intercept ± SE	r^2	df	*F*	*P*
Carvacrol	-	-	-	6	4.11	0.098
1,8-Cineole	-	-	-	6	0.00	0.974
Trans-cinnamaldehyde	-21.46 ± 2.73	35.13 ± 0.81	0.93	6	61.95	0.001
Citronellic acid	-23.58 ± 7.72	31.20 ± 2.04	0.65	6	9.34	0.028
Eugenol	-13.45 ± 3.41	30.49 ± 1.03	0.76	6	15.52	0.011
Geraniol	-23.38 ± 7.67	28.04 ± 1.94	0.65	6	9.30	0.028
S-(-)-Limonene	-	-	-	6	0.45	0.533
(-)-Linalool	-	-	-	6	5.78	0.061
(-)-Menthone	-25.07 ± 8.38	28.04 ± 2.35	0.64	6	8.95	0.030
(+)-α-Pinene	-	-	-	6	0.58	0.482
(-)-β-Pinene	-	-	-	6	1.63	0.258
Thymol	-9.81 ± 3.68	27.08 ± 1.03	0.59	6	7.11	0.045

Table 10. Relationship between doses applied to gravid females and percentage of dropped oothecae

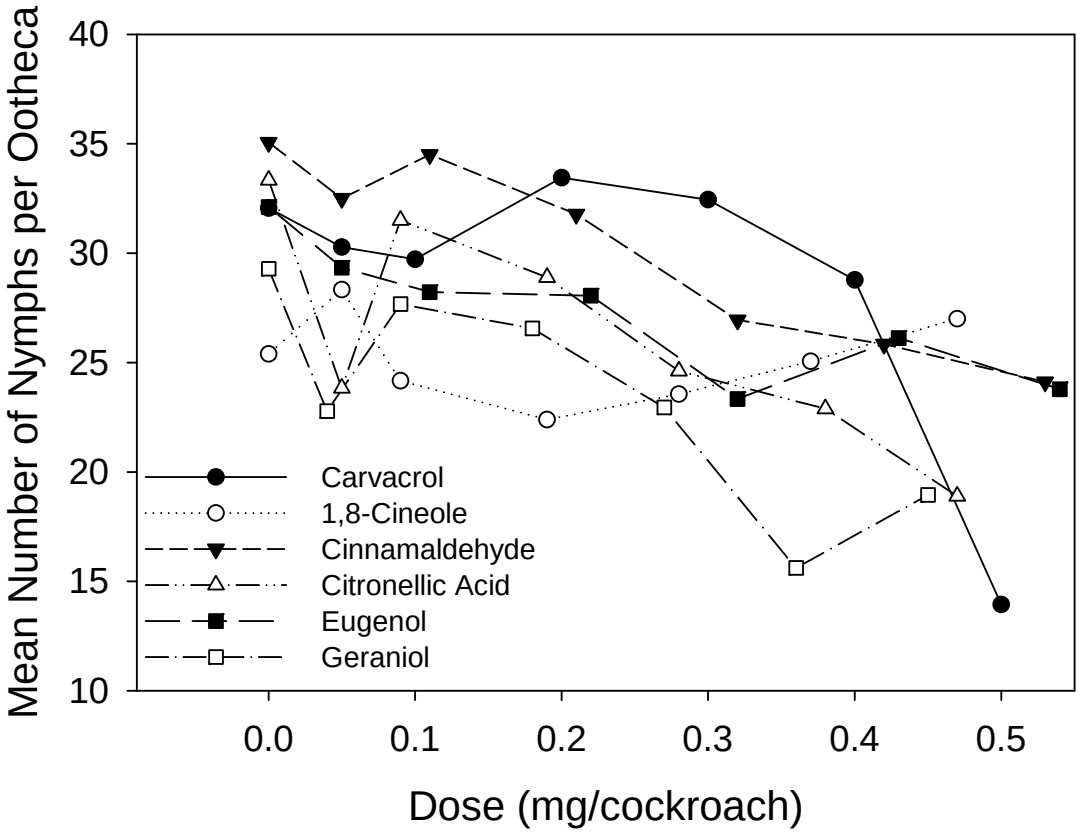
TMT	Slope ± SE	Intercept ± SE	r^2	df	*F*	*P*
Carvacrol	-135.54 ± 27.96	97.66 ± 7.86	0.83	6	23.50	0.005
1,8-Cineole	-	-	-	6	0.02	0.890
Trans-cinnamaldehyde	-188.44 ± 57.24	90.97 ± 17.01	0.68	6	10.84	0.022
Citronellic acid	-138.24 ± 35.81	97.88 ± 9.48	0.75	6	14.91	0.012
Eugenol	-200.61 ± 39.61	90.72 ± 12.00	0.84	6	25.65	0.004
Geraniol	-167.74 ± 24.93	95.85 ± 6.30	0.90	6	45.28	0.001
S-(-)-Limonene	-	-	-	6	1.87	0.229
(-)-Linalool	-73.97 ± 24.74	93.27 ± 6.26	0.64	6	8.94	0.030
(-)-Menthone	-	-	-	6	1.08	0.346
(+)-α-Pinene	-	-	-	6	0.35	0.579
(-)-β-Pinene	-	-	-	6	3.26	0.131
Thymol	-190.17 ± 31.52	83.05 ± 8.85	0.88	6	36.41	0.002

Table 11. Relationship between doses applied to gravid females and percentage of hatched oothecae

TMT	Slope ± SE	Intercept ± SE	r^2	df	*F*	*P*
Carvacrol	-74.06 ± 23.74	100.90 ± 6.67	0.66	6	9.73	0.026
1,8-Cineole	-	-	-	6	0.03	0.865
Trans-cinnamaldehyde	-30.44 ± 8.42	98.40 ± 2.50	0.72	6	13.07	0.015
Citronellic acid	-61.06 ± 12.86	100.05 ± 3.40	0.82	6	22.56	0.005
Eugenol	-	-	-	6	1.70	0.250
Geraniol	-	-	-	6	3.27	0.130
S-(-)-Limonene	-	-	-	6	0.17	0.700
(-)-Linalool	-	-	-	6	2.53	0.173
(-)-Menthone	-65.22 ± 24.64	90.63 ± 6.92	0.58	6	7.01	0.046
(+)-α-Pinene	-	-	-	6	1.71	0.248
(-)-β-Pinene	-	-	-	6	0.06	0.822
Thymol	-	-		6	0.17	0.700

Fig. 1. The effect of dose on the mean number of nymphs per ootheca

a.



b.

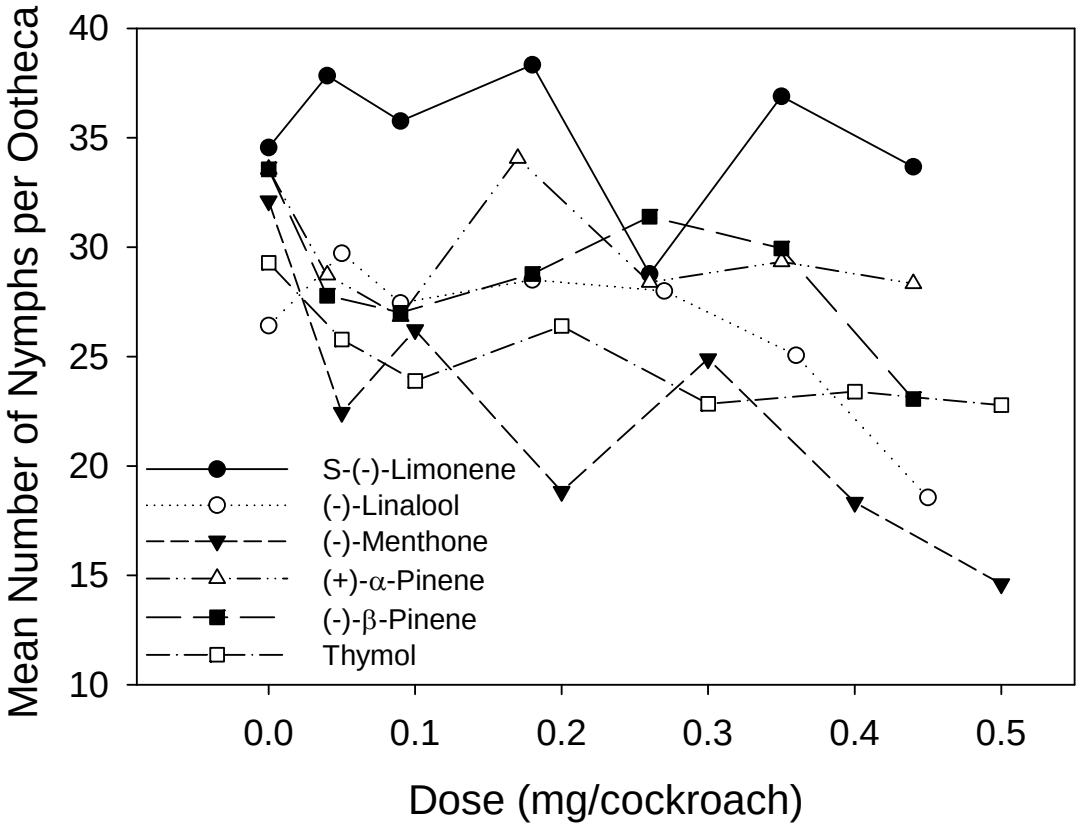
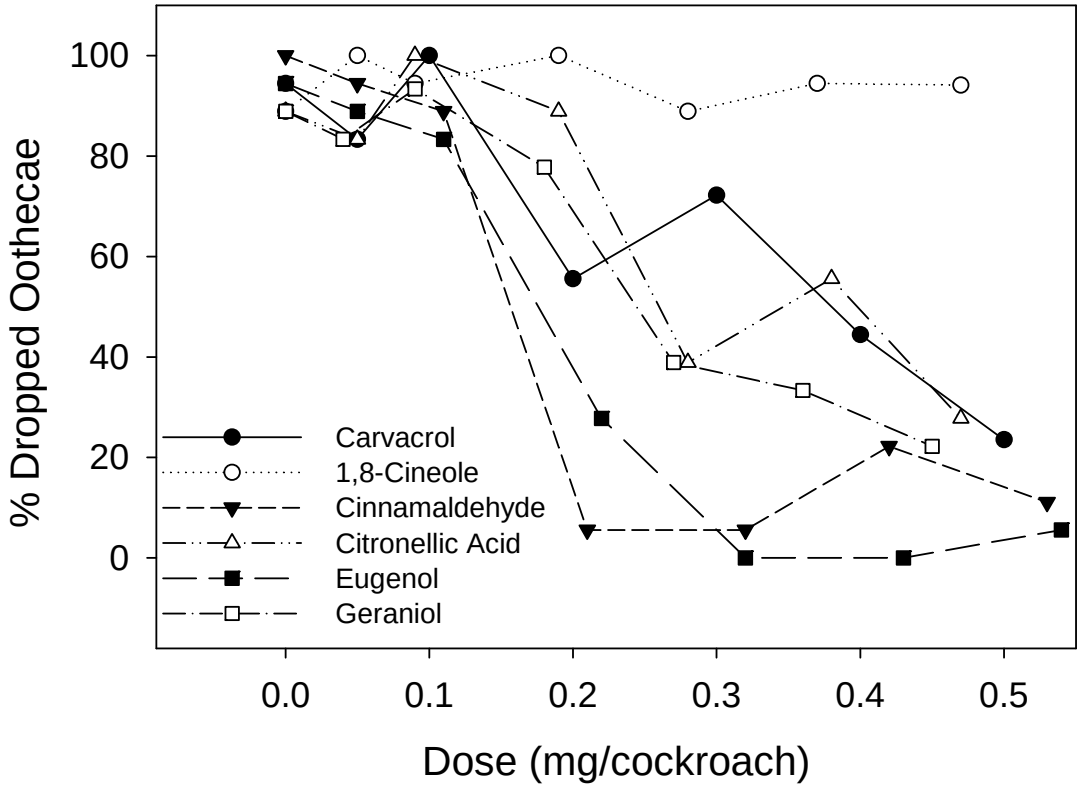

Mean Number of Nymphs per Ootheca
40
35
30
25
20
15
10
0.0
0.1
0.2
0.3
0.4
0.5
Dose (mg/cockroach)
S-(-)-Limonene
(-)-Linalool
(-)-Menthone
(+)-α-Pinene
(-)-β-Pinene
Thymol

Fig. 2. The effect of dose on the percentage of dropped oothecae

a.



b.

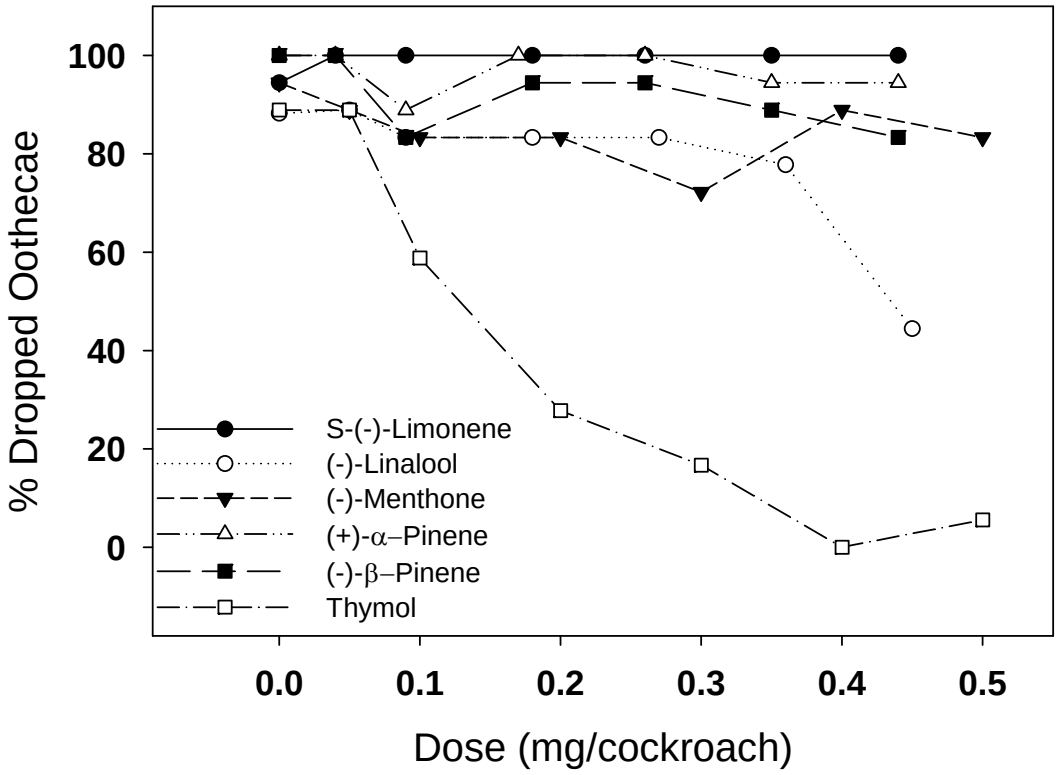
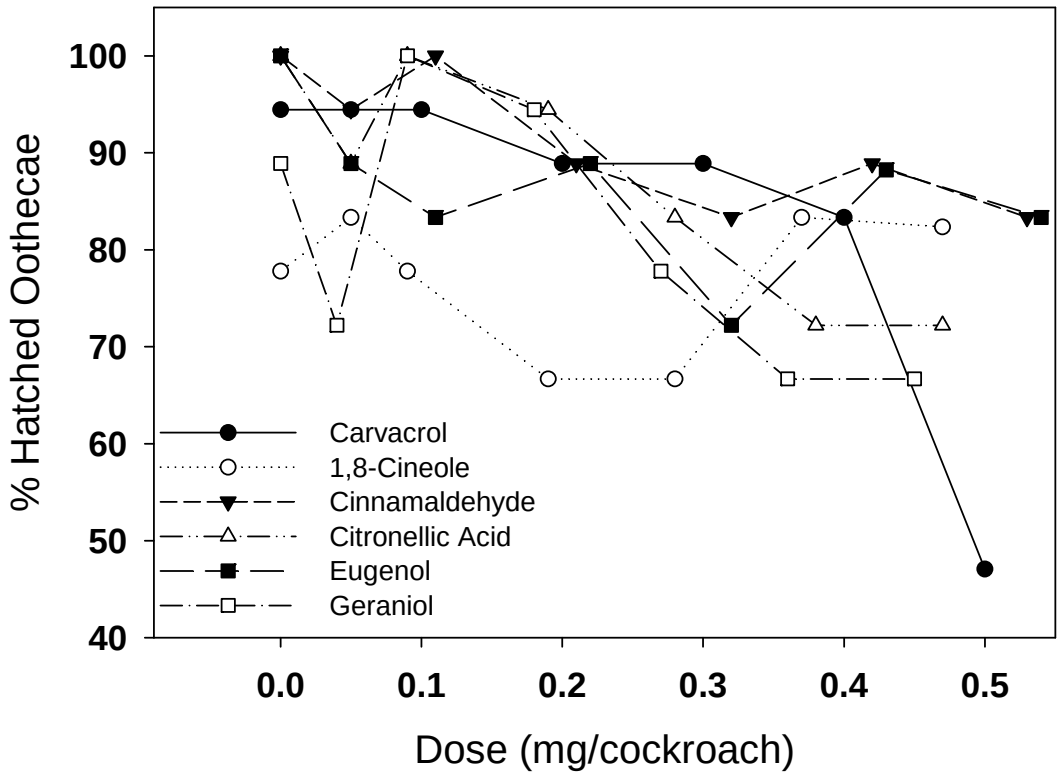

% Dropped Oothecae
100
80
60
40
20
0
0.0
0.1
0.2
0.3
0.4
0.5
Dose (mg/cockroach)
S-(-)-Limonene
(-)-Linalool
(-)-Menthone
(+)-α–Pinene
(-)-β–Pinene
Thymol

Fig. 3. The effect of dose on the percentage of hatched oothecae

a.



b.

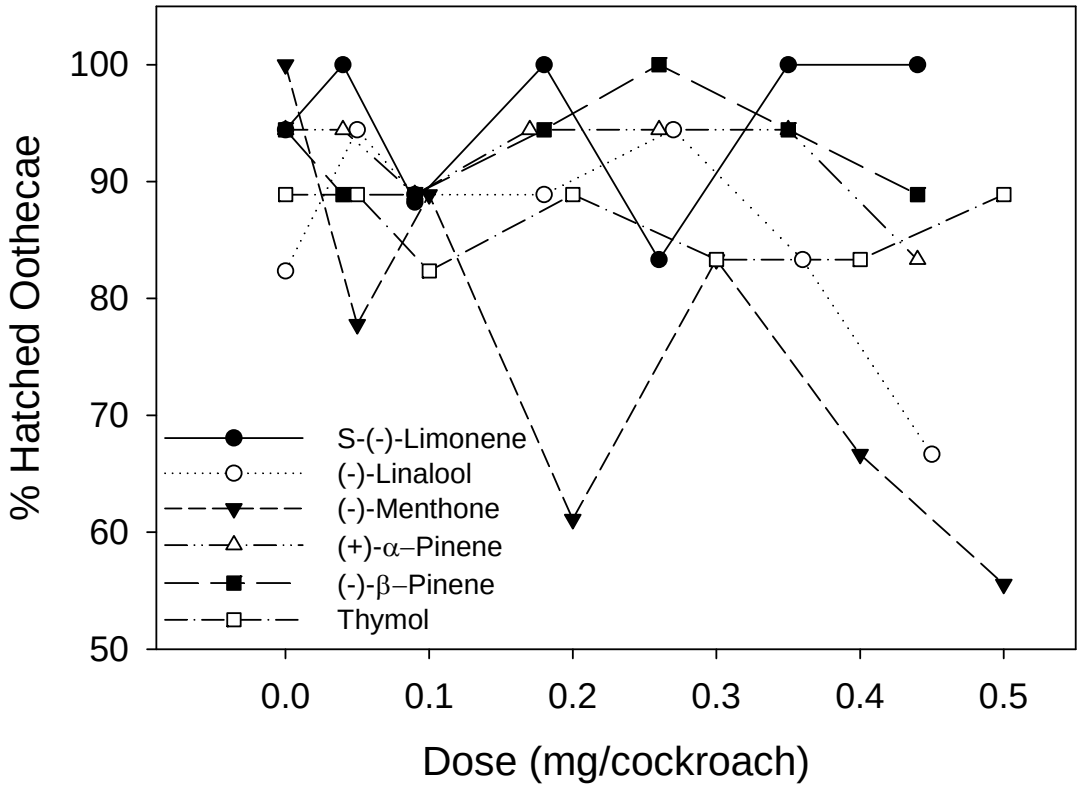

% Hatched Oothecae
100
90
80
70
60
50
0.0
0.1
0.2
0.3
0.4
0.5
Dose (mg/cockroach)
S-(-)-Limonene
(-)-Linalool
(-)-Menthone
(+)-α–Pinene
(-)-β–Pinene
Thymol

Fig. 4. The effect of essential oil density on toxicity

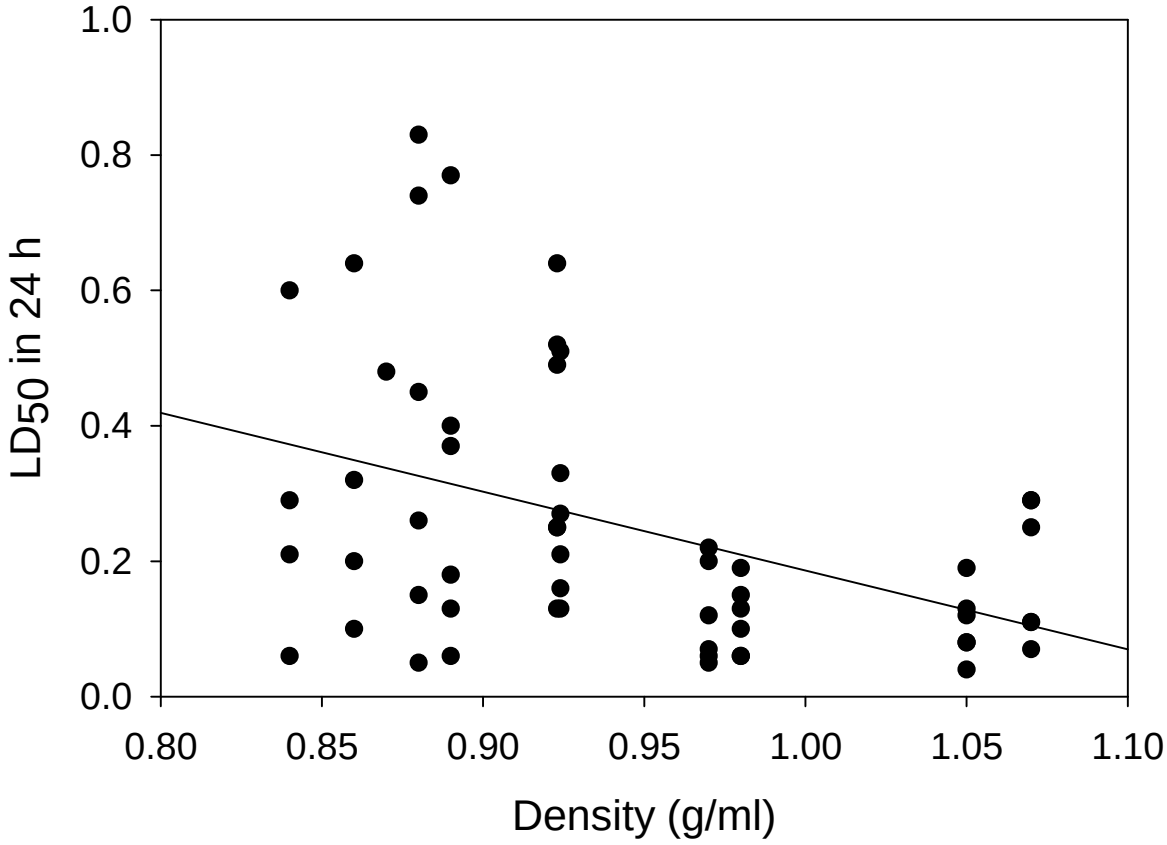
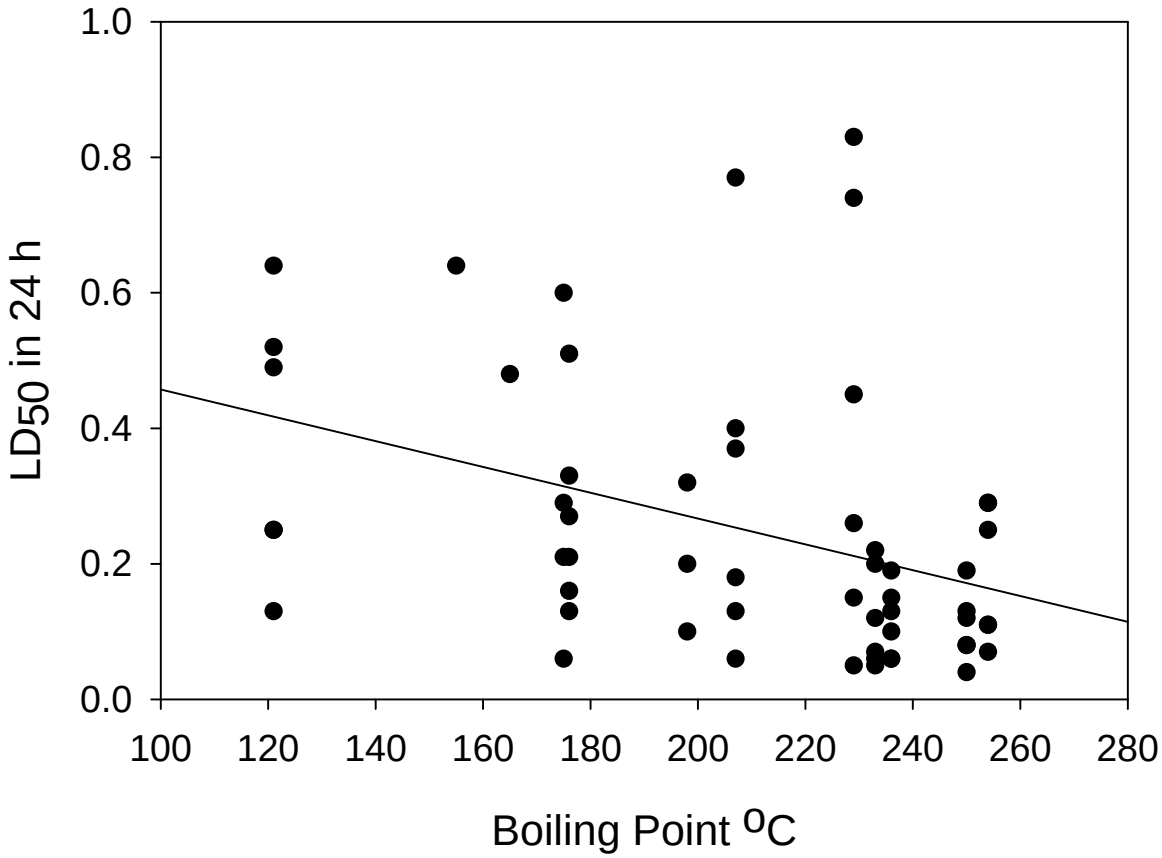


Fig. 5. The effect of essential oil boiling point on toxicity



Fumigant Toxicity of Essential Oils to the German Cockroach (Dictyoptera: Blattellidae)

The German cockroach, *Blattella germanica* (L.), is an important household and industrial pest. Its feces and exuviae can cause allergic reactions in sensitive people (Schal and Hamilton 1990), and they can vector numerous microorganisms that are pathogenic to humans and wildlife, including viruses, bacteria, protozoa, and helminthes (Roth and Willis 1957, 1960). In addition, German cockroaches are disgusting to most people and indicate an unsanitary environment. German cockroaches have a short generation time and high fecundity which increases their chance of developing resistance to the insecticides used to manage populations (Barcay 2004).

The public's increasing concern about potentially negative effects of traditional fumigants, such as methyl bromide and sulfuryl fluoride, and the future prohibition of methyl bromide by the United States Environmental Protection Agency (EPA), has stimulated the investigation of botanical alternatives. Essential oils are safer alternatives to traditional fumigants and could potentially be used in areas or on objects that are isolated or can be tightly sealed, such as kitchens, ships, transport vehicles, sewer systems, sensitive equipment, and storage and household items. Essential oils are secondary plant substances (Isman 2006) comprised of many compounds, including monoterpenoids, which are responsible for a plant's aromatic characteristics. They have been used in the past and are still used as fragrances for perfumes and flavorings for food items (Isman 2006).

Essential oils are an excellent alternative to traditional fumigants because of their low toxicity to humans and wildlife and short residual period (Isman 2006). Unlike methyl bromide (Bell et al. 1996), no studies have shown that essential oils deplete the ozone. Minimum risk pesticides, which contain one or more essential oils, are currently exempt from EPA registration requirements (EPA PR Notice 2000-6). Fumigants that are exempt from EPA registration requirements can reach the market faster than conventional fumigants (Isman 2000).

Fumigation is the most common method used to control stored product pests because it is effective against most insect pests, can easily penetrate the product to reach the insect inside the grain, and leaves little residual (VanRyckeghem 2004). Because the use of methyl bromide and phosphine (the two primary fumigants used against stored product insects) is likely to be limited in the future (Lee et al. 2004), several studies have investigated the feasibility of essential oils for stored product fumigation. These include work by Stamopoulos et al. (2007) on the confused flour beetle, *Tribolium confusum* (du Val); Lee et al. (2004) on the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); Kordali et al. (2006) on the granary weevil, *Sitophilus granarius* (L.); and Rozman el al. (2007) on the lesser grain borer, *Rhyzopertha dominica* (F.), rice weevil, *Sitophilus oryzae* (L.), and red flour beetle, *Tribolium castaneum* (Herbst). Percentage mortality ranged from 0 for the rice weevil to 100 for the sawtoothed grain beetle treated with 50 µg/ml air of linalool.

Constituents of marjoram oil were tested against female German cockroaches to determine if they could be used as insecticides (Jang et al. 2005). Results demonstrated that thymol, α-terpineol, and linalool, the major constituents of marjoram oil, had

fumigant toxicity to female German cockroaches, but were less toxic than dichlorvos (Jang et al. 2005). The fumigant activity of corn mint, *Mentha arvensis* L., oil to American, *Periplaneta americana* (L.), and German cockroaches was determined by Appel et al. (2001). Corn mint oil, containing menthol and menthone as main components, had fumigant activity against both species. KT_{50} values for 46.45 μg/cm^3 corn mint oil were 7.38 and 9.21 h for American and German cockroaches, respectively (Appel et al. 2001).

Essential oils and their constituents have also been tested, for fumigant toxicity, against a variety of other insects. In the following studies, units used to measure fumigation were μl/ml, μl/L, μg/cm^3, mg/cm^2, and μg/cm^2; however, the standard U.S. unit for fumigation is oz·h/1000 ft^3 (Thoms and Scheffrahn 1994). Insects used to study the fumigant activity of essential oils include cat flea, *Ctenocephalides felis* Bouché, (Hink and Fee 1986); copra mite, *Tyrophagus putrescentiae* (Schrank), (Kim et al. 2004); click beetle, *Agriotes obscurus* L., (Waliwitiya et al. 2005); Japanese termite, *Reticulitermes speratus* Kolbe, (Park and Shin 2005); house fly, *Musca domestica* L., (Tarelli et al. 2009); and the human head louse, *Pediculus humanus capitis* De Geer, (Yang et al. 2004). Results demonstrated that all of the above species were susceptible to several of the essential oils and their constituents.

Because true fumigants are gases, they posses certain features that make them a unique method for insect control. They target a broad spectrum of pests because fumigants have a respiratory mode of action (Thoms and Phillips 2004). The gas will enter the tracheae of all insect species infesting the area or object. Unlike other insecticides, fumigants penetrate hard to reach areas, such as wall voids and equipment.

They leave little to no residual, which is convenient for commercial kitchens. Fumigation is the fastest method of pest control (Thoms and Phillips 2004).

Methyl bromide, one of the primary fumigants used for controlling the German cockroach, depletes the ozone layer (Bell et al. 1996); therefore, the EPA will eventually phase out its use in the United States and internationally too. Because essential oils can volatilize rapidly and do not leave a residual, the potential efficacy of these materials as alternative fumigants for control of the German cockroach was investigated. The purpose of this study was to determine and compare the fumigant toxicity of several pure essential oils to several life stages of the German cockroach.

Materials and Methods

Chemicals. Essential oil components (Table 1) were obtained from Sigma-Aldrich (St. Louis, MO). Some of the essential oil components were chosen because they are present in the essential oil extracts of numerous plant species, while others were chosen because they occur at high concentrations in the essential oils of selected plants. Both aromatic and aliphatic hydrocarbons were tested, and the functional groups represented in the chosen essential oil components included acids, alcohols, aldehydes, ketones, and ethers. Physical and chemical properties of essential oil components were either obtained from Sigma-Aldrich or estimated using Advanced Chemistry Development software version 12.0 (ACD/Labs 2008).

Insects. An insecticide susceptible strain of the German cockroach was used in all experiments. This strain (American Cyanamid, Clifton, NJ) has been in continuous laboratory culture for >35 years. The stages used were adult males, adult females, gravid females, large nymphs (5^{th}-7^{th} instar, ≥8.5 mm long), medium nymphs (3^{rd}-4^{th} instar, 5-8

mm long), and small nymphs (1^{st}-2^{nd} instar, ≤4.5 mm long). Laboratory cultures were maintained at 28 ± 2ºC, 40-55% RH, and a photoperiod of 12:12 (L:D) h. Colonies were provided water and dog chow (Purina) as needed. Cockroaches were briefly (<5 min) anesthetized with CO_2 to facilitate handling.

Fumigations. Fumigant activity was assessed by sealing groups of 10 German cockroaches in 0.95 liter Ball® glass jars (Jarden Corporation, Cleveland, OH) with 0.05-1,000 µl of an essential oil component spread evenly on the underside of the lids. Filter paper (2 qualitative, 12.5 cm diameter) (Whatman Group, Maidstone, UK) was hot glued to the underside of the lid for essential oil deposits exceeding 100 µl. This provided a larger surface area for a greater volume of essential oil to absorb. Water was used as the control. Prior to putting the cockroaches in the jar, the top, inner portion of the jar was coated with fluon (Bioquip, Rancho Dominguez, CA) to prevent the cockroaches from directly contacting the oil. Fluon treated jars were air-dried for 24 h at room temperature to allow offgassing of the fluon. Three replicate jars were used for each essential oil concentration tested. The jars were maintained in an incubator at ≈28ºC. No food, water, or harborage was provided. The room air that was sealed in the jar was ≈40% RH. Mortality was assessed at 24 h.

The standard unit for fumigation is oz·h/1000 ft^3 (Thoms and Scheffrahn 1994); however, in our experiments mg/L air at 24 h was used to measure fumigant toxicity of the essential oil components. These measurements can easily be converted into oz·h/1000 ft^3 by multiplying our values by 23.97

Effects of essential oils on ootheca hatch. After mortality was recorded for the fumigation tests, the live and dead gravid females and the dropped oothecae were held in

10.16 cm, 12 oz transparent plastic containers (Packaging With Perfection, Vernon, CA) and observed every 7 d for 30 d. Mortality, ootheca drop, ootheca hatch, and the number of nymphs present in each container were recorded. Cockroaches were supplied with carrot slices *ad libitum* and maintained in an incubator at ≈80% RH and ≈28ºC. The carrot slice provided both food and moisture.

Data analysis. Probit analysis for independent data was used to estimate toxicity in the fumigation tests (LC_{50}) (Proc Probit, SAS 9.1, SAS Institute 2003). Non-overlap of the 95% confidence intervals (CI) was used to estimate significant differences among LC_{50} values. A t-test was used to test for significant differences between the percentage hatched oothecae for control and treated females (Proc Ttest, SAS 9.1, SAS Institute 2003). One-way ANOVA and Tukey's multiple comparison tests were used to study differences in the percentage of hatched oothecae responding to concentrations of individual essential oils (Proc GLM, SAS 9.1, SAS Institute 2003). Regression analysis was used to examine the linear relationship between concentrations tested and the mean number of hatched nymphs, percentage of dropped oothecae, and percentage of hatched oothecae (SigmaPlot 11.0; SPSS 2008). Correlation analysis was used to relate essential oil toxicity (LC_{50}) with physical and chemical properties (SigmaPlot 11.0; SPSS 2008).

Results

Fumigations. No control mortality was observed for any stage during the study. LC_{50} values of carvacrol ranged from 3.63 to >1,000 mg/L air for small nymphs (Table 17) and adult females (Table 13), respectively. Homogeneity of response (slope of the log-dose probit relationship) was similar among most stages ranging from 0.75 to 0.92

for adult females (Table 13) and small nymphs (Table 17), respectively. The slope was significantly greater (6.40) for adult males (Table 12).

1,8-Cineole was highly toxic to most stages of the German cockroach; LC_{50} values ranged from 4.04 mg/L air for small nymphs (Table 17) to 11.60 mg/L air for medium nymphs (Table 16). Homogeneity of response was greatest for adult males (19.97) (Table 12) and least for small nymphs (2.30) (Table 17).

LC_{50} values of *trans*-cinnamaldehyde ranged from 5.75 to 46.70 mg/L air for small (Table 17) and large nymphs (Table 15), respectively. Homogeneity of response was greatest for adult males (10.47) (Table 12) and least for small nymphs (Table 17) (0.99). Citronellic acid was not toxic to any stages of the German cockroach.

LC_{50} values for eugenol ranged from 14.48 mg/L air for small nymphs (Table 17) to >1,000 mg/L air for adult females (Table 13). Homogeneity of response was similar for adult males, gravid females, medium nymphs, and small nymphs (2.01, 2.53, 2.07, and 1.72 for adult males, gravid females, medium nymphs, and small nymphs, respectively) (Tables 12, 14, 16, and 17).

Geraniol was slightly toxic to small and medium nymphs, but it was not toxic to adult males, adult females, gravid females, or large nymphs. LC_{50} values of geraniol ranged from 149.54 to 833.99 mg/L air for small nymphs (Table 17) and medium nymphs (Table 16), respectively. Homogeneity of response was similar for small (0.85) (Table 17) and medium nymphs (1.60) (Table 16).

S-(-)-Limonene was moderately toxic to all stages of the German cockroach. LC_{50} values for S-(-)-limonene ranged from 13.01 mg/L air for adult males (Table 12) to

25.64 mg/L air for large nymphs (Table 15). Homogeneity of response was greatest for large nymphs (13.56) (Table 15) and least for adult males (1.38) (Table 12).

LC_{50} values for (-)-linalool ranged 9.56 mg/L air for small nymphs (Table 17) to 157.75 mg/L air for large nymphs (Table 15). Homogeneity of response was greatest for adult males (6.00) (Table 12) and least for adult females (1.26) (Table 13).

LC_{50} values of (-)-menthone ranged from 5.75 to 18.36 mg/L air for small (Table 17) and large (Table 15) nymphs, respectively. Homogeneity of response was greatest for adult males (9.42) (Table 12) and least for small nymphs (2.58) (Table 17).

(+)-α-Pinene was moderately toxic to all stages of the German cockroach, with similar LC_{50} values for most stages. LC_{50} values ranges from 11.75 mg/L air for adult males (Table 12) to 30.42 mg/L air for medium (Table 16) nymphs. Homogeneity of response was greatest for gravid females (15.72) (Table 14) and least for small nymphs (2.72) (Table 17).

(-)-β-Pinene was moderately toxic to all stages of the German cockroach, with similar LC_{50} values for most stages. LC_{50} values ranges from 12.35 mg/L air for adult males (Table 12) to 28.49 mg/L air for gravid females (Table 14). Homogeneity of response was similar among most stages ranging from 4.01 to 8.57 for small nymphs (Table 17) and adult males, respectively (Table 12).

LC_{50} values of thymol ranged from 19.09 to 142.94 mg/L air for small nymphs (Table 17) and adult females (Table 13), respectively. Homogeneity of response was similar among most stages ranging from 1.59 for large nymphs (Table 15) to 5.60 for gravid females (Table 14).

Ootheca hatch. Combining all concentrations for each essential oil component, there were significant differences in percentage of hatched oothecae attached to gravid females treated with essential oil. Percentage of oothecae dropped before hatch for the control treatment and the percentage oothecae hatched for the control treatment was 93.33 ± 0.05 and 100, respectively. Percentage of oothecae dropped before hatch for essential oil components ranged from 31.33 ± 0.06 for (-)-linalool (Fig. 7b) to 95.71 ± 0.01 for 1,8-cineole (Fig. 7a). Percentage of oothecae hatched for essential oil components ranged from 73.33 ± 0.06 for (-)-menthone (Fig. 8b) to 100 for citronellic acid (Fig. 8a). Oothecae attached to females treated with citronellic acid had significantly greater percentage hatch (100) than oothecae attached to females treated with other essential oil components (Figs. 8a and 8b). Citronellic acid had only 20.55% fewer hatched nymphs than the control (Fig. 6a); therefore, citronellic acid treatment had the least effect on ootheca hatch. Oothecae that were attached to females treated with (-)-menthone had a significantly lower percentage hatch (73.33 ± 0.06) than oothecae attached to females treated with the other essential oil components; therefore, (-)-menthone treatment had the greatest effect on ootheca hatch (Figs. 8a and 8b). The essential oil components having the greatest to least effect on ootheca hatch were (-)-menthone>(-)-linalool>*trans*-cinnamaldehyde>S-(-)-limonene>carvacrol>eugenol>(-)-β-pinene>(+)-α-pinene>thymol>1,8-cineole>geraniol >citronellic acid (Figs. 8a and 8b).

Percentage of oothecae hatched from S-(-)-limonene-treated females ranged from 100 for 0, 8.52, and 12.78 mg/L air to 63.33 ± 0.18 for 34.08 mg/L air (Fig. 8b). Significantly fewer hatched for 34.08 mg/L air (63.33%) than 0 mg/L air (100%) (Fig. 8b). The percentage of hatched oothecae decreased linearly with increasing

concentration: percentage hatched= 106.57 (± 4.40) – 1.19 (± 0.22) concentration, r^2 = 0.88 (F = 28.37, df = 1, 4, P = 0.006) (Table 20). S-(-)-Limonene had 50.46% fewer hatched nymphs than the control (Fig. 6b)

Percentage of oothecae hatched from (-)-linalool-treated females ranged from 100 to 66.67 ± 0.07 for 0 and 51.78 mg/L air, respectively (Fig. 8a). Significantly fewer hatched for 51.78 (66.67%), 69.04 (66.67%), and 86.31 (66.67%) mg/L air than 0 (100%) mg/L air (Fig. 8a). (-)-Linalool had 26.1% fewer hatched nymphs than the control (Fig. 6a)

The percentage of oothecae hatched from (-)-menthone-treated females ranged from 100 to 56.67 ± 0.22 for 0 and 25.38 mg/L air, respectively (Fig. 8b). The percentage of hatched oothecae decreased linearly with increasing concentration: percentage hatched= 99.03 (± 4.96) – 1.51 (± 0.30) concentration, r^2 = 0.86 (F = 24.77, df = 1, 4, P = 0.008) (Table 20). (-)-Menthone had 51.78% fewer hatched nymphs than the control (Fig. 6b)

Percentage of oothecae hatched from (+)-α-pinene-treated females ranged from 100 for 0 and 8.85 mg/L air to 80.00 ± 0.06 for 35.4 mg/L air (Fig. 8b). Significantly fewer hatched for 35.4 mg/L air (80.00%) than 0 (100%) and 8.85 (100%) mg/L air (Fig. 8b). Percentage of hatched oothecae decreased linearly with increasing concentration: percentage hatched= 103.74 (± 3.40) – 0.50 (± 0.16) concentration, r^2 = 0.72 (F = 10.40, df = 1, 4, P = 0.032) (Table 20). (+)-α-Pinene had 21.79% fewer hatched nymphs than the control (Fig. 6b).

Percentage of oothecae hatched from (-)-β-pinene-treated females ranged from 100 for 0 and 9.08 mg/L air to 80.00 ± 0.06 for 36.41 mg/L air (Fig. 8b). Significantly

fewer hatched for 36.41 mg/L air (80.00%) than 0 (100%) and 9.08 (100%) mg/L air (Fig. 8b). Percentage of hatched oothecae decreased linearly with increasing concentration: percentage hatched= 103.87 (± 3.25) – 0.56 (± 0.15) concentration, r^2 = 0.79 (F = 14.66, df = 1, 4, P = 0.019) (Table 20). (-)-β-Pinene had 28.75% fewer hatched nymphs than the control (Fig. 6b). There were no significant ($P > 0.05$) effects of carvacrol, 1,8-cineole, *trans*-cinnamaldehyde, citronellic acid, eugenol, geraniol, and thymol on ootheca hatch (Figs. 8a and 8b).

Discussion

Toxicity. 1,8-Cineole, (-)-menthone, (+)-α-pinene, and (-)-β-pinene were the most toxic essential oil components to adult males and large nymphs of the German cockroach. The most toxic essential oil components to adult females were 1,8-cineole, (-)-menthone, S-(-)-limonene, and (-)-β-pinene. 1,8-Cineole, (-)-menthone, *trans*-cinnamaldehyde, and S-(-)-limonene were the most toxic essential oil components to gravid females and medium nymphs, and the most toxic components to small nymphs were carvacrol, 1,8-cineole, *trans*-cinnamaldehyde, and (-)-menthone. The fumigant toxicity of 1,8-cineole and (-)-menthone, two essential oil components highly toxic to all stages, was less than that of the conventional fumigant sulfuryl fluoride (LC_{50} = 0.938 mg/L air at 24 h) against adult German cockroaches (Thoms and Scheffrahn 1994). 1,8-Cineole and (-)-menthone were also less toxic than the air-borne insecticide, dichlorvos (LC_{50} = 0.007 mg/L air at 24 h), to adult female German cockroaches (Jang et al. 2005).

The Pearson product-moment correlation was used to relate toxicity (LC_{50}) and physical properties of the essential oils. The Log_{10} of the LC_{50} values of all stages were correlated negatively with the Log_{10} of the vapor pressure (mmHg at 25ºC) of the

essential oil components (r = -0.41, P = 0.0003) (Fig. 9). The vapor pressure of the essential oil components may affect the ability of the compounds to volatilize and become available to the tracheal system during respiration. Essential oil components with high vapor pressures can volatilize easily and were generally more toxic than those with low vapor pressures. The Log_{10} of the LC_{50} values of all stages were correlated positively with the molecular weight of the essential oil components (r = 0.58, P = <0.0001) (Fig. 10). Lighter compounds may be able to volatilize more easily than the heavier compounds. Boiling point, density, and solubility of essential oil components were not correlated with toxicity (P>0.05).

We used one-way ANOVA and Tukey's multiple comparison tests to verify that there were significant differences in body mass among stages (P < 0.0001) (Phillips et al. 2009). However, we did not find a consistent relationship between body mass and toxicity for any of the essential oils, suggesting that there are other factors contributing to the difference in toxicity among the stages in addition to body mass. For example, the metabolic rate of each stage may affect toxicity (Yu 2008). The smaller stages have a relatively greater metabolic rate than larger stages, so they should have a higher respiratory rate because they require more O_2 (Appel 2008). If the smaller stages are respiring at a faster rate, the essential oil vapors are entering the insect trachea at a faster rate. Toxicity may also be affected by the behavior of each stage. Some stages are more active than others. For example, adult male cockroaches are more mobile than nymphs and adult females are more mobile than gravid females (Metzger 1995). The more active an insect is, the more rapidly they must respire, which increases the intake of essential oil vapors (Appel 2008). The German cockroach has the ability to breathe discontinuously

(Dingha et al. 2005). The length of the closed-phase of the spiracles may affect toxicity; however, Woodman et al. (2007) reported that discontinuous gas exchange in the American cockroach was disrupted when exposed to phosphine vapors, so this is an unlikely hypothesis. Because larger stages have a larger tracheal system, they can store more O_2 than smaller stages (Appel 2008). The spiracles must open more often in smaller stages to replenish the O_2 supply, during which, essential oil vapors may enter the system.

Structural characteristics such as chemical class, ring size of cyclic aliphatic hydrocarbons, and presence of a carbonyl functional group may also contribute to the toxicity of compounds. The most toxic essential oil components to the majority of cockroach stages were cyclic aliphatic hydrocarbons rather than aromatic or open-chain hydrocarbons. These compounds included 1,8-cineole, (-)-menthone, (+)-α-pinene, (-)-β-pinene, and S-(-)-limonene, all of which contain six-member carbon rings (cyclohexane or its unsaturated equivalents). Compared with other alicyclic hydrocarbons (containing <6 carbons), cyclohexane is the most stable because it is free of angle (carbon bond angle = 109.5º) and torsional strain (Morrison and Boyd 1992). Because the hydrogen atoms on adjacent carbons are equal distance apart, cyclohexane is also free of Van der Waals strain (Morrison and Boyd 1992). The open-chain hydrocarbons used in the experiments were less toxic than the alicyclic hydrocarbons because they were less stable and may not have been able to retain their structural integrity as they traveled to the target site. Although benzene is also a very stable molecule, due to the lack of angle strain (carbon bond angle = 109.5º) and delocalization of electrons (Morrison and Boyd 1992), aromatic compounds were less toxic than alicyclic compounds to the majority of cockroach stages.

The conformation of the two molecules may have attributed to the difference in toxicities. Cyclohexane is a molecule that maintains a staggered chair conformation and has twelve bonded hydrogen atoms (Morrison and Boyd 1992). Benzene is a two dimensional molecule with only six bonded hydrogen atoms (Morrison and Boyd 1992), which would provide more locations for enzyme attachment than cyclohexane in phase one of the metabolism of xenobiotics (Hodgson 1987). Cyclohexane is very soluble in water (871 g/l water) because it is a polar compound; however, benzene is not (0.93 g/l water) because the delocalization of electrons in the ring make it non-polar (ACD/Labs 11.0 2008, Morrison and Boyd 1992). Alicyclic compounds may pass through the fluid layer that separates the tracheoles from the cells (Nation 2008) more easily than aromatic compounds. Our results are consistent with those of Lee et al. (2003) who found that adult male German cockroaches fumigated with 50 mg/L of menthone, cineole, and limonene for 14 h resulted in 100% mortality.

The ring size of the compounds may have attributed to the toxicity of the oils. 1,8-Cineole is a bicyclic compound consisting of cyclohexane and a 5-carbon cyclic ether. The cyclic ether is similar to cyclohexane because the oxygen atom has bond angles similar to carbon, which permits it to exist in a corresponding conformation (Morrison and Boyd 1992). 1,8-Cineole is more toxic than (+)-α-pinene and (-)-β-pinene to all cockroach stages. Like 1,8-cineole, (+)-α-pinene and (-)-β-pinene are bicyclic compounds; however, they consist of one 6-carbon ring and cyclobutane (4-carbon ring). Cyclobutane quickly changes between two folded conformations to reduce torsional strain; however, angle strain (bond angles ~90º) can not be eliminated (Morrison and Boyd 1992). Due to the lack of flexibility caused by angle strain, (+)-α-pinene and (-)-β-

pinene may have been unable to retain their structural integrity; the chemical bonds may be inclined to break more easily in response to detoxifying enzyme activity, which could lead to faster degradation in the insect body. Jang et al. (2005) also found that 1,8-cineole was more toxic than (+)-α-pinene and (-)-β-pinene.

Our results agreed with those of Lee et al. (2003) who reported that the presence of a carbonyl functional group may have increased the fumigant toxicity of the monoterpenoids tested. We found that (-)-menthone (cyclic ketone) was highly toxic to all stages, and *trans*-cinnamaldehyde (aromatic aldehyde) was the most toxic aromatic compound to all stages. The oxygen atom in the carbonyl group is a hydrogen bond acceptor because it is an electronegative atom. This allows the carbonyl group to form intermolecular hydrogen bonds. If the carbonyl groups can form hydrogen bonds with the water molecules present in the fluid layer between the tracheoles and the cells, they may be able to pass through the fluid layer and contact the cell at a faster rate than other compounds.

The most toxic essential oil components (by fumigation) in this study differed from the most toxic essential components in a previous study (Phillips et al. 2009), where we applied the oils topically to the cockroaches. 1,8-Cineole, (-)-menthone, (+)-α-pinene, and (-)-β-pinene had the greatest fumigant toxicity to the German cockroach; however, *trans*-cinnamaldehyde, thymol, carvacrol, and eugenol had the greatest topical toxicity. These differences are associated with the route of entry into the insect. Fumigants reach the target site by entering the tracheal system through the spiracles, and contact insecticides must pass through the cuticle, fat body, and other tissues before reaching the target site (Yu 2008). Because of the different routes, the physical and

chemical properties and structural characteristics that affect the toxicity of the compounds also differed between the two application methods. Traditional fumigants, such as methyl bromide, are broad spectrum insecticides (Bell et al. 1996). They have great fumigant and topical toxicity, compared to essential oil components which are more specific. The most toxic essential oil components used for fumigation will likely differ from those oils that are most effective when used in contact kill spray formulations.

Ootheca hatch. Our results showed that an ootheca attached to a dead female can hatch, which is consistent with the results of Abd-Elghafar and Appel (1992). Although tiny air spaces present in the keel of the ootheca provide air to developing embryos, the egg case apparently protected embryos from essential oil vapors. Unlike essential oils, oxygen is able to pass through the tiny spaces, which are smaller than spiracles, because it is a small molecule. Four essential oil components had a significant effect on ootheca hatch. Significantly fewer oothecae hatched for the higher concentrations for S-(-)-limonene, (-)-menthone, (+)-α-pinene, and (-)-β-pinene, and fewer oothecae hatched from dead females. These results are consistent with those of Abd-Elghafar et al. (1991), who found that the percentage of oothecae hatched declined as insecticide concentration increased. We also found that significantly fewer oothecae dropped from treated than control females. These results demonstrate that S-(-)-limonene, (-)-menthone, (+)-α-pinene, and (-)-β-pinene reduced ootheca hatch, in part, because the high concentrations killed the females before they had time to deposit their oothecae, a naturally occurring process for the German cockroach before ootheca hatch (Ross and Mullins 1995). Oothecae receive nutrients and water while attached to their living mother's body (Roth 1970a). Contamination with essential oil components or the

lack of nutrients and water from dead females may also have contributed to nymph mortality. It is also possible that the body of the dead females absorbed water from the developing embryos by a passive wicking action (Phillips et al. 2009). No essential oil components completely prevented ootheca hatch; however, even with traditional fumigants, such as sulfuryl fluoride, not all eggs are killed (Thoms and Scheffrahn 1994). From a practical standpoint, additional treatments of these oils would be required in the field to prevent reinfestation from hatching nymphs.

When fumigating an area or object, sealing tape and poly tarps and sheets are necessary to make all windows, doorways, vents, and other small openings air tight (Wood 1987). Fans should be placed at fumigant release sites to circulate the gas (Wood 1987). The temperature at which fumigation should occur depends upon the boiling point (temperature at which a chemical enters the gas phase) of the essential oils (Thoms and Phillips 2004). Research on the effect of temperature on the fumigant activity of essential oils would be required to determine the optimum temperature for fumigation with essential oils. As temperature decreases, adsorption of compounds to surfaces increases (Bell et al. 1996), which would make the essential oil unavailable to the pests. The temperature at which each essential oil component denatures would need to be determined to ensure that fumigations occur below that temperature. Because essential oils are lipophilic, absorption of the fumigants into lipophilic foods or residues in the kitchen, such as fats and grease, should be considered. Laboratory fumigations in the presence of lipophilic food items will determine the effects of essential oil fumigations on common kitchen products and byproducts. If the oils are absorbed by the foods, there will be less available during the fumigations. This will have to be factored in when

determining the appropriate concentration. After the required exposure time, the area or object should be aerated (Wood 1987). Aeration is accomplished by removing sealing materials and opening windows, doorways, and vents. Fans should be used to circulate fresh air around the fumigated area or object.

1,8-Cineole, (-)-menthone, (+)-α-pinene, and (-)-β-pinene are good candidates for fumigants against the German cockroach. Because they are used for flavorings in food items and have little residual activity (Isman 2006), food preparation areas, food, and utensils will not be contaminated by the essential oils. The essential oils will leave an odor on the premises, but it will degas quickly. Like traditional fumigants, no insecticidal activity will remain after the fumigated area or object has aerated (Wood 1987); therefore, preventative measures should be taken to avoid reinfestation, such as the application of a repellent, residual insecticide. Because no essential oil prevented ootheca hatch (not uncommon with traditional fumigants), follow up treatment would be necessary to prevent reinfestation by the hatched nymphs. The time required to effectively fumigate an area or object with 1,8-cineole, (-)-menthone, (+)-α-pinene, or (-)-β-pinene would be at least 8 h; however, the time increases for the less toxic essential oil components (unpublished data). Based on our study, essential oil fumigations should occur at ≥28ºC to prevent adsorption of oils to surfaces. The use of essential oil components along with other integrated pest management techniques can be an effective method for controlling German cockroaches that have infested kitchens, ships, transport vehicles, sewer systems, sensitive equipment, and storage and household items. For example, fumigation with an essential oil can be used for fast cleanout of the infestation, leaving behind little residual. Then cultural control should be implemented (good

sanitation: clean up and eliminate harborages). After the area has been thoroughly cleaned, gel and solid baits can be used to kill any cockroaches that come in after the fumigation. Repellent insecticides can be sprayed for preventative measures, and then the area should the monitored (scouting and trapping) to determine the effectiveness of the IPM program.

Table 12. Fumigant toxicity of essential oils to adult male German cockroaches

Essential oil	*n*	Slope ± SE	$LC_{50,}$ mg/L air (95%CI)	χ^2	*P*
Carvacrol	150	6.40 ± 1.94	80.67 (65.50-109.66)	10.82	0.0010
1,8-Cineole	150	19.97 ± 2.90	6.84 (6.49-7.16)	47.45	0.0001
Trans-cinnamaldehyde	150	10.47 ± 2.47	32.03 (29.89-36.34)	17.99	0.0001
Citronellic acid	270	-	>1,000 *a*	-	0.9907
Eugenol	240	2.01 ± 0.52	95.89 (50.30-148.58)	15.12	0.0001
Geraniol	510	-	>1,000 *a*	-	0.9204
S-(-)-Limonene	150	1.38 ± 0.44	13.01 (6.08-147.12)	9.99	0.0016
(-)-Linalool	150	6.00 ± 1.05	15.73 (12.80-19.21)	32.60	0.0001
Menthone	150	9.42 ± 1.85	7.36 (6.34-8.16)	25.81	0.0001
(+)-α-Pinene	150	10.34 ± 1.73	11.75 (10.39-13.43)	35.67	0.0001
(-)-β-Pinene	150	8.57 ± 1.24	12.35 (10.94-13.77)	48.01	0.0001
Thymol	150	2.97 ± 0.85	19.33 (11.71-25.93)	12.21	0.0005

[a] Probit model did not work because < 20% mortality occurred

Table 13. Fumigant toxicity of essential oils to adult female German cockroaches

Essential oil	*n*	Slope ± SE	$LC_{50,}$ mg/L air (95%CI)	χ^2	*P*
Carvacrol	540	0.75 ± 0.25	>1,000	9.17	0.0025
1,8-Cineole	150	6.08 ± 0.86	8.43 (7.37-9.50)	49.83	0.0001
Trans-cinnamaldehyde		1.47 ± 0.32	34.44 (21.91-48.01)	21.66	0.0001
Citronellic acid	150	-	>1,000 *a*	-	0.7280
Eugenol	150	-	>1,000 *a*	-	0.0945
Geraniol	150	-	>1,000 *a*	-	0.9065
S-(-)-Limonene	150	4.32 ± 0.59	15.26 (12.87-17.54)	52.91	0.0001
(-)-Linalool	150	1.26 ± 0.89	141.98 (82.76-854.72)	10.71	0.0011
Menthone	150	5.39 ± 1.20	13.88 (11.13-16.55)	20.16	0.0001
(+)-α-Pinene	150	11.16 ± 1.77	26.12 (24.44-27.53)	39.82	0.0001
(-)-β-Pinene	150	4.44 ± 0.71	20.13 (17.56-22.67)	39.32	0.0001
Thymol	240	2.04 ± 0.71	142.94 (88.91-810.42)	8.18	0.0042

[a] Probit model did not work because < 20% mortality occurred

Table 14. Fumigant toxicity of essential oils to gravid female German cockroaches

Essential oil	*n*	Slope ± SE	$LC_{50,}$ mg/L air (95%CI)	χ^2	*P*
Carvacrol	150	0.87 ± 0.44	>1,000	3.90	0.0484
1,8-Cineole	150	5.04 ± 0.96	5.31 (4.19-6.22)	27.67	0.0001
Trans-cinnamaldehyde	150	4.89 ± 1.24	20.39 (11.90-29.57)	15.61	0.0001
Citronellic acid	150	-	>1,000 *a*	-	77.23
Eugenol	150	2.53 ± 0.48	624.47 (507.04-772.33)	27.34	0.0001
Geraniol	150	-	>1,000 *a*	-	0.1633
S-(-)-Limonene	150	11.08 ± 1.70	23.19 (21.47-24.99)	42.61	0.0001
(-)-Linalool	150	4.01 ± 0.68	33.72 (26.31-39.81)	34.56	0.0001
Menthone	150	7.43 ± 1.78	9.89 (7.73-11.46)	17.44	0.0001
(+)-α-Pinene	150	15.72 ± 5.23	27.07 (23.74-33.98)	9.03	0.0027
(-)-β-Pinene	150	6.01 ± 2.52	28.49 (20.81-103.22)	5.71	0.0168
Thymol	150	5.60 ± 2.23	119.67 (78.10-165.41)	6.28	0.0122

[a] Probit model did not work because < 20% mortality occurred

Table 15. Fumigant toxicity of essential oils to large nymph German cockroaches

Essential oil	*n*	Slope ± SE	$LC_{50,}$ mg/L air (95%CI)	χ^2	*P*
Carvacrol	210	-	>1,000 *a*	-	0.8105
1,8-Cineole	150	13.15 ± 2.18	11.44 (10.60-12.29)	36.57	0.0001
Trans-cinnamaldehyde	150	1.06 ± 0.43	46.70 (29.96-376.65)	6.00	0.0143
Citronellic acid	150	-	>1,000 *a*	-	0.8517
Eugenol	210	-	>1,000 *a*	-	0.6857
Geraniol	150	-	>1,000 *a*	-	0.9999
S-(-)-Limonene	150	13.56 ± 2.49	25.64 (23.49-26.99)	29.65	0.0001
(-)-Linalool	150	1.52 ± 0.43	157.75 (88.87-938.30)	12.35	0.0004
Menthone	150	3.86 ± 0.57	18.36 (15.66-21.83)	45.74	0.0001
(+)-α-Pinene	150	3.46 ± 0.82	22.01 (16.25-32.12)	17.85	0.0001
(-)-β-Pinene	150	6.41 ± 1.66	21.93 (16.74-27.64)	14.84	0.0001
Thymol	150	1.59 ± 0.42	110.97 (72.65-330.97)	14.50	0.0001

[a] Probit model did not work because < 20% mortality occurred

Table 16. Fumigant toxicity of essential oils to medium nymph German cockroaches

Essential oil	*n*	Slope ± SE	$LC_{50,}$ mg/L air (95%CI)	χ^2	*P*
Carvacrol	150	0.98 ± 0.32	144.66 (76.95-1,356.00)	9.71	0.0018
1,8-Cineole	150	2.60 ± 1.07	11.60 (6.94-2,092.00)	5.88	0.0153
Trans-cinnamaldehyde	150	4.02 ± 1.61	22.70 (3.13-28.21)	6.24	0.0125
Citronellic acid	150	-	>1,000 *a*	-	0.1422
Eugenol	150	2.07 ± 0.54	120.43 (82.22-233.80)	16.92	0.0001
Geraniol	150	1.60 ± 0.38	833.99 (521.97-2,509.00)	17.44	0.0001
S-(-)-Limonene	150	6.41 ± 1.65	17.34 (15.35-22.58)	15.07	0.0001
(-)-Linalool	150	2.34 ± 0.58	34.77 (24.62-82.81)	16.04	0.0001
Menthone	150	2.94 ± 0.54	8.96 (7.26-10.91)	29.52	0.0001
(+)-α-Pinene	150	6.19 ± 2.77	30.42 (24.05-629,386.21)	4.98	0.0256
(-)-β-Pinene	150	6.35 ± 1.20	24.14 (22.00-27.71)	27.95	0.0001
Thymol	150	3.57 ± 1.00	23.89 (19.25-42.73)	12.85	0.0003

[a] Probit model did not work because < 20% mortality occurred

Table 17. Fumigant toxicity of essential oils to small nymph German cockroaches

Essential oil	*n*	Slope ± SE	$LC_{50,}$ mg/L air (95%CI)	χ^2	*P*
Carvacrol	300	0.92 ± 0.28	3.63 (0.16-8.56)	10.96	0.0009
1,8-Cineole	150	2.30 ± 0.42	4.04 (2.42-5.87)	30.37	0.0001
Trans-cinnamaldehyde	150	0.99 ± 0.30	5.43 (1.55-11.42)	10.69	0.0011
Citronellic acid	300	-	>1,000 *a*	-	0.0837
Eugenol	300	1.72 ± 0.23	14.48 (10.67-18.15)	54.23	0.0001
Geraniol	240	0.85 ± 0.29	149.54 (23.49-274.54)	8.77	0.0031
S-(-)-Limonene	150	3.38 ± 0.58	13.74 (11.76-16.35)	33.45	0.0001
(-)-Linalool	150	2.74 ± 0.67	9.59 (5.88-11.92)	16.63	0.0001
Menthone	150	2.58 ± 0.52	5.75 (3.68-8.04)	24.26	0.0001
(+)-α-Pinene	150	2.72 ± 0.72	24.50 (20.20-37.59)	14.20	0.0002
(-)-β-Pinene	150	4.01 ± 1.01	23.63 (19.63-334.38)	15.77	0.0001
Thymol	150	3.91 ± 1.15	19.09 (12.74-28.05)	11.50	0.0007

[a] Probit model did not work because < 20% mortality occurred

Table 18. Relationship between fumigant concentrations exposed to gravid females and the mean number of hatched nymphs

TMT	Slope ± SE	Intercept ± SE	r^2	df	*F*	*P*
Carvacrol	-0.00 ± 0.00	12.95 ± 0.85	0.64	5	6.98	0.058
1,8-Cineole	-0.39 ± 0.09	11.02 ± 1.07	0.81	5	17.42	0.014
Trans-cinnamaldehyde	-	-	-	5	1.65	0.269
Citronellic acid	-	-	-	5	0.02	0.884
Eugenol	-0.00 ± 0.00	11.87 ± 0.74	0.58	5	5.54	0.078
Geraniol	-	-	-	5	1.93	0.238
S-(-)-Limonene	-	-	-	5	2.28	0.205
(-)-Linalool	-	-	-	5	0.01	0.917
(-)-Menthone	-0.24 ± 0.08	10.20 ± 1.33	0.78	5	8.45	0.044
(+)-α-Pinene	-	-	-	5	0.14	0.731
(-)-β-Pinene	-0.14 ± 0.06	11.77 ± 1.34	0.57	5	5.19	0.085
Thymol	-	-	-	5	4.27	0.108

Table 19. Relationship between fumigant concentrations exposed to gravid females and percentage of dropped oothecae

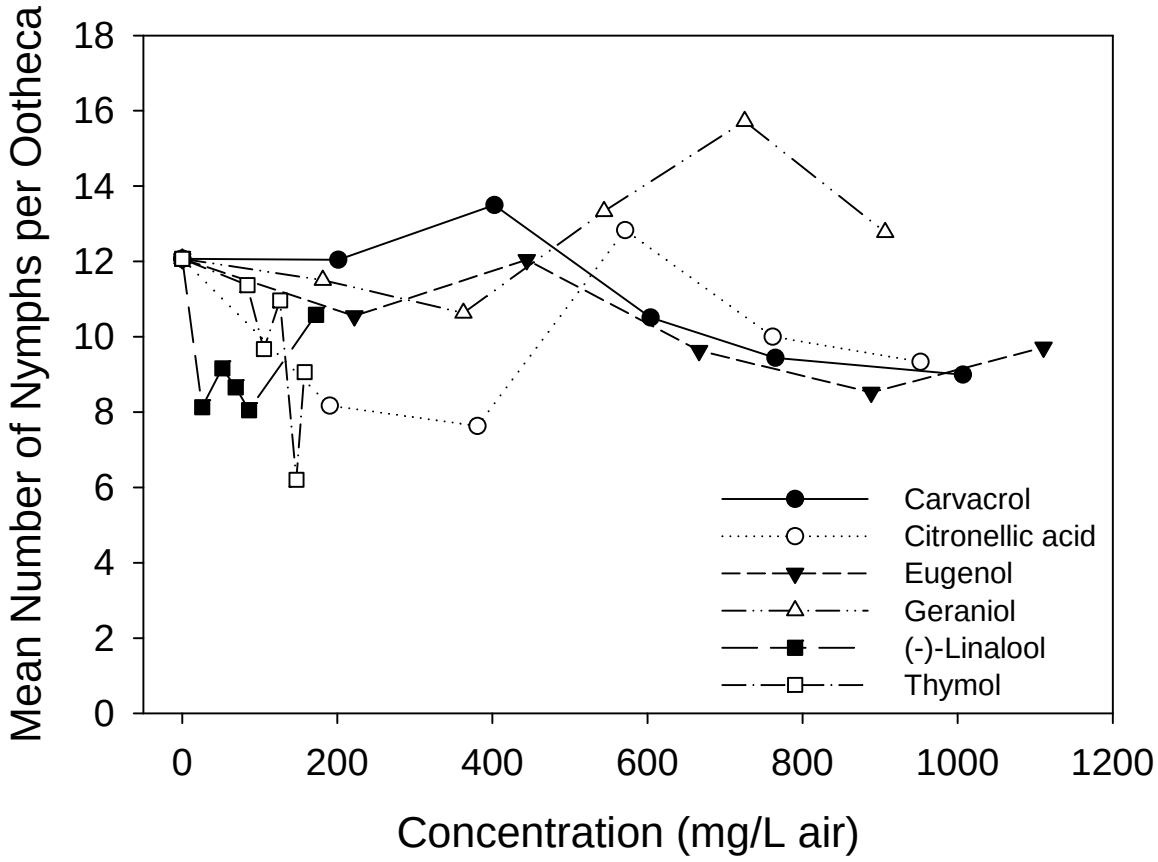
TMT	Slope ± SE	Intercept ± SE	r^2	df	*F*	*P*
Carvacrol	-	-	-	5	0.93	0.390
1,8-Cineole	-	-	-	5	0.01	0.927
Trans-cinnamaldehyde	-0.52 ± 0.15	79.45 ± 8.48	0.76	5	12.58	0.024
Citronellic acid	-	-	-	5	2.76	0.172
Eugenol	-0.05 ± 0.02	74.44 ± 11.09	0.73	5	10.73	0.031
Geraniol	-	-	-	5	1.51	0.286
S-(-)-Limonene	-	-	-	5	0.03	0.867
(-)-Linalool	-0.45 ± 0.17	72.18 ± 14.45	0.65	5	7.38	0.053
(-)-Menthone	-	-	-	5	0.32	0.599
(+)-α-Pinene	-	-	-	5	3.90	0.119
(-)-β-Pinene	-0.40 ± 0.18	97.49 ± 4.07	0.54	5	4.71	0.096
Thymol	-0.38 ± 0.09	97.64 ±10.37	0.82	5	18.06	0.013

Table 20. Relationship between fumigant concentrations exposed to gravid females and percentage of hatched oothecae

TMT	Slope ± SE	Intercept ± SE	r^2	df	*F*	*P*
Carvacrol	-	-	-	5	1.53	0.284
1,8-Cineole	-	-	-	5	1.50	0.287
Trans-cinnamaldehyde	-	-	-	5	1.74	0.258
Citronellic acid	-	-	-	5	-	-
Eugenol	-	-	-	5	1.06	0.361
Geraniol	-	-	-	5	0.73	0.441
S-(-)-Limonene	-1.19 ± 0.22	106.57 ± 4.40	0.88	5	28.37	0.006
(-)-Linalool	-	-	-	5	1.48	0.290
(-)-Menthone	-1.51 ± 0.30	99.03 ± 4.96	0.86	5	24.77	0.008
(+)-α-Pinene	-0.50 ± 0.16	103.74 ± 3.40	0.72	5	10.40	0.032
(-)-β-Pinene	-0.56 ± 0.15	103.87 ± 3.25	0.79	5	14.66	0.019
Thymol	-	-	-	5	1.99	0.231

Fig. 6. The effect of concentration on the mean number of nymphs per ootheca

a.



b.

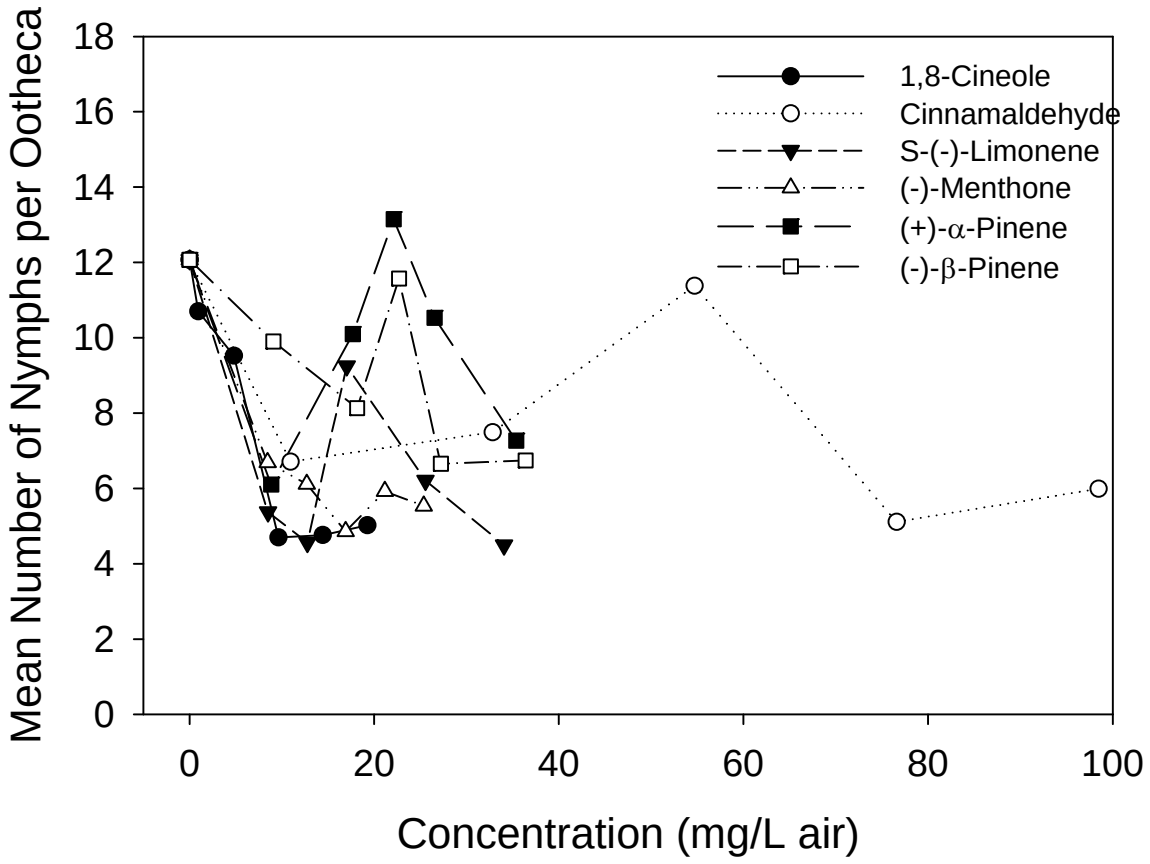
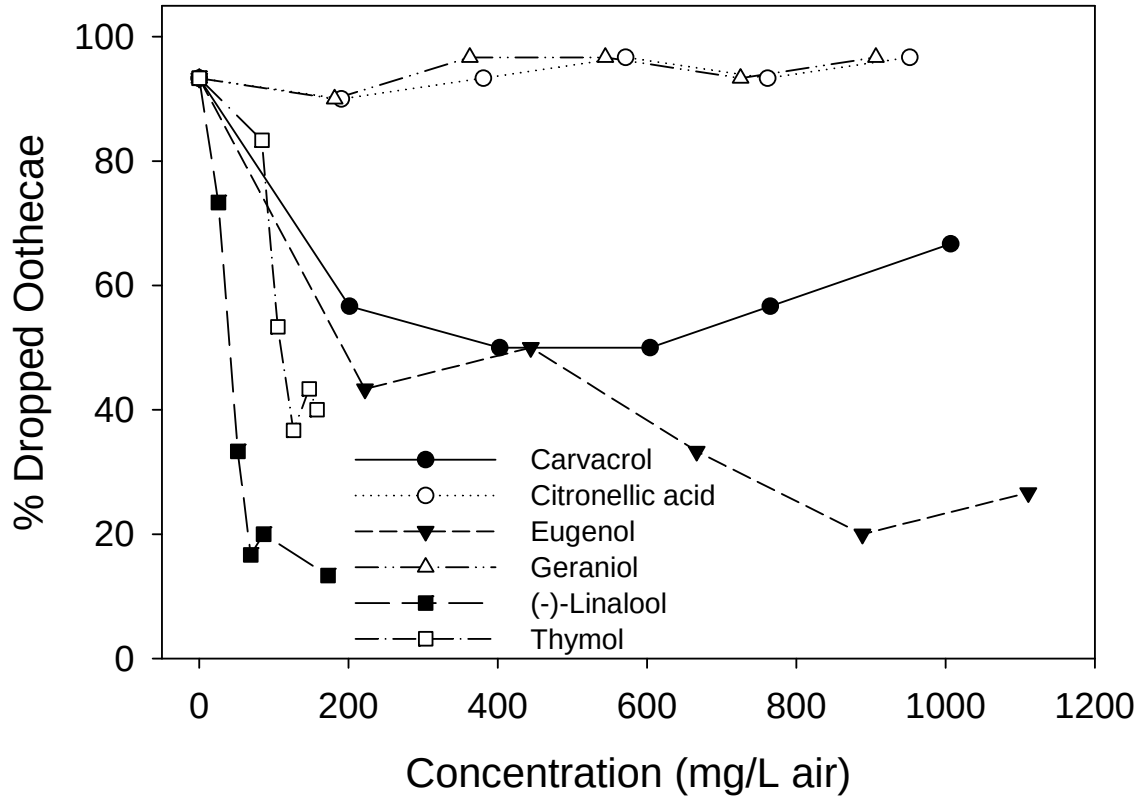

Mean Number of Nymphs per Ootheca
18
16
14
12
10
8
6
4
2
0
0
20
40
60
80
100
Concentration (mg/L air)
1,8-Cineole
Cinnamaldehyde
S-(-)-Limonene
(-)-Menthone
(+)-α-Pinene
(-)-β-Pinene

Fig. 7. The effect of concentration on the percentage of dropped oothecae

a.



b.

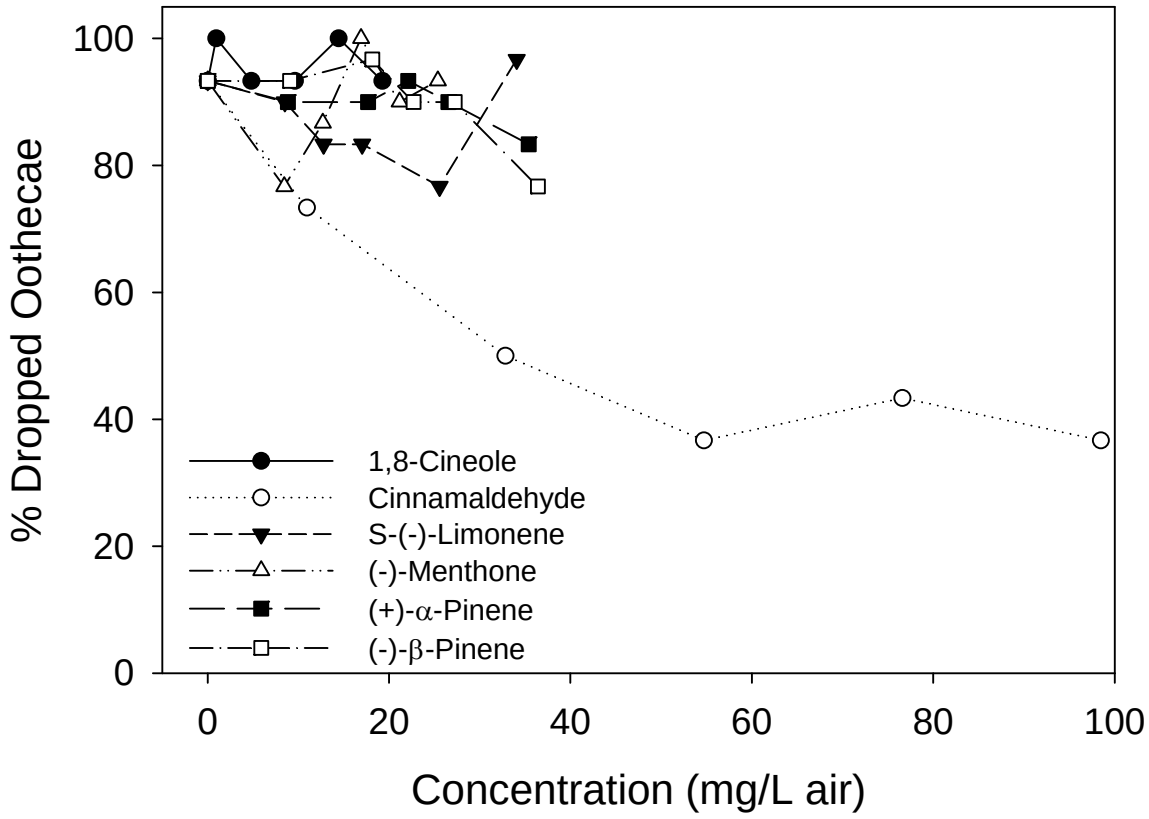
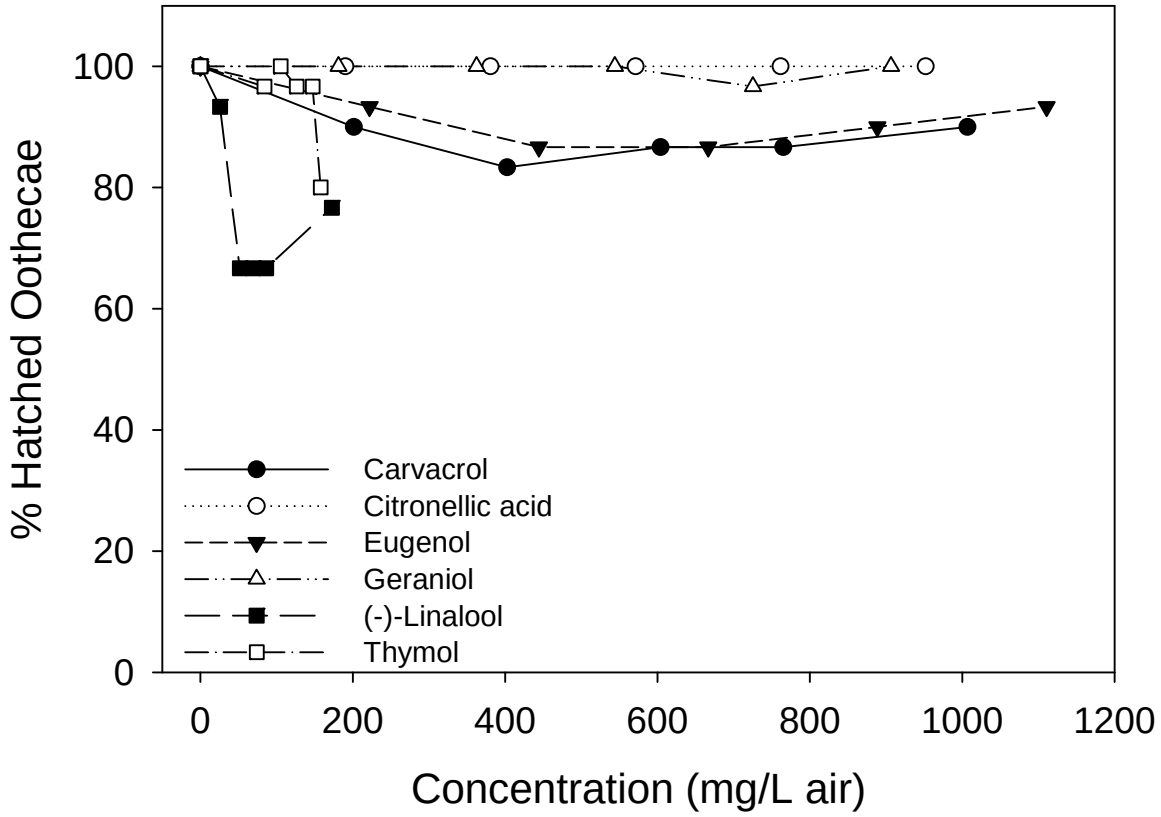

% Dropped Oothecae
100
80
60
40
20
0
0
20
40
60
80
100
Concentration (mg/L air)
1,8-Cineole
Cinnamaldehyde
S-(-)-Limonene
(-)-Menthone
(+)-α-Pinene
(-)-β-Pinene

Fig. 8. The effect of concentration on the percentage of hatched oothecae

a.



b.

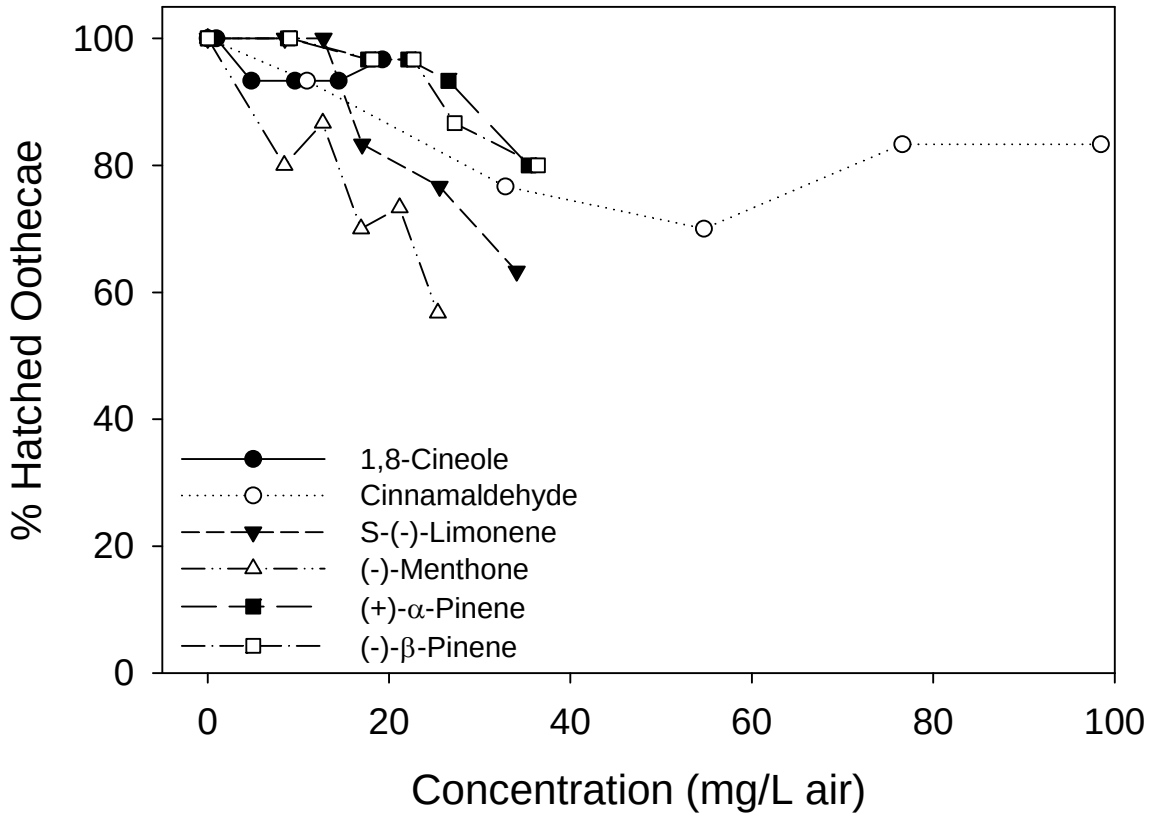

% Hatched Oothecae
100
80
60
40
20
0
0
20
40
60
80
100
Concentration (mg/L air)
1,8-Cineole
Cinnamaldehyde
S-(-)-Limonene
(-)-Menthone
(+)-α-Pinene
(-)-β-Pinene

Fig. 9. The effect of essential oil vapor pressure on toxicity

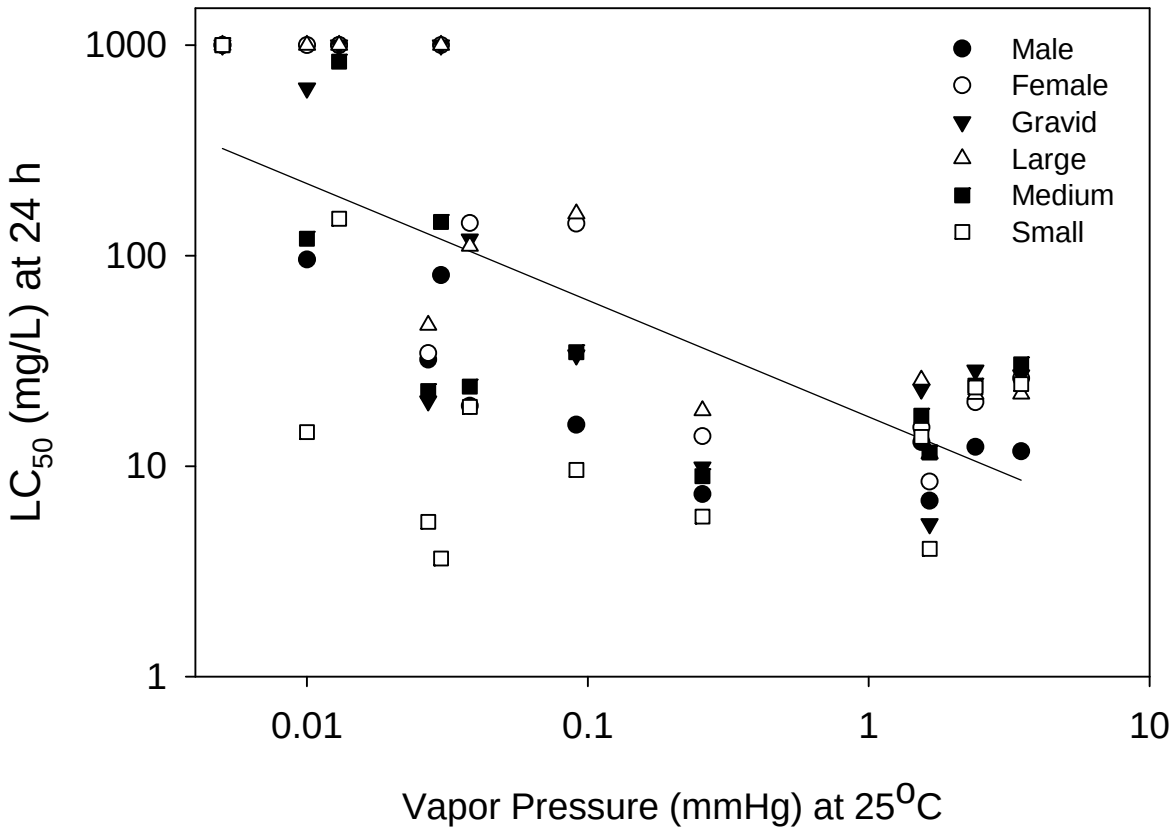
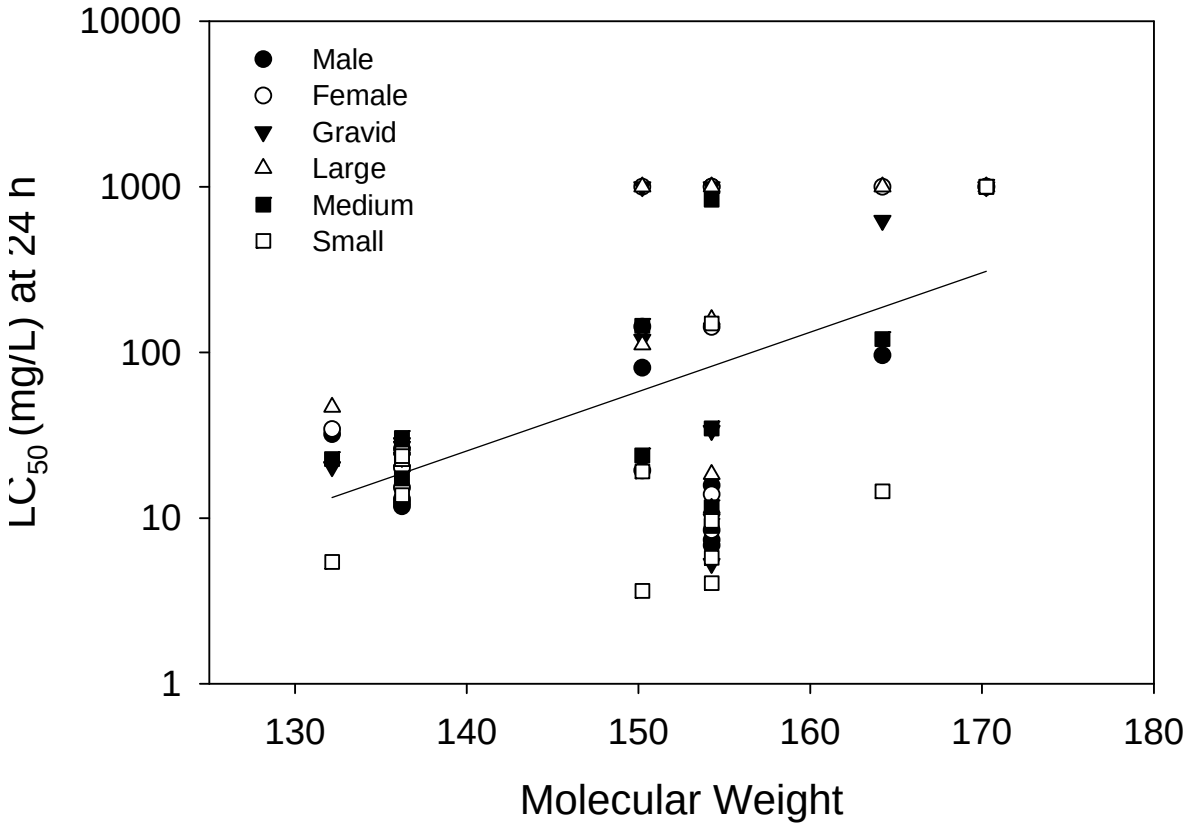


Fig. 10. The effect of essential oil molecular weight on toxicity



Repellency of Essential Oils to the German Cockroach (Dictyoptera: Blattellidae)

The German cockroach, *Blattella germanica* (L.), is an important economic pest because it can cause allergic reactions (Schal and Hamilton 1990), vector numerous pathogenic microorganisms (Roth and Willis 1957, 1960), and cause psychological problems (Grace and Wood 1987) in humans. The German cockroach has a short generation time and high fecundity which increases the chance of it developing resistance to insecticides (Barcay 2004).

Public concern about the negative effects of traditional insecticides has stimulated the investigation of botanical alternatives. Essential oils have historically been and are currently still used as flavoring and fragrances (Isman 2006). They are safer alternatives that could be used in areas where traditional insecticides are prohibited, such as food preparation areas. Essential oils are considered to be secondary plant substances (Isman 2006) consisting of many compounds, including monoterpenoids, that are responsible for the aromatic characteristics of plants.

Their low toxicity to humans and wildlife and short residual period make essential oils an excellent alternative to traditional insecticides (Isman 2006). Because they are commonly used as flavors and fragrances, the active ingredients of many essential oils are reasonably priced, compared with other botanical insecticides, such as neem and pyrethrum (Isman 2006). The United States Environmental Protection Agency considers several of the essential oils minimum risk pesticides, and these are currently exempt from

registration requirements (EPA PR Notice 2000-6). Exempt pesticides can be commercialized and would reach the market faster than conventional pesticides (Isman 2000).

The toxicity and repellency of corn mint, *Mentha arvensis* L., oil to American, *Periplaneta americana* (L.), and German cockroaches was determined by Appel et al. (2001). Corn mint oil, containing menthol and menthone as main components, was repellent and toxic to both species. Mean repellency to 2 ml of mint oil deposited on an aluminum foil insert in an Ebeling choice-box ranged from 93% to 100% for adult male German cockroaches and 100% for adult male American cockroaches (Appel et al. 2001).

Essential oils and their constituents have also been tested, for repellency, against a variety of other insects including the yellow fever mosquito, *Aedes aegypti* (L.), anopheles mosquito, *Anopheles albimanus* Wiedemann, (Barnard 1999); African malaria mosquito, *Anopheles gambiae* Giles, (Omolo et al. 2004); bloodsucking bug, *Rhodnius prolixus* Stahl, (Sfara et al. 2009); sheep tick, *Ixodes ricinus* (L.), (Palsson et al. 2008); red imported fire ant, *Solenopsis invicta* (Buren), (Appel et al. 2004); human body louse, *Pediculus humanus humanus* L., (Mumcuoglu et al. 1996); cigarette beetle, *Lasioderma serricorne* (Fab.), (Hori 2003); red flour beetle, *Tribolium castaneum* (Herbst), and cowpea weevil, *Callosobruchus maculatus* (L.), (Tripathi et al. 2000). Results from these studies demonstrated that all of the above species were repelled by several of the essential oils and their constituents.

Repellency is the result of associative learning (Ebeling et al. 1966). When the cockroach is exposed to a negative stimulus, such as neurological effects from an insecticide, the cockroach (if it survives) learns to avoid the area and stay in the

insecticide free area because it is associating the effects of the insecticide (negative stimulus) with the treated area. An insecticide can become repellent if the insect does not receive a lethal dose (Ebeling et al. 1966). If an insecticide is moderate in toxicity, or a very toxic insecticide degrades over time, the cockroaches will receive a sublethal dose, which will only make them sick. The cockroaches will learn to avoid the insecticide once they recover.

The repellency of an insecticide will determine its use and effectiveness for controlling the German cockroach (Ebeling et al. 1966). If used incorrectly, repellent insecticides can spread an infestation to other parts of the home or building (Ebeling et al. 1966). Repellent insecticides can also contaminate baits by making them repellent (Appel 2004); however, if used correctly, they are useful for prevention and inspection (Oswalt et al. 1997). They can be used to eliminate harborages, which will increase the chances of the cockroaches contacting the non-repellent treatment (Steltenkamp et al. 1992). Repellent insecticides that are exempt from registration can also be used to prevent infestations in and around items that can not be treated with traditional insecticides, such as sensitive equipment, important documents, and stored food items (Ngoh et al. 1998). To determine the degree of an infestation, repellent insecticides can be used as flushing agents during inspections (Barcay 2004).

The potential efficacy of essential oils as repellents for control of the German cockroach was investigated. The purpose of this study was to determine the repellency of several pure essential oils to the German cockroach using two methods to measure repellency and comparing the efficacy of those methods.

Materials and Methods

Chemicals. Essential oil components were obtained from Sigma-Aldrich (St. Louis, MO). Some of the essential oil components were chosen because they are present in the essential oil extracts of numerous plant species, while others were chosen because they occur at high concentrations in the essential oils of selected plants. Both aromatic and aliphatic hydrocarbons were tested; the functional groups represented included acids, alcohols, aldehydes, ketones, and ethers. Physical and chemical properties of essential oil components, obtained from Sigma-Aldrich or estimated using Advanced Chemistry Development software version 12.0 (ACD/Labs 2008), are described in a previous manuscript by Phillips and Appel (2009).

Insects. An insecticide susceptible strain of the German cockroach was used in all experiments. This strain (American Cyanamid, Clifton, NJ) has been in continuous laboratory culture for >35 years. Adult males were used for both the choice box and the harborage-choice bioassays. Laboratory cultures were maintained at 28 ± 2ºC, 40-55% RH, and a photoperiod of 12:12 (L:D) h. Colonies were provided water and dog chow (Purina) as needed. Cockroaches were briefly (<5 min.) anesthetized with CO_2 to facilitate handling during the repellency experiments.

Ebeling choice box. The repellency of essential oils to German cockroaches was determined in Ebeling choice boxes (Ebeling et al. 1966) (Fig. 11). As described by Appel (1992), the choice box is a square box divided by a partition into two equal compartments. A hole in the partition allows the cockroaches to move freely between compartments. Food and water were placed in one of the compartments and safety glass was used to cover the compartment. This is the untreated, light side, which represents,

for example, a kitchen counter. The hole in the partition was then plugged with a cork from the treatment side. Twenty adult male German cockroaches were released into the untreated, light compartment and were allowed to acclimate for 2 h. The essential oil was placed in the other compartment (the treated, dark side). After 2 h the cockroaches were allowed to move between the untreated, light compartment and the treated, dark compartment by removing the cork. A transparent safety glass and an opaque sheet were used to cover the treated side. This side represents, for example, the void under a kitchen counter where cockroaches harbor in the dark and where insecticides may be deposited. The choice boxes were exposed to a photoperiod of 12:12 (L:D) h at 28ºC. The number of live and dead cockroaches in each compartment was recorded at 4-5 h into the photophase daily for 14 d. The percentage of live cockroaches found in the untreated, light compartment of each choice box during the photophase was defined as the percentage of repellency.

Treatments consisted of 2 ml of a 1% essential oil solution in Fisher Scientific Certified ACS acetone (99.7% purity; Fisher Scientific, Fair Lawn, NJ) uniformly deposited onto aluminum foil-covered inserts that fit tightly onto the floor of the dark compartment. Each treatment had 6 replicates. Controls were treated with 2 ml of acetone.

Harborage-choice method. A modification of the harborage-choice method of Steltenkamp et al. (1992) was used to determine the repellency of essential oils to the German cockroach (Fig. 12). One hour before the assay, 20 adult male German cockroaches were placed in each 20 qt. plastic test container (Sterilite Corporation, Townsend, MA) and allowed to acclimate. Food and water were placed in the center of

each container. The top perimeter of each container was coated with petroleum jelly (Unilever, Greenwich, CT) to prevent escape. Three 1.5 cm holes were cut around the lip of 8 oz unwaxed paper cartons (SOLO Cup Company, Highland Park, IL) to allow the cockroaches access to the inside. Two milliliters of a 1% essential oil solution in Fisher Scientific Certified ACS acetone (99.7% purity; Fisher Scientific, Fair Lawn, NJ) was uniformly deposited onto the underside of each treatment carton. Control cartons were treated with acetone. The treated cartons were allowed to dry. Then one treated and one untreated carton were inverted and placed in each container. The number of cockroaches on the inside and outside of each carton was recorded at 4-5 h into the photophase daily for 14 d. The percentage of cockroaches that avoided the treated carton during the photophase was defined as the percentage of repellency.

Data Analysis. For the choice box method repellency (percent of live cockroaches in the light side of the choice box) was analyzed using repeated measures ANOVA (Proc Mixed, SAS 9.1, SAS Institute 2003). Prior to data analysis, an arc sine square root transformation was performed. Linear regression and exponential linear combination was used to analyze the change in repellency over the 14 d experimental period (SigmaPlot 11.0; SPSS 2008). For the harborage-choice method, repellency (percent of cockroaches that avoided the treated cup) was analyzed using repeated measures ANOVA (Proc Mixed, SAS 9.1, SAS Institute 2003). Percent repellency was calculated using the following equation: % Repellency= 100 – [(100 x TA) / (TA + UA)], where TA equals the number of cockroaches under the treated cup and UA equals the number of cockroaches under the untreated cup. Prior to data analysis, an arc sine square root transformation was performed. Linear regression was used to analyze the

change in repellency over the 7 d experimental period (SigmaPlot 11.0; SPSS 2008). Pearson product-moment correlation was used to relate repellency with physical and chemical properties (SigmaPlot 11.0; SPSS 2008).

Results

Ebeling choice-box. Combining the data from all 14 d of the experiment, citronellic acid (44.99 ± 2.19%) was significantly more repellent than all of the other essential oil components (Table 21). (-)-β-Pinene (22.04 ± 2.25%), the second most repellent compound, was not significantly more repellent than geraniol, (+)-α-pinene, carvacrol, or (-)-linalool (Table 21). S-(-)-Limonene (5.55 ± 2.19%), the least repellent compound, was not significantly less repellent than (-)-menthone or 1,8-cineole (Table 21). Neither S-(-)-limonene or 1,8-cineole were significantly different from the control (3.89 ± 2.19%) (Table 21).

Mean repellency decreased from 34.93 ± 5.08 to 62.73 ± 6.93% for citronellic acid for days 14 and 1, respectively (Fig. 14). Combining all 14 d of the experiment, citronellic acid had a mean repellency of 44.99 ± 2.19% ($P < 0.0001$) (Table 21). Repellency in (-)-β-pinene treated choice boxes ranged from 13.33 ± 6.74% for day 12 to 42.36 ± 10.42% for day 1 (Fig. 16). (-)-β-Pinene had a mean repellency of 22.04 ± 2.35% ($P < 0.0001$) over the 14 d experimental period (Table 21). Mean repellency ranged from 7.95 ± 4.85 to 41.67 ± 10.54% for geraniol for days 6 and 1, respectively (Fig. 14). Combining all 14 d of the experiment, geraniol had a mean repellency of 19.89 ± 2.19% ($P < 0.0001$) (Table 21). Repellency in carvacrol treated choice boxes ranged from 9.21 ± 3.95% for day 4 to 41.36 ± 11.12% for day 1 (Fig. 13). Carvacrol had a mean repellency of 16.01 ± 2.19% ($P < 0.0001$) over the 14 d experimental period (Table

21). Control repellency ranged from 0.83 ± 0.83 to 6.89 ± 2.59% for days 6 and 14, respectively (Fig. 13). Combining all 14 d of the experiment, control had a mean repellency of 3.89 ± 2.19% ($P = 0.0003$) (Table 21).

ANOVA showed a significant effect of day on repellency ($P < 0.0001$); therefore linear and exponential linear combination models were used to analyze the change in repellency over the 14 d experimental period. Exponential linear combination was used because repellency decreased exponentially during the first 2-3 days of the experiment and then decreased linearly thereafter; however, citronellic acid, (+)-α-pinene, and thymol did not decay in this manner, so they were analyzed using linear regression (Figs. 13-16). The following function was used to fit our results to the exponential linear combination model: $y = y_0 + ae^{-bt} + cx$, where y = % repelled, y_0 = y-intercept, a = initial repellency, b = decay rate, t = time, c = slope, and x = day.

The results from all essential oil components were significant when analyzed using one of the above models ($P < 0.05$) (Table 22). Slopes ranged from -2.1 ± 0.61 for (+)-α-pinene to 1.88 ±1.41 for geraniol (Table 22). R^2 values were high (ranging from 0.42 to 0.97 for thymol and (-)-linalool, respectively) indicating that variation in repellency can be explained by the day.

Harborage-choice method. Combining all 7 d of the experiment, carvacrol (76.15 ± 3.63%) was the most repellent compound to the German cockroach using the harborage-choice method. However, carvacrol was not significantly more repellent than citronellic acid, thymol, eugenol, geraniol, *trans*-cinnamaldehyde, (-)-β-pinene , (-)-linalool, or S-(-)-limonene (Table 23). 1,8-Cineole (43.07 ± 3.63%), the least repellent compound, was not significantly less repellent than (+)-α-pinene or (-)-menthone (Table

23). *Trans*-cinnamaldehyde, (-)-β-pinene, (-)-linalool, S-(-)-limonene, (+)-α-pinene, (-)-menthone, and 1,8-cineole were not significantly different from the control (52.25 ± 3.17%) (Table 23).

Mean repellency ranged from 65.08 ± 3.97 to 92.98 ± 3.51% for carvacrol for days 7 and 1, respectively (Fig. 17). Combining all 7 d of the experiment, carvacrol had a mean repellency of 76.15 ± 3.63% ($P < 0.0001$) (Table 23). Repellency of citronellic acid treated cups ranged from 62.23 ± 4.82% for day 5 to 86.84 ± 8.35% for day 1 (Fig. 18). Citronellic acid had a mean repellency of 75.39 ± 3.63% ($P < 0.0001$) over the 7 d experimental period (Table 23). Mean repellency ranged from 57.16 ± 7.91 to 95.70 ± 2.82% for thymol for days 7 and 1, respectively (Fig. 20). Combining all 7 d of the experiment, thymol had a mean repellency of 72.71 ± 3.63% ($P < 0.0001$) (Table 23). Repellency in eugenol treated cups ranged from 63.29 ± 4.70% for day 7 to 94.50 ± 2.49% for day 1 (Fig. 18). Eugenol had a mean repellency of 72.90 ± 3.63% ($P < 0.0001$) over the 7 d experimental period (Table 23). Control repellency ranged from 32.39 ± 9.63 to 66.31 ± 9.90% for days 1 and 7, respectively (Fig. 17). Combining all 7 d of the experiment, control had a mean repellency of 52.25 ± 3.17% ($P < 0.0001$) (Table 23).

ANOVA showed a significant effect of day on repellency ($P < 0.0001$); therefore linear regression was used to analyze the change in repellency over the 7 d experimental period. Exponential linear combination was not used because repellency did not decrease exponentially during the first few days of the experiment (Figs. 17-20). Only carvacrol, geraniol, and thymol produced significant results when analyzed using linear regression ($P < 0.05$) (Table 24). Slopes ranged from -4.00 ± 0.80 for carvacrol to -5.77 ± 0.55 for

geraniol (Table 24). R^2 values were high for the above three compounds (ranging from 0.83 to 0.96 for carvacrol and geraniol, respectively) indicating that variation in repellency can be explained by the day.

Discussion

We used the Ebeling choice box and harborage-choice method to measure repellency of essential oil components to the German cockroach. The Ebeling choice box simulates real world cockroach habitat. It measures repellency relative to the repellency of light (Ebeling et al. 1966). Because the German cockroach is repelled by light, it prefers conditions on the dark side of the choice box. If cockroaches remain on the light side of a treated choice box, the treatment must be repellent. The harborage-choice method does not simulate normal cockroach habitat. Unlike the choice boxes, there is no variable, such as light or darkness, that would make one harborage more desirable than the other. The possibility exists that cockroaches found under the untreated harborage may not be repelled by the essential oil component; they have failed to leave the untreated harborage to explore other areas because there is no stimulus encouraging them to move.

The results from the Ebeling choice box experiments indicated that citronellic acid, (-)-β-pinene, geraniol, and carvacrol were the most repellent essential oil components to the German cockroach, and S-(-)-limonene was the least repellent. Repellency in the choice boxes was positively correlated with the Log_{10} of the LC_{50} values (Phillips and Appel 2009) ($r = 0.75$, $P = 0.0055$) (Fig. 21). Compounds with the greatest fumigant toxicity were less repellent than those with the least fumigant toxicity. The results from the harborage-choice method showed that carvacrol, citronellic acid,

thymol, and eugenol were the most repellent compounds, and 1,8-cineole was the least repellent. Repellency for the harborage-choice method was negatively correlated with vapor pressure ($r = -0.58$, $P = 0.0505$) (Fig. 22). Compounds with high vapor pressures were less repellent than those with low vapor pressures. These correlations are consistent with those for the choice boxes because fumigant activity is positively correlated with vapor pressure (Phillips and Appel 2009).

Repellency was greater for the harborage-choice method than the choice boxes. This is probably because more essential oil component was applied per square centimeter in the harborage-choice method. Appel and Mack (1989) also found that repellency increased when more active ingredient was applied per square centimeter. Therefore, the amount of essential oil component applied in the field can be increased to enhance the repellency of the compound. Non exploring cockroaches in the untreated cup also explain the greater repellency found in the harborage-choice method. Unlike the Ebeling choice boxes, the harborage-choice method lacks a stimulus (such as light) to encourage the movement of cockroaches to the treated cup; therefore, even if the treated cup was not repellent, it may have appeared repellent because some cockroaches failed to explore the area outside the untreated cup during the 7 d experimental period.

For the majority of the oils, percentage of cockroaches repelled in the Ebeling choice boxes decreased dramatically during the first 2-3 days of the experiment (Figs. 13-16). This dramatic decrease did not occur for the harborage-choice method (Figs. 17-20), so degradation of the compound would not be a probable explanation for this occurrence. However, the sharp decline could be attributed to prolonged acclimation of the cockroaches to the choice boxes.

The harborage-choice method used by Steltenkamp et al. (1992) was designed to measure the repellency life of alkyl and aryl neoalkanamides (synthetic compounds). They studied the repellency duration of their compounds. Unlike our study, Steltenkamp et al. (1992) analyzed their data using only probit analysis. Probit analysis in our study did not yield significant results ($P > 0.05$). Using linear regression, we determined that the repellency of the essential oil components did not change significantly over the 7 days, which is why probit analysis results were not significant. Of the two methods used in this study, the Ebeling choice box is the superior method for determining the repellency of short lived compounds, such as essential oil components, because it is a better approximation of normal cockroach habitat, and they are designed to measure the percentage of repelled cockroaches, rather than repellency persistence.

Essential oil components, such as citronellic acid, carvacrol, and geraniol, can potentially be used as repellents for cockroach prevention. These repellents could be useful for eliminating harborages, which would increase the efficacy of the non-repellent, insecticide treated areas (Steltenkamp et al. 1992). Essential oils do not conduct electricity, so they would be effective in preventing infestations in hard-to-reach places, such as electrical systems (Ngoh et al. 1998). They would be effective around items that cannot be treated with traditional insecticides, such as sensitive equipment, important documents, and stored food items (Ngoh et al. 1998). Also, essential oils may be added to cleaning solutions, which could then be used to deter cockroaches from food preparation surfaces (Steltenkamp et al. 1992).

Repellent essential oils could also be used as flushing agents during inspections to determine the degree of infestation (Barcay 2004). An essential oil component

formulated as an aerosol would be sprayed into harborage areas, such as cracks and crevices, and the number of cockroaches that flee from the sprayed area would be counted (Koehler et al. 1995). This information would indicate the level of infestation, as well as the location where the pest control operator should focus control efforts. Flushing cockroaches from their harborages can also move the cockroaches toward residual insecticides, resulting in higher mortality (Koehler et al. 1995).

Repellents should not be applied on or near insecticidal baits, bait stations, and traps because this could reduce their attractiveness to German cockroaches. Baits that are contaminated with essential oils or placed on surfaces contaminated with essential oils suffer reduced performance (Appel 2004). The repellent oils could delay or prevent cockroaches from eating the baits (Appel 2004). Appel (2004) reported in his study on bait contamination that consumption of each formulation (solid, gel, and dust) contaminated with mint oil was reduced; however, some of each bait formulation was eventually consumed and toxic to the German cockroach. To prevent delayed mortality, a pest control operator should avoid spraying the repellent essential oils on or around the baits.

Previous studies have shown that essential oil components can be toxic and repellent to the German cockroach (Phillips et al. 2009, Phillips and Appel 2009). The 12 essential oil components used in this study have different routes of entry into the German cockroach; therefore, some can serve as fumigants (Phillips and Appel 2009), direct contact sprays (Phillips et al. 2009), or repellents. Along with other integrated pest management techniques, such as proper sanitation, the use of baits, and monitoring techniques, essential oil components can provide a safer, more environmentally friendly

approach to German cockroach control where traditional insecticides are prohibited and human health is the greatest concern.

Fig. 11. Ebeling choice box

Fig. 12. Harborage-choice method

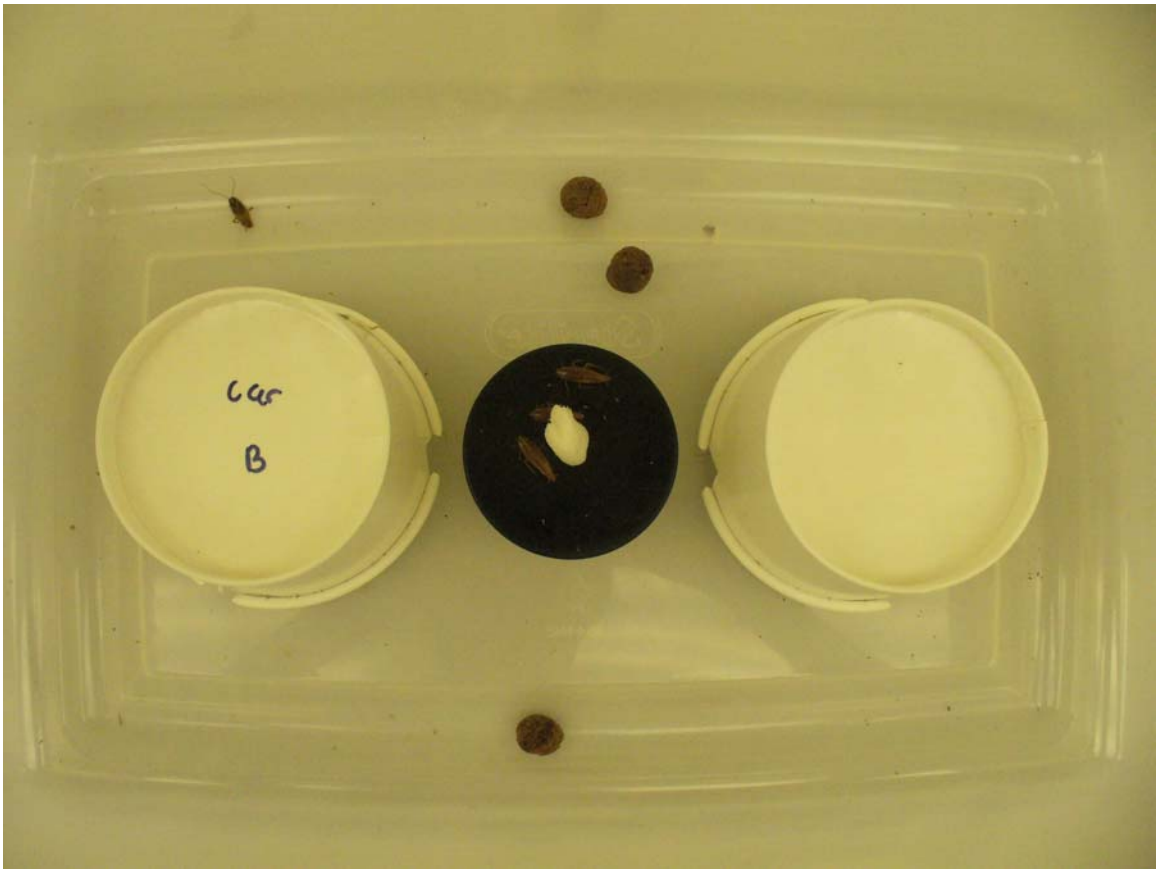


Table 21. Repellency of essential oil components to the German cockroach determined in Ebeling choice boxes

TMT	*n*	% Alive in light[a]	Range
		Mean ± SE	
Citronellic acid	120	44.99 ± 2.19a	34.93-62.73
(-)-β-Pinene	120	22.04 ± 2.25b	13.33-42.36
Geraniol	120	19.89 ± 2.19cb	7.95-41.67
Carvacrol	120	16.01 ± 2.19cbd	9.21-41.36
(+)-α-Pinene	120	15.92 ± 2.19cbd	6.32-56.10
(-)-Linalool	120	14.91 ± 2.19 cbd	6.97-52.02
Eugenol	120	14.47 ± 2.19ced	4.46-40.10
Thymol	120	13.10 ± 2.29ed	7.39-21.70
trans-Cinnamaldehyde	120	12.05 ± 2.19ed	6.02-27.59
(-)-Menthone	120	12.41 ± 2.19fed	5.92-37.50
1,8-Cineole	120	9.62 ± 2.19feg	1.63-24.94
S-(-)-limonene	120	5.55 ± 2.19fg	1.71-17.19
Control	120	3.89 ± 2.19g	0.83-6.89

[a] Mean over the entire test. Means followed by the same letters were not significantly different. ($P < 0.05$).

Table 22. Relationship between day and percentage of cockroaches repelled in Ebeling choice boxes.

TMT	Slope ± SE	Intercept ± SE	b ± SE	a ± SE	r^2	df	*F*	*P*
Carvacrol	0.59 ± 0.25	9.34 ± 2.33	4.54 ± 10.60	2953.10 ± 31225.83	0.89	13	27.69	0.0001
1,8-Cineole	0.98 ± 0.26	-0.31 ± 2.57	1.12 ± 0.37	75.07 ± 26.03	0.86	13	21.06	0.0001
trans-Cinnamaldehyde	-0.56 ± 0.33	15.13 ± 3.15	1.91 ± 2.29	87.55 ± 194.22	0.72	13	8.41	0.0044
Citronellic acid	-1.26 ± 0.31	54.44 ± 2.65	-	-	0.58	13	16.57	0.0020
Eugenol	1.46 ± 0.35	-0.93 ± 3.47	1.03 ± 0.26	112.63 ± 27.55	0.91	13	34.06	0.0001
Geraniol	1.88 ±1.41	-3.64 ± 17.84	0.40 ± 0.23	64.88 ± 13.68	0.75	13	10.04	0.0023

S-(-)-Limonene	0.23 ± 0.18	2.76 ± 1.67	3.49 ± 5.87	467.57 ± 2719.47	0.77	13	11.35	0.0015
(-)-Linalool	0.14 ± 0.19	10.42 ± 1.79	2.14 ± 0.53	353.21 ± 181.78	0.97	13	110.49	0.0001
(-)-Menthone	0.93 ± 0.17	2.39 ± 1.60	1.66 ± 0.33	179.54 ± 57.49	0.96	13	83.14	0.0001
(+)-α-Pinene	-2.1 ± 0.61	31.61 ± 5.19	-	-	0.50	13	11.88	0.0050
(-)-β-Pinene	-0.52 ± 0.34	24.54 ± 3.28	1.54 ± 1.08	86.17 ± 89.56	0.81	13	14.17	0.0006
Thymol	0.76 ± 0.26	7.82 ± 2.20	-	-	0.42	13	0.69	0.0120

Fig. 13. Repellency of carvacrol, 1,8-cineole, *trans*-cinnamaldehyde, and control to the German cockroach determined in Ebeling choice boxes

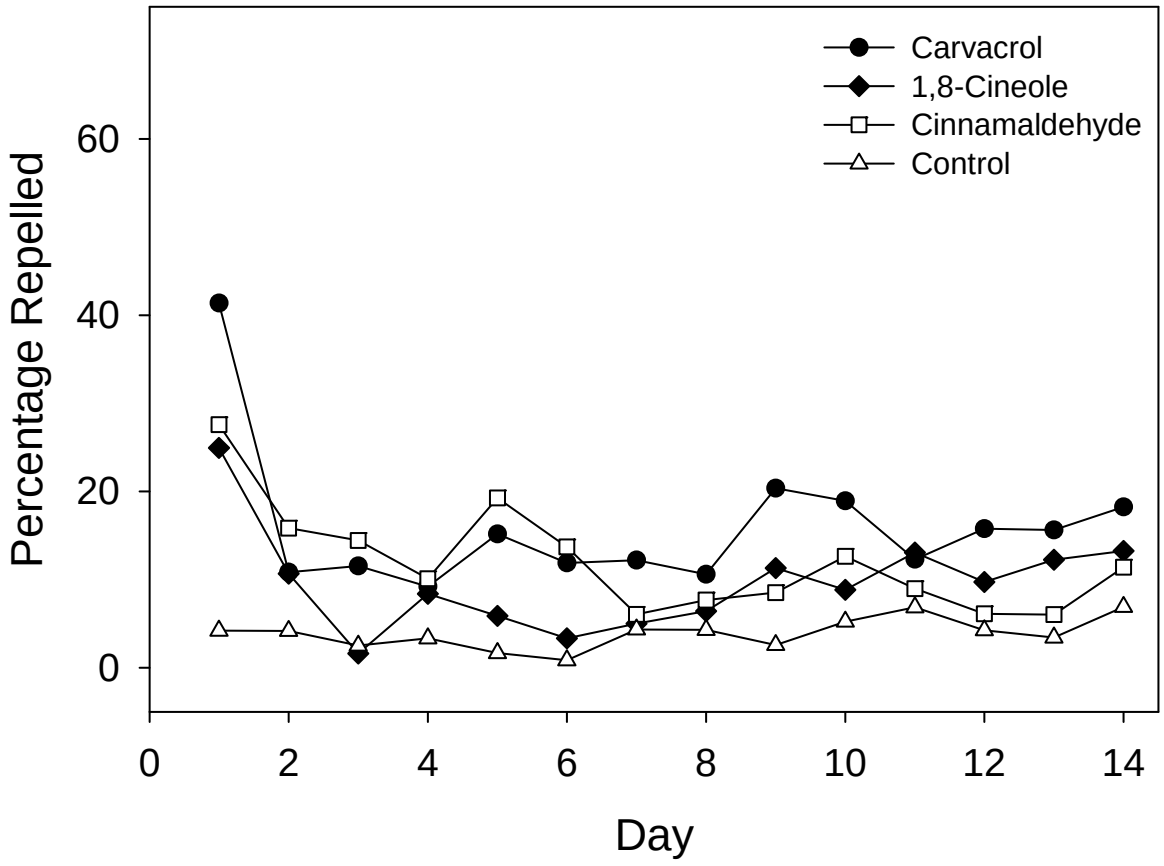


Fig. 14. Repellency of citronellic acid, eugenol, and geraniol to the German cockroach determined in Ebeling choice boxes

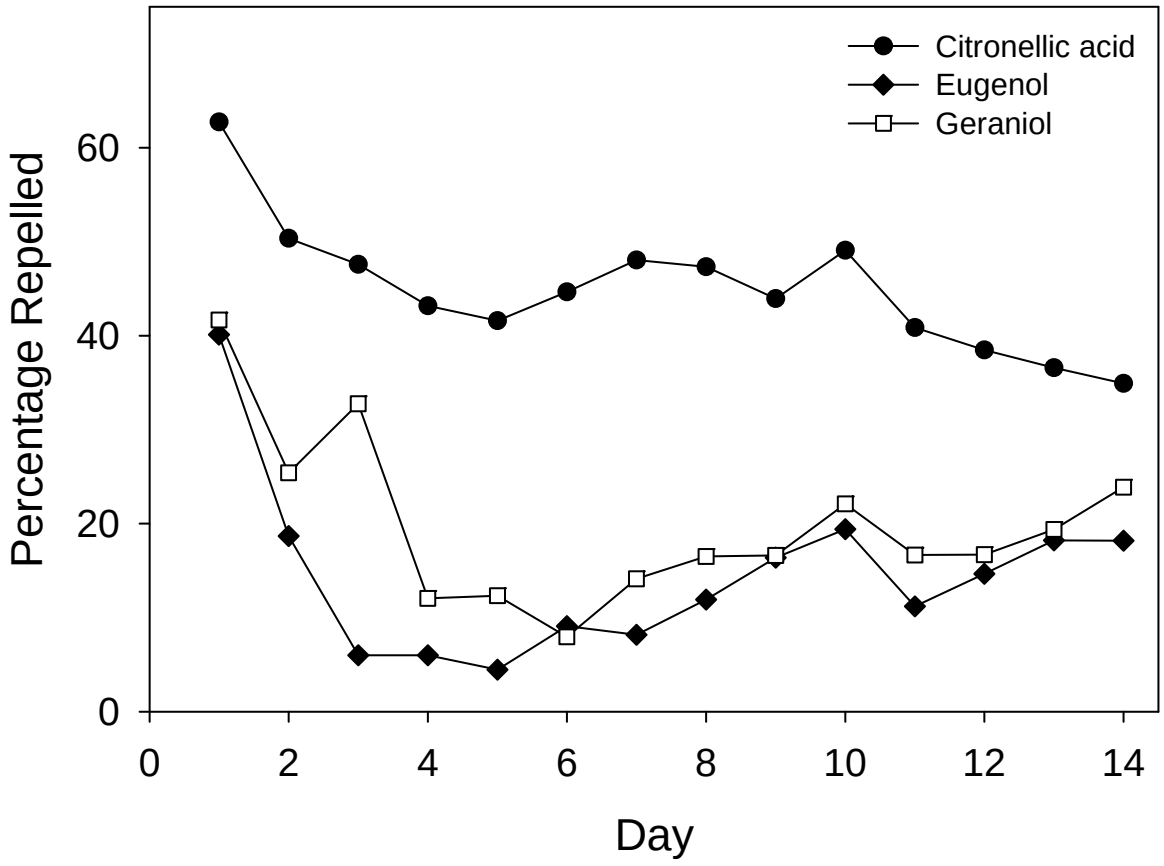


Fig. 15. Repellency of S-(-)-limonene, (-)-linalool, and (-)-menthone to the German cockroach determined in Ebeling choice boxes

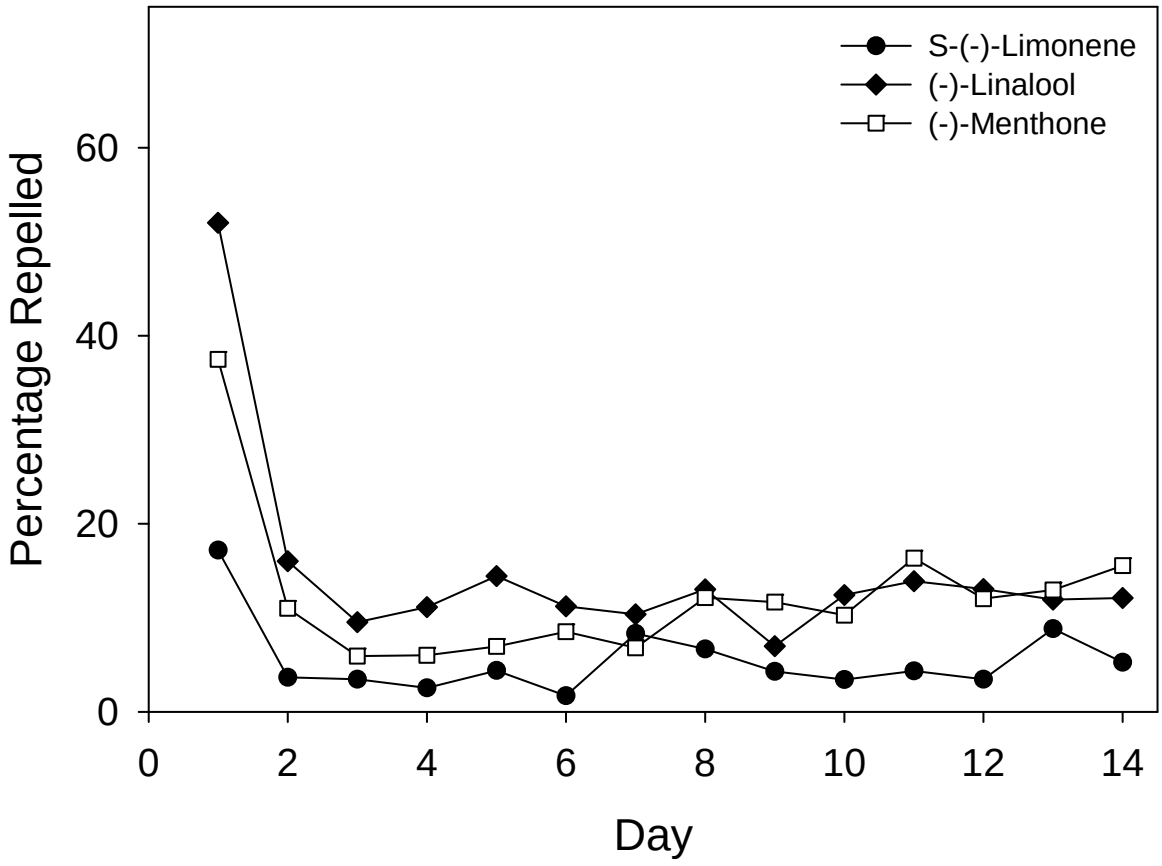


Fig. 16. Repellency of (+)-α-pinene, (-)-β-pinene, and thymol to the German cockroach determined in Ebeling choice boxes

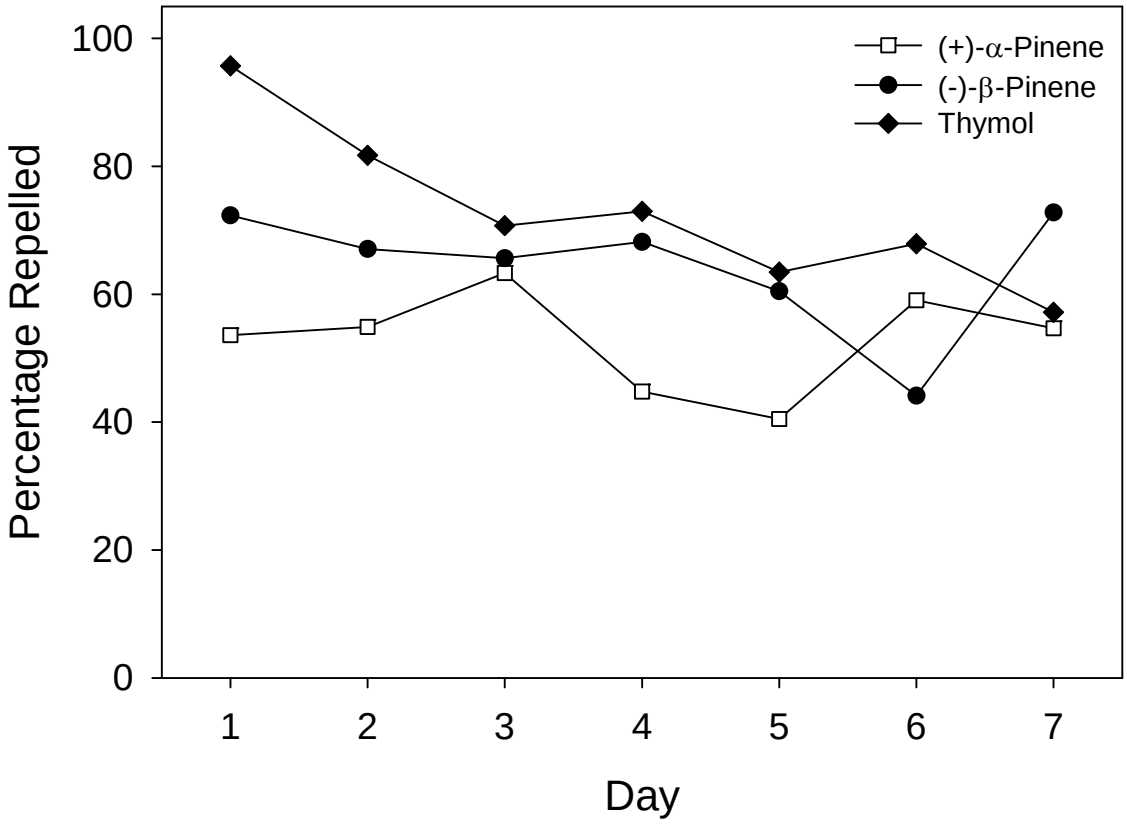


Table 23. Repellency of essential oil components to the German cockroach determined using the harborage-choice method

TMT	*n*	% Alive Under UA[a]	Range
		Mean ± SE	
Carvacrol	120	76.15 ± 3.63a	65.08-92.98
Citronellic acid	120	75.39 ± 3.63a	62.23-76.18
Thymol	120	72.71 ± 3.63a	57.16-95.70
Eugenol	120	72.90 ± 3.63a	63.29-94.50
Geraniol	120	70.86 ± 3.63a	56.97-89.78
trans-Cinnamaldehyde	120	65.90 ± 3.63ba	54.38-92.29
(-)-β-Pinene	120	64.29 ± 3.63ba	44.13-72.78
(-)-Linalool	120	64.78 ± 3.63bac	55.79-74.08
S-(-)-Limonene	120	62.28 ± 3.63bac	50.44-72.39
(+)-α-Pinene	120	52.89 ± 3.63bdc	40.49-63.31
Control	120	52.25 ± 3.17bdc	32.39-66.31
(-)-Menthone	120	49.68 ± 3.63dc	47.31-52.29
1,8-Cineole	120	43.07 ± 3.63d	34.44-51.66

[a] Mean over the entire test. Means followed by the same letters were not significantly different. ($P < 0.05$).

Table 24. Relationship between day and percentage of cockroaches repelled for harborage-choice method

TMT	Slope ± SE	Intercept ± SE	r^2	df	*F*	*P*
Carvacrol	-4.00 ± 0.80	92.22 ± 3.58	0.83	6	24.87	0.0040
1,8-Cineole	-	-	-	6	1.08	0.3460
trans-Cinnamaldehyde	-	-	-	6	7.50	0.0660
Citronellic acid	-	-	-	6	5.36	0.0680
Eugenol	-	-	-	6	3.84	0.1070
Geraniol	-5.77 ± 0.55	94.04 ± 2.45	0.96	6	111.43	0.0010
S-(-)-Limonene	-	-	-	6	0.10	0.7650
(-)-Linalool	-	-	-	6	1.10	0.342
(-)-Menthone	-	-	-	6	0.03	0.8520
(+)-α-Pinene	-	-	-	6	0.06	0.8150
(-)-β-Pinene	-	-	-	6	0.89	0.389
Thymol	-5.38 ± 1.05	94.32 ± 4.70	0.84	6	26.23	0.0040

Fig. 17. Repellency of carvacrol, 1,8-cineole, *trans*-cinnamaldehyde, and control to the German cockroach determined using harborage-choice method

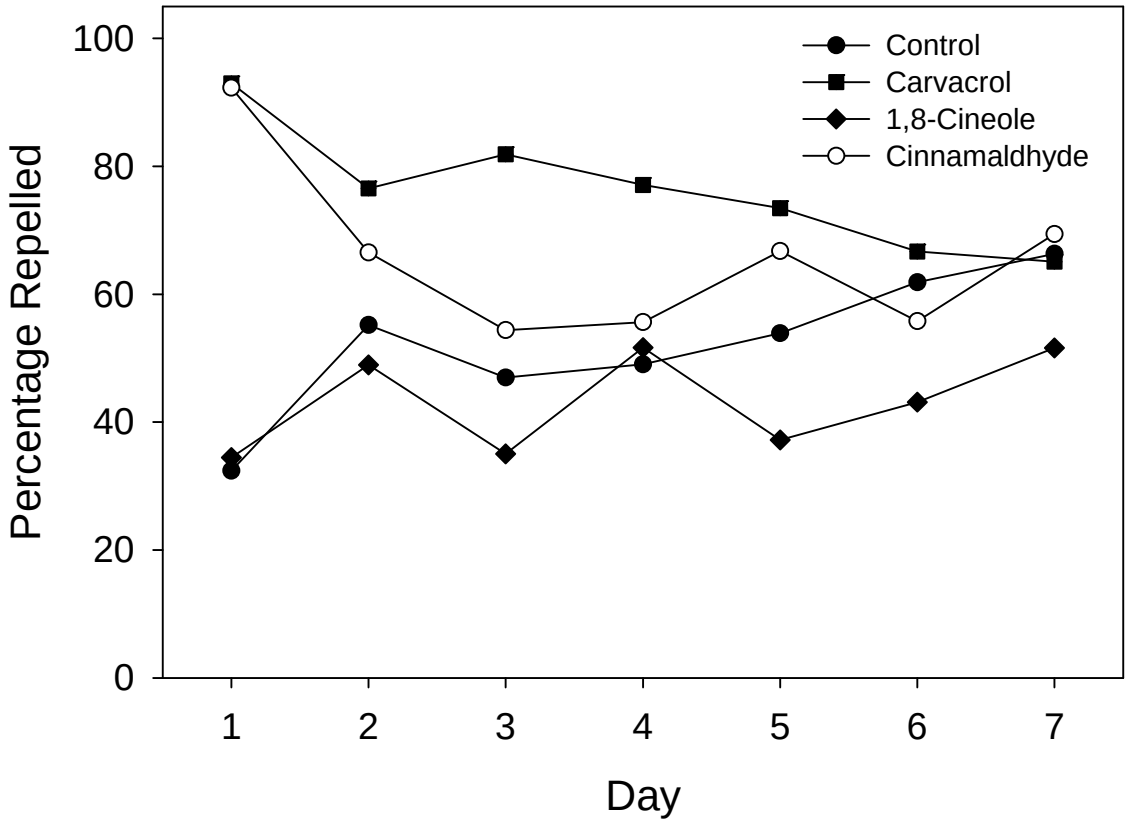


Fig. 18. Repellency of citronellic acid, eugenol, and geraniol to the German cockroach determined using harborage-choice method

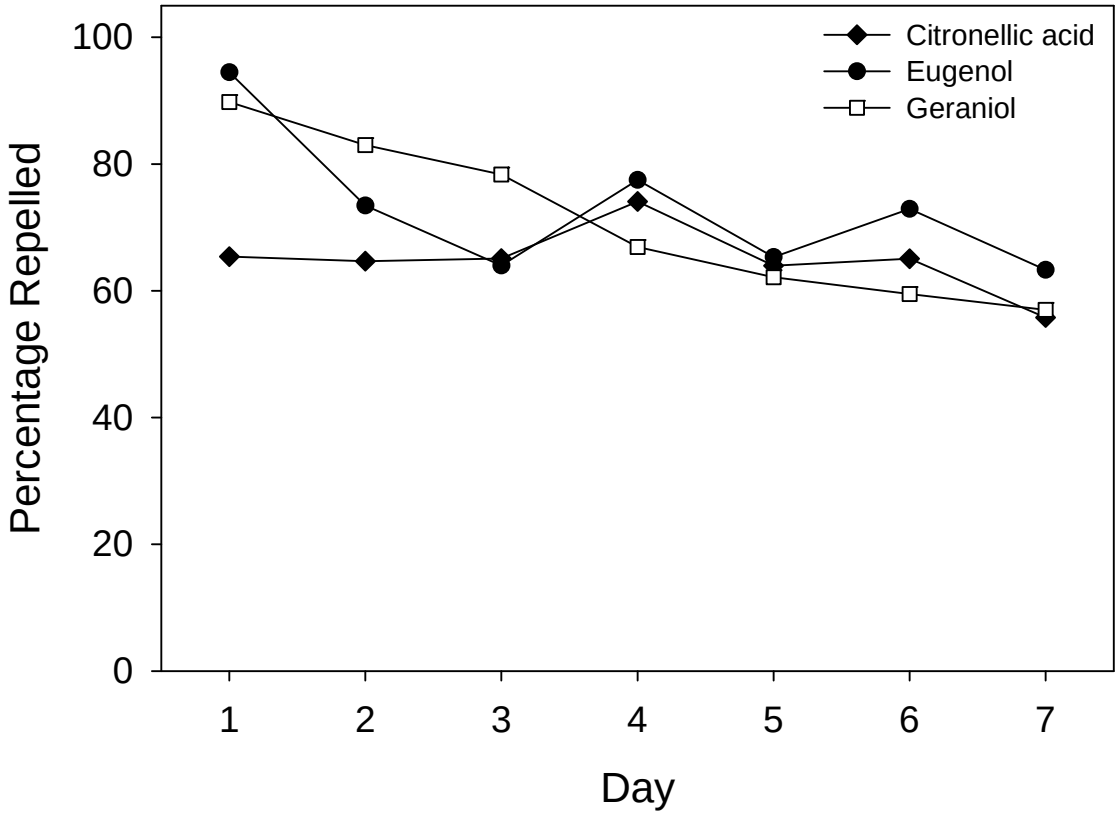


Fig. 19. Repellency of S-(-)-limonene, (-)-linalool, and (-)-menthone to the German cockroach determined using harborage-choice method

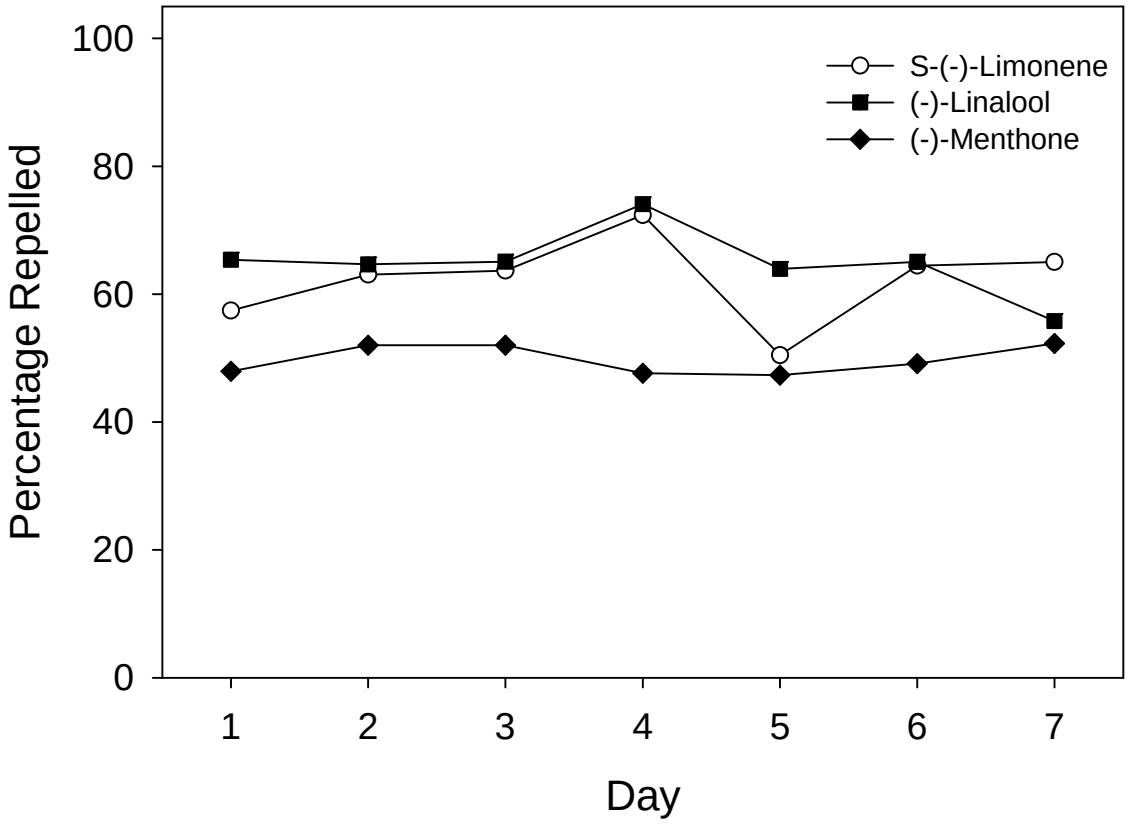


Fig. 20. Repellency of (+)-α-pinene, (-)-β-pinene, and thymol to the German cockroach determined using harborage-choice method

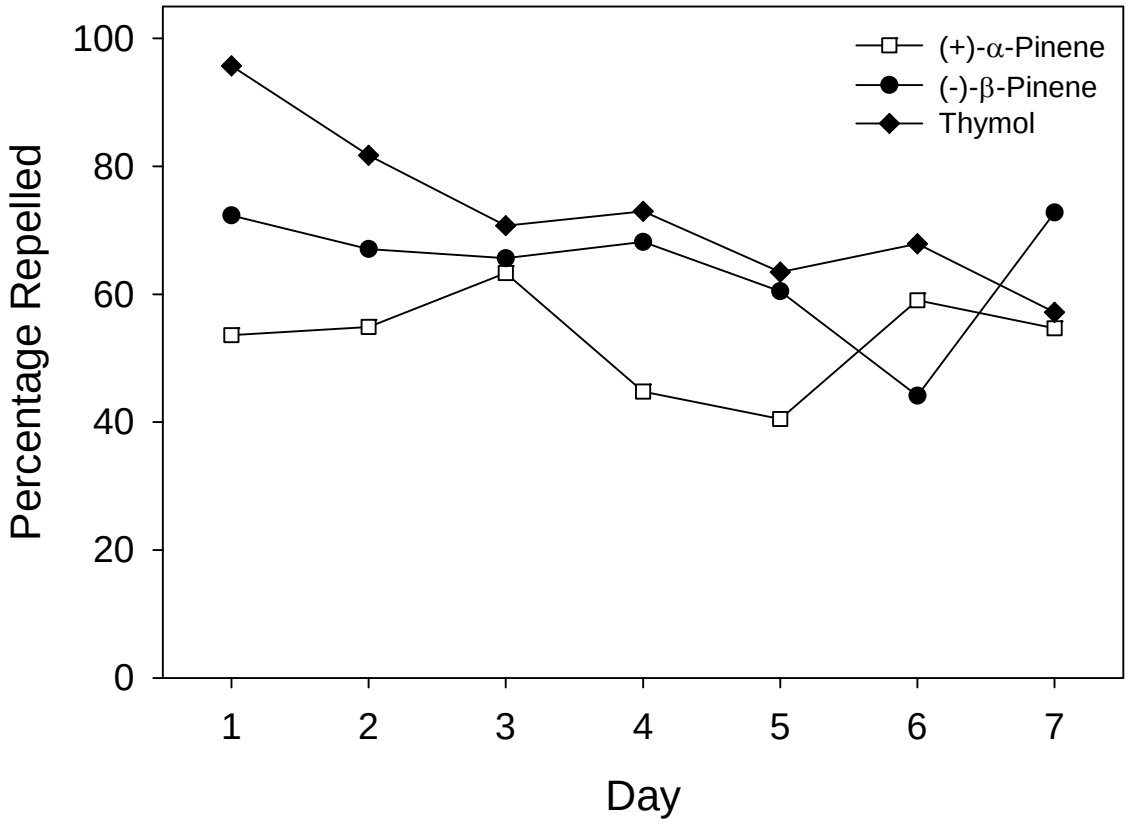


Fig. 21. The effect of essential oil fumigant toxicity on repellency determined using Ebeling choice boxes

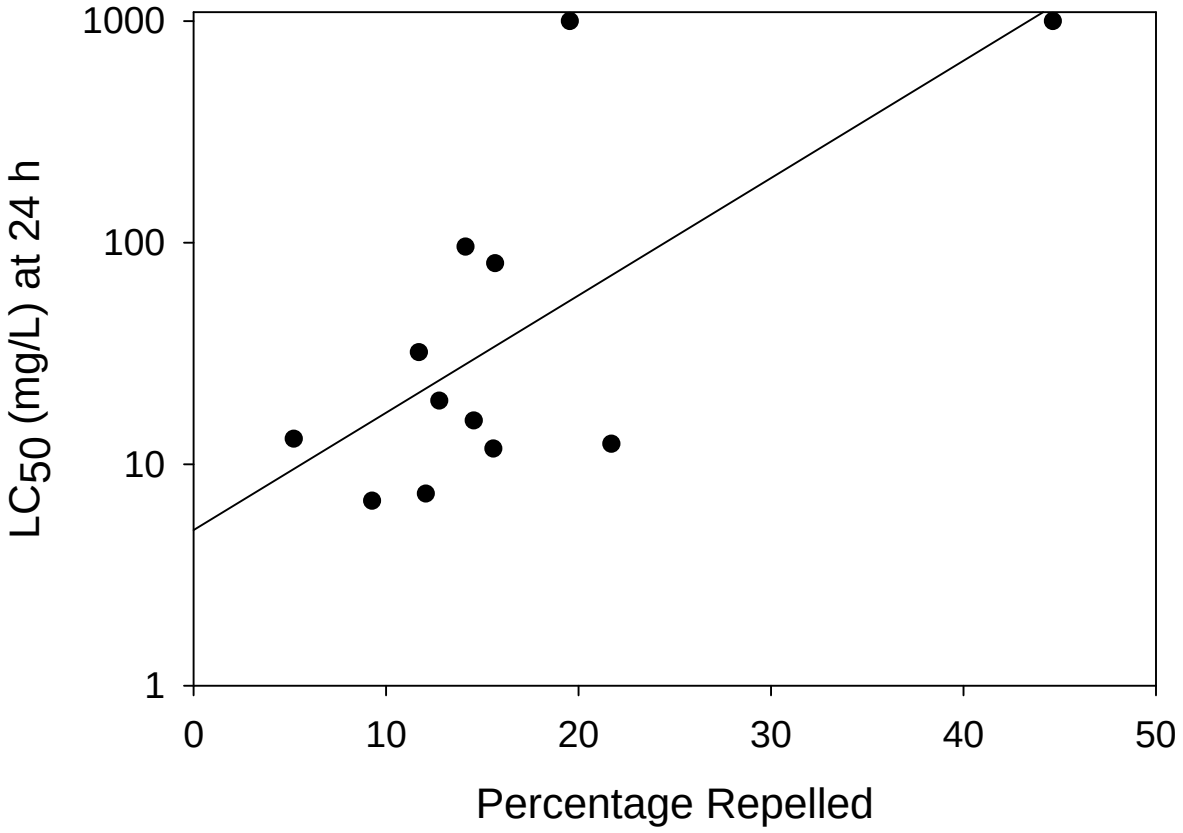
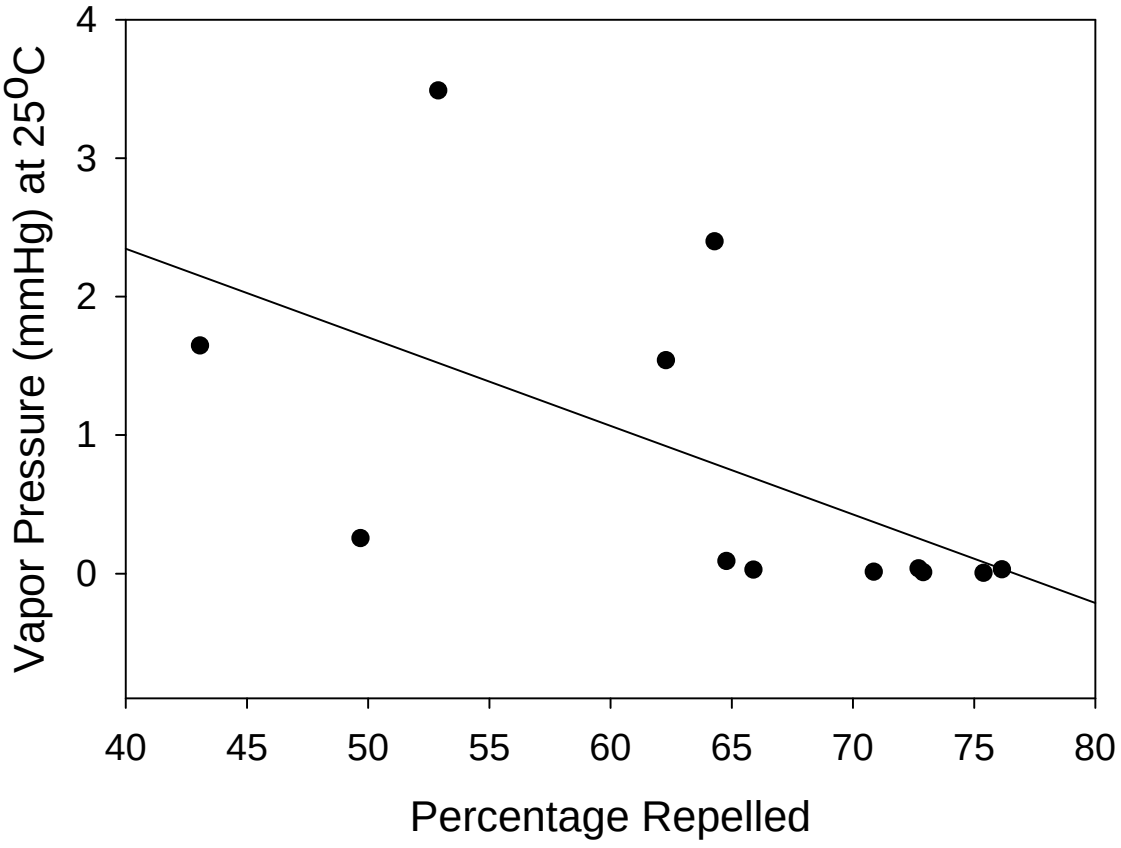


Fig. 22. The effect of essential oil vapor pressure on repellency determined using the harborage-choice method



References

Abd-Elghafar, S. F., and A. G. Appel. 1992. Sublethal effects of insecticides on adult longevity and fecundity of German cockroaches (Dictyoptera: Blattellidae). J. Econ. Entomol. 85: 1809-1817.

Abd-Elghafar, S. F., A. G. Appel, and T. P. Mack. 1990. Toxicity of several insecticide formulations against adult German cockroaches (Dictyoptera: Blattellidae). J. Econ. Entomol. 83: 2290-2294.

Abd-Elghafar, S. F., A. G. Appel, and T. P. Mack. 1991. Effects of several insecticide formulations on oothecal drop and hatchability in German cockroaches (Dictyoptera: Blattellidae). J. Econ. Entomol. 84: 502-509.

ACD/Labs. 2008. Version 11.0. Advanced Chemistry Development, Toronto ON, Canada.

Antonelli, A., C. Fabbri, M. E. Giorgioni, and I. Bazzocchi. 1997. Characterization of 24 old garden roses from their volatile compositions. J. Agric. Food Chem. 45: 4435-4439.

Appel, A. G. 1992. Performance of gel and paste bait products for German cockroach (Dictyoptera: Blattellidae) control: laboratory and field studies. J. Econ. Entomol. 85: 1176-1183.

Appel, A. G. 2004. Effects of contamination on the laboratory performance of insecticidal baits against German cockroaches (Dictyoptera: Blattellidae). J. Econ. Entomol. 97: 2035-2042.

Appel, A. G. 1997. Nonchemical approaches to cockroach control. J. Agric. Entomol. 14: 271-280.

Appel, A. G. 2008. Behavioral and physiological aspects of energetics and respiration patterns of cockroaches, pp. 1-10. In N. Liu (ed.), Recent advances in insect physiology, toxicology and molecular biology. Research Signpost, Kerala, India.

Appel, A. G., and T. P. Mack. 1989. Repellency of milled aromatic eastern red cedar to domiciliary cockroaches (Dictyoptera: Blattellidae and Blattidae). J. Econ. Entomol. 82: 152-155.

Appel, A. G., M. J. Gehret, and M. J. Tanley. 2001. Repellency and toxicity of mint oil to American and German cockroaches (Dictyoptera: Blattidae and Blattellidae). J. Agric. Urban Entomol. 18: 149-156.

Appel, A. G., M. J. Gehret, and M. J. Tanley. 2004. Repellency and toxicity of mint oil granules to red imported fire ants (Hymenoptera: Formicidae). J. Econ. Entomol. 97: 575-580.

Baldinger, L. H. 1942. The analysis of peppermint oil. Ind. Eng. Chem. 14: 15-20.

Barcay, S. J. 2004. Cockroaches, pp. 121-215. In S. A. Hedges (ed.), Handbook of Pest Control. GIE Media, Inc., Richfield, OH.

Barnard, D. R. 1999. Repellency of essential oils to mosquitoes (Diptera: Culicidae). J. Med. Entomol. 36: 625-629.

Bell, C. H., N. Price, and B. Chakrabarti. 1996. The methyl bromide issue. John Wiley and Sons. Chichester, England.

Brenner, R. J. 1995. Medical and Economic Significance, pp. 77-92. In M. K. Rust, J. M. Owens, and D. A. Reierson (eds.), Understanding and controlling the German cockroach. Oxford University Press, New York.

Caredda, A., B. Marongiu, S. Porcedda, and C. Soro. 2002. Supercritical carbon dioxide extraction and characterization of *Laurus nobilis* essential oil. J. Agric Food Chem. 50: 1492-1496.

Chang, R. 2003. General chemistry: the essential concepts, 3rd ed. McGraw-Hill, New York, NY.

Dingha, B. N., A. G. Appel, and M. D. Eubanks. 2005. Discontinuous carbon dioxide release in the German cockroach, *Blattella germanica* (Dictyoptera: Blattellidae), and its effect on respiratory transpiration. J. Insect Physiol. 51: 825–836.

Dudai, N., Z. G. Weinburg, O. Larkov, U. Ravid, G. Ashbell, and E. Putievsky. 2001. Changes in essential oil during enzyme-assisted ensiling of lemongrass (*Cymbopogon citratus* Stapf.) and lemon eucalyptus (*Eucalyptus citriodora* Hook). J. Agric. Food Chem. 49: 2262-2266.

Ebeling, W., R. E. Wagner, and D. A. Reierson. 1966. Influence of repellency on the blatticides. I. learned modification of behavior of the German cockroach. J. Econ. Entomol. 59: 1374-1388.

(EPA) U. S. Environmental Protection Agency. 2000. Pesticide Registration (PR) Notice 2000-6. EPA, Washington, D.C. http://www.epa.gov/PR_Notices/pr2000-6.pdf.

Gish, C. D., and N. J. Chura. 1970. Toxicity of DDT to Japanese Quail as influenced by body weight, breeding condition, and sex. Toxicol. Appl. Pharmacol. 17: 740-751.

Grace, K. J., and D. L. Wood. 1987. Delusory cleptoparasitosis: delusion of arthropod infestation in the home. Pan-Pacif. Entomol. 63: 1-4.

Hertel, W., W. Hertel, and P. J. Muller. 2006. Physiological effects of natural products quassin, cinnamaldehyde and azadirachtin on *Periplaneta americana* (L.). J. Appl. Entomol. 130: 323-328.

Hink, W. F., and B. J. Fee. 1986. Toxicity of D-limonene, the major component of citrus peel oil, to all life stages of the cat flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae). J. Med. Entomol. 23: 400-404.

Huang, Y., and S. H. Ho. 1998. Toxicity and antifeedant activities of cinnamaldehyde against the grain storage insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. J. Stored Prod. Res. 34: 11-17.

Hodgson, E. 1987. Metabolism of toxicants, pp. 51-83. In E. Hodgson and P. E. Levi (eds.), A Textbook of Modern Toxicology. Elsevier, New York.

Hori, M. 2003. Repellency of essential oils against the cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae). Appl. Entomol. Zool. 38: 467-473.

Ishii, S., and Y. Kuwahara. 1967. An aggregation pheromone of the German cockroach *Blattella germanica* (L.) (Orthoptera: Blattellidae). Appl. Entomol. Zool. 2: 203–217.

Isman, M. B. 2000. Plant essential oils for pest and disease management. Crop Protect. 19: 603-608.

Isman, M. B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Ann. Rev. Entomol. 51: 45-66.

Izutsu, M., S. Ueda, and S. Ishii. 1970. Aggregation effects on the growth of the German cockroach, *Blattella germanica* (L.) (Blattaria: Blattellidae). Appl. Entomol. Zool. 5: 159-171.

Jang, Y. S., Y. C. Yang, D. S. Choi, and Y. J. Ahn. 2005. Vapor phase toxicity to marjoram oil compounds and their related monoterpenoids to *Blattella germanica* (Orthoptera: Blattellidae). J. Agric. Food Chem. 53: 7892-7898.

Kang, B. 1976. Study on cockroach antigen as a probable causative agent in bronchial asthma. J. Allergy Clin. Immunol. 58: 357-365.

Kilic, A., H. Kollmannsberger, and S. Nitz. 2005. Glycosidically bound volatiles and flavor precursors in *Laurus nobilis* L. J. Agric. Food Chem. 53: 2231-2235.

Kim, H. K., J. R. Kim, and Y. J. Ahn. 2004. Acaricidal activity of cinnamaldehyde and its congeners against *Tyrophagus putrescentiae* (Acari: Acaridae). J. Stored Prod. Res. 40: 55-63.

Koehler, P. G., R. S. Patterson, J. M. Owens. 1995. Chemical systems approach to German cockroach control, pp. 287-324. In M. K. Rust, J. M. Owens, and D. A. Reierson (eds.), Understanding and controlling the German cockroach. Oxford University Press, New York.

Kordali, S., **I. Aslan, O. Calmasur, and A. Cakir. 2006.** Toxicity of essential oils isolated from three *Artemisia* species and some of their major components to

granary weevil, *Sitophilus granaries* (L.) (Coleoptera: Curculionidae). Ind. Crop. Prod. 23: 162-170.

Lee, S., C. J. Peterson, and J. R. Coats. 2003. Fumigation toxicity of monoterpenoids to several stored product insects. J. Stored Prod. Res. 39: 77-85.

Lee, B. H., P. C. Annis, F. Tumaalii, and W. S. Choi. 2004. Fumigant toxicity of essential oils from the Myrtaceae family and 1,8-cineole against 3 major stored-grain insects. J. Stored Prod. Res. 40: 553-564.

Matsumura, F. 1985. Toxicology of Insecticides. 2nd ed. Plenum Press. New York, NY.

Metzger. 1995. Behavior, pp. 49-76. In M. K. Rust, J. M. Owens, and D. A. Reierson (eds.), Understanding and controlling the German cockroach. Oxford University Press, New York.

Mockute D., and G. Bernotiene. 1999. The main citral-geraniol and carvacrol chemotypes of the essential oil *Thymus pulegioides* L. growing wild in Vilnius district (Lithuania). J. Agric. Food Chem. 47: 3787-3790.

Morrison, R. T., and R. N. Boyd. 1992. Organic Chemistry. 6th ed. Prentice Hall. Englewood Cliffs, New Jersey.

Mullins, D. E., C. B. Keil, and R. H. White. 1992. Maternal and paternal nitrogen investment in *Blattella germanica* (L.) (Dictyoptera; Blattellidae). J. exp. Biol. 162: 55-72.

Mumcuoglu, K. Y., R. Galun, U. Bach, J. Miller, and S. Magdassi. 1996. Repellency of essential oils and their components to the human body louse *Pediculus humanus humanus*. Entomologia Experimentalis et Applicata. 78: 309-314.

Nakahara, K., N. S. Alzoreky, T. Yoshihashi, H. T. T. Nguyen, and G. Trakoontivakorn. 2003. Chemical composition and antifungal activity of essential oil from *Cymbopogon nardus* (Citronella grass). JARQ. 37: 249-252.

Nation, J. L. 2008. Insect physiology and biochemistry. 2nd ed. CRC Press, New York.

Ngoh, S. P., L. E. W. Choo, F. Y. Pang, Y. Huang, M. R. Kini, and S. H. Ho. 1998. Insecticidal and repellent properties of nine volatile constituents of essential oils against the American cockroach, *Periplaneta Americana* (L.). Pestic. Sci. 54: 261-268.

O'Connor, G. T., and R. D. Gold. 1999. Cockroach allergy and asthma in a 30-year-old man. Environmental Health Perspectives. 107: 243-247.

Omolo, M. O., D. Okinyo, I. O. Ndiege, W. Lwande, and A. Hassanali. 2004. Repellency of essential oils of some Kenyan plants against *Anopheles gambiae*. Phytochemistry. 65: 2797-2802.

O'Neil, M. J. (ed.). 2006. The Merck Index, 14 ed. Whitehouse Station, NJ.

Oswalt, D. A., A. G. Appel, and L. M. Smith II. 1997. Water loss and desiccation tolerance of German cockroaches (Dictyoptera: Blattellidae) exposed to moving air. Comp. Biochem. Physiol. 117A: 477-486.

Palmer, R. C. 1942. Developments in terpene chemicals. Ind. Eng. Chem. 34: 1028-1034.

Pålsson, K., T. G. T. Jaenson, P. Bæckström, and A. K. Borg-Karlson. 2008. Tick repellent substances in the essential oil of *Tanacetum vulgare*. J. Med. Entomol. 45: 88-93.

Panella, N. A., M. C. Dolan, J. J. Karchesy, and Y. Xiong. 2005. Use of novel compounds for pest control: insecticidal and acaricidal activity of essential oil components from heartwood of Alaska yellow cedar. J. Med. Entomol. 42: 352-358.

Park, I., and S. Shin. 2005. Fumigant activity of plant essential oils and components from garlic (*Allium sativum*) and clove bud (*Eugenia caryophyllata*) oils against the Japanese termite (*Reticulitermes speratus* Kolbe). J. Agric. Food Chem. 53: 4388- 4392.

Phillips, A. K., and A. G. Appel. 2009. Fumigant toxicity of essential oils to the German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol. Submitted.

Phillips, A. K., A. G. Appel, and S. R. Sims. 2009. Topical toxicity of essential oils to the German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol. Submitted.

Pridgeon, J. W., A. G. Appel, W. J. Moar, and N. Liu. 2002. Variability of resistance mechanisms in pyrethroid resistant German cockroaches (Dictyoptera: Blattellidae). Pestic. Biochem. Physiol. 73: 149-157.

Reierson, D. A., and M. K. Rust. 1984. Insecticidal baits and repellency in relation to control of the German cockroach, *Blattella germanica* (L.) Pest Manag. Sci. 2: 26-32.

Rice, P. J., and J. R. Coats. 1994. Insecticidal properties of several monoterpenoids to the house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae), and southern corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 87: 1172-1179.

Ross, M. H., and D. E. Mullins. 1995. Biology, pp. 21-47. In M. K. Rust, J. M. Owens, and D. A. Reierson (eds.), Understanding and controlling the German cockroach. Oxford University Press, New York.

Roth, L. M. 1970a. Evolution and taxonomic significance of reproduction in Blattaria. Annu. Rev. Entomol. 15: 75-96.

Roth, L. M. 1970b. The stimuli regulating reproduction in cockroaches. Int. Cent. Nat. Res. Sci. Paris. 267-286.

Roth, L. M., and E. R. Willis. 1957. The medical and veterinary importance of cockroaches. Smithsonian Institution, Washington, D.C.

Roth, L. M., and E. R. Willis. 1960. The biotic associations of cockroaches. Smithsonian Institution, Washington, D.C.

Rozman, V., **I. Kalinovic, and Z. Korunic. 2007.** Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to three stored-product insects. J. Stored Prod. Res. 43: 349-355.

Sampson, B. J., N. Tabanca, N. Kirimer, B. Demirci, K. H. C. Baser, I. A. Khan, J. M. Spiers, and D. E. Wedge. 2005. Insecticidal activity of 23 essential oils and their major compounds against adult *Lipaphis pseudobrassicae* (Davis) (Aphididae: Homoptera). Pest Manag. Sci. 61: 1122-1128.

SAS Institute. 2003. SAS user's guide: statistics, version 9.1. SAS Institute, Cary, N.C.

Schal, C., and R. L. Hamilton. 1990. Integrated suppression of synanthropic cockroaches. Annu. Rev. Entomol. 35: 521-551.

Scott, J. G., D. G. Cochran, and B. D. Siegfried. 1990. Insecticide toxicity, synergism, and resistance in the German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol. 83: 1698-1703.

Senanayake, U. M., T. H. Lee, and R. B. H. Willis. 1978. Volatile constituents of cinnamon (*Cinnamomum zeylanicum*) oils. J. Agric. Food Chem. 26: 822-824.

Sfara, V., E. N. Zerba, and R. A. Alzogaray. 2009. Fumigant insecticidal activity and repellent effect of five essential oils and seven monoterpenes on first-instar nymphs of *Rhodnius prolixus*. J. Med. Entomol. 46: 511-515.

Sigma-Aldrich. 2008. (http://www.sigmaaldrich.com).

Sotomayor, J. A., R. M. Martinez, A. J. Garcia, and M. J. Jordan. 2004. *Thymus zygis* subsp. *Gracilis*: watering level effect on phytomass production and essential oil quality. J. Agric. Food Chem. 52: 5418-5424.

SPSS Inc. 2008. SigmaPlot 2008, version 11.0. SPSS Inc., Chicago, IL.

Stamopoulos, D. C., P. Damos, and G. Karagianidou. 2007. Bioactivity of five monoterpenoid vapors to *Tribolium confusum* (du Val) (Coleoptera: Tenebrionidae). J. Stored Prod. Res. 43: 571-577.

Steltenkamp, R. J., R. L. Hamilton, R. A. Cooper, and C. Schal. 1992. Alkyl and aryl neoalkanamides: highly effective insect repellents. J. Med. Entomol. 29: 141-149.

Tarelli, G., E. N. Zerba, and R. A. Alzogaray. 2009. Toxicity to vapor exposure and topical application of essential oils and monoterpenes on *Musca domestica* (Diptera: Muscidae). J. Econ. Entomol. 102: 1383-1388.

Thoms, E. M., and R. H. Scheffrahn. 1994. Control of pests by fumigation with Vikane gas fumigant (sulfuryl fluoride). Down to Earth. 49: 23-30.

Thoms, E. M., and T. W. Phillips. 2004. Fumigation, pp. 1165-1217. In S. A. Hedges (ed.), Handbook of Pest Control. GIE Media, Inc., Richfield, OH.

Timmer, R., R. Heide, P. J. Valois, and H. J. Wobben. 1971. Qualitative analysis of the most volatile neutral components of reunion geranium oil (*Pelargonium roseum* Bourbon). J. Agric. Food Chem. 19: 1066-1068.

Tortora, G. J., B. R. Funke, and C. L. Case. 2007. Microbiology: an introduction, 9th ed. Benjamin Cummings, San Francisco, CA.

Tripathi, A. K., V. Prajapati, K. K. Aggarwal, S. P S. Khanuja, and S. Kumar. 2000. Repellency and toxicity of oil from *Artemisia annua* to certain stored-product beetles. J. Econ. Entomol. 93: 43-47.

Usai, M., **G. Arras, and F. Fronteddu. 1992.** Effects of cold storage on essential oils of peel of Thompson naval oranges. J. Agric. Food Chem. 40: 271-275.

VanRyckeghem, A. 2004. Stored Product Pests, pp. 747-823. In S. A. Hedges (ed.), Handbook of Pest Control. GIE Media, Inc., Richfield, OH.

Vogt, J. T., T. G. Shelton, M. E. Merchant, S. A. Russell, M. J. Tanley, and A. G. Appel. 2002. Efficacy of three citrus oil formulations against *Solenopsis invicta* Buren (Hymenoptera: Formicidae), the red imported fire ant. J. Agric. Urban Entomol. 19: 159-171.

Waliwitiya, R., **M. B. Isman, R. S. Vernon, and A. Riseman. 2005.** Insecticidal activity of selected monoterpenoids and rosemary oil to *Agriotes obscurus* (Coleoptera: Elateridae). J. Econ. Entomol. 98: 1560-1565.

Wood M. 1987. Restaurant fumigation. Pest Manag. 6: 12-14

Woodman, J. D., V. S. Haritos, and P. D. Cooper. 2007. Effects of phosphine on the neural regulation of gas exchange in *Periplaneta americana*. Comp. Biochem. Physiol. C. 147: 271-277.

Yang, Y. C., H. S. Lee, J. M. Clark, and Y. J. Ahn. 2004. Insecticidal activity of plant essential oils against *Pediculus humanus capitis* (Anoplura: Pediculidae). J. Med. Entomol. 41: 699-704.

Yousif, A. N., C. H. Scaman, T. D. Durance and B. Girard. 1999. Flavor volatiles and physical properties of vacuum-microwave and air-dried sweet basil (*Ocimum basilicum* L.). J. Agric. Food Chem. 47: 4777-4781.

Yu, S. J. 2008. The toxicology and biochemistry of insecticides. Taylor and Francis Group. Bacon Raton, FL.