TEMPERATURE EFFECTS ON VAPOR TOXICITY OF VOLATILE COMPOUNDS TO BED BUGS, *Cimex lectularius* L.

By

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To Madeline, Rylan, and Aaralyn

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The increase of bed bug *Cimex lectularius* L. (Hemiptera: Cimicidae) infestations and pesticide resistance has resulted in a constant search for new alternative control tools. Temperature effects on bed bug toxicity were investigated for the organophosphate dichlorvos, the essential oil anise seed, and the secondary essential oil components linalool, trans-anethole, and eucalyptol. A novel method of heating dichlorvos resin strips was tested in an unoccupied apartment. Bed bugs were most resistant to dichlorvos vapor at 36°C, followed by 28°C.

Bed bugs were most susceptible at 17°C. Toxicity of the essential oil anise seed to bed bugs was positively correlated with temperature. The interaction of temperature and concentration affected dichlorvos, trans-anethole, eucalyptol, and anise seed oil.. These results suggest that essential oils and their components have insecticidal properties that are toxic to bed bugs, and greater toxicity can be achieved at smaller active ingredient concentrations by manipulating exposure temperatures.

Field tests using resin-embedded dichlorvos resulted in 100% bed bug mortality in 12 h at ½, ¾, and the label rate by accelerating the vapor release of resin-embedded dichlorvos using a novel application method and exposing bed bugs for 6 h in a118 m3 apartment. These results 10 suggest application techniques and factors including temperature, concentration, air circulation,

exposure time, and post-treatment time can influence the efficacy of resin-embedded dichlorvos for bed bug control.

CHAPTER 1
LITERATURE REVIEW

Brief History of Bed Bugs and the Evolution of Infestations

Bed bugs, *Cimex lectularius* L. (Hemiptera: Cimicidae), have contributed to human suffering and misery throughout their hypothesized co-evolution (Panagiotakopulu & Buckland 1999). Usinger (1966) deduced that the original hosts of *C. lectularius* were Palearctic cave-dwelling bats that took advantage of cave-dwelling hominids. Bed bugs may have interacted with Neanderthals in the Middle East that lived in caves ~100,000 years ago. Humans and bed bugs gradually transitioned together from cave life to city life, resulting in the bed bug's preference for human blood (Adams 1960, Usinger 1966, Panagiotakopulu & Buckland 1999). Despite these earlier studies, Reinhardt and Siva-Jothy (2007) suggested that the origins of the relationship between bed bugs and humans are not clear and recommended that molecular tools should provide greater inference than ancient writings and folktales.

Historically, the increase in population and spread of bed bugs coincided with the increase in human population density and world trade. Bed bug infestations were known since ancient times. Aristotle (384-322 B.C.) documented problems which were caused by bed bugs. Dioscorides of Cilicia, a Greek army surgeon during the reign of the Roman emperor Nero (54-68 A.D.), speculated that the bed bug possessed magical powers that could heal poisonous snake bites by neutralizing venom and could expel leeches from horses. Early Jewish and Christian writings also indicate the presence of bed bugs (Usinger 1966). Bed bugs arrived in the United States aboard ships that brought the first European explorers and settlers who referred to the insects simply as "bugs" (Potter 2008a). Bed bug populations were high in the early 20th century and new control tactics were needed. The bed bug resurgence in the early 1900s was thought to have been fueled by the upsurge of centralized heating and other urban living advancements

(Johnson 1941). After World War II, there was a dramatic decrease in bed bug infestations which was the direct result of widespread use of DDT (dichlorodiphenyltrichloroethane), and then organophosphates and carbamates. The first reported resurgence of bed bug populations occurred in large cities between 1998 and 2000, and is now thought to be the first sign of bed bug resurgence in the United States (Krueger 2000). Subsequently, all 50 U.S states have reported bed bug infestations (Potter 2008a).

Biology

The insect family Cimicidae (Order: Hemiptera), are true bugs that contains at least 91 identified species, all of which are flightless parasites feeding exclusively on blood. Cimicids are a sister group to the family Anthocoridae, containing predatory bugs that prey on insects and mites, and the family Polyctenidae, containing bugs that have adapted to parasitic lifestyle attacking bats. The two species in Cimicidae which commonly feed on humans are the common bed bug and the tropical bed bug, *Cimex lectularius* L., and *Cimex hemipterus* L. (Usinger 1966; Ryckman et al. 1981; Krinsky 2002). The generic name *Cimex* is the Latin word for "bug", while *lectularius* refers to a "couch" or "bed".

Adult bed bugs are 3-5 mm long, oval, and reddish brown. The bodies of non-engorged adult bed bugs are broad, oval, and dorsoventrally flattened and become longer and inflated while they take a blood meal. Bed bug gender is most easily distinguished by the shape of the abdomen. The male bed bug abdomen tip is usually pointed while the female abdomen tip is rounded. However, it is difficult to sex fully engorged bed bugs. Eggs are about 1mm in length, white, and usually glued in clusters to a surface. Bed bug nymphs closely resemble smaller versions of adults; however, they are of yellowish transparent color.

Bed bugs have seven life stages including the egg, five nymph instars, and adult. All life stages of bed bugs, except the egg stage, feed solely on blood. Nymphs require at least a blood

meal to molt into a new instar, and reach maturity after five molts. Feeding length and frequency affects blood meal size and egg production (Pereira et al. Unpublished Data). Bed bugs reared in a laboratory take about 10-15 minutes to feed and can feed multiple days each week. Egg production increases with more frequent feedings. An adult female can produce up to 500 eggs in her lifetime (Usinger 1966). Suitable conditions (e.g., 75-80% RH; 28-32°C) trigger bed bug eggs to hatch in 5-12 days, and allow the development and molting of nymphs to the next stage in 6-14 days. At temperatures maintained below 10°C, each nymphal instar may take 3 months (Romero 2009).

Bed bugs prefer to feed on sleeping or immobile victims. Bed bugs are only attracted to host stimuli when they are not engorged Bed bugs feed to repletion, and then return to a secluded hideaway. Engorgement acts as a trigger to switch from positive thermotaxis to negative thermotaxis (Reinhardt and Siva-Jothy 2006).

Bed bugs must feed to drive ontogeny, fecundity, and mating. Bed bugs have a unique mating behavior known as hemocoelic insemination. Investigations of the bed bug mating behavior serve as models to elucidate sexual conflict and cryptic female choice. Adult male bed bugs mount recently fed females, and then probe the underside of the female's abdomen with their intromittant organ. The female paragenital system consists of the mesospermalege, which is the site of penetration. Inside the female, the spermatozoa move through the paragenital system, which leads to the ovaries. Females may encounter many hemocoelic inseminations by one or more males after feeding. The first male to mate injects the most semen and later males reduce their ejaculatory volume by up to 75% (Stutt and Siva-Jothy 2001), suggesting that the volume of semen being released is affected by an antagonistic feedback system. Later males are rewarded

with up to 68% paternity despite injecting less semen into the female. Repeated mating may be costly to the female (Stutt and Siva-Jothy 2001).

Nymph and adult bed bugs aggregate in cracks and crevices that usually also contain eggs, cast skins, and fecal matter. Nymphs and adult males are found aggregating more often than females (Pfeister et al. 2009). The primary advantage of bed bug aggregation is unknown; however, aggregation could be a defensive strategy against desiccation (Benoit et al. 2007), or a strategy which increases sexual encounters (Sutt and Siva-Jothy 2001).

Environments which are comfortable to humans are normally ideal habitats for bed bugs. In natural settings, bed bugs are most active in hours before dawn. The mechanism that attracts bed bugs to the host is not fully understood. However, Reinhardt (2007) hypothesized that they locate their host by random searching, followed by orientation to heat, CO_2, and host odors from perspiration and other secretions.

Bed Bug Impacts on Human Health

Bed bugs affect human hosts in many ways including psychological distress, secondary skin infections, physiological changes in the host, and sleeplessness (Goddard 2009). Components in the bed bug saliva may cause severe skin reactions in humans; however, some humans do not react at all (Feingold et al. 1968, Reinhardt and Siva-Jothy 2006). Bed bug bites and other skin irritations often are indistinguishable. Limited studies have been conducted on systemic reactions from bed bug bites, including asthma, generalized urticaria, and anaphylaxis (Goddard 2009). A model on blood meal size and frequency predicts harmful levels of human blood loss occurring in 11-15 weeks after initial bed bug population establishment with constant availability of blood meals (Pereira et al. Unpublished Data). Psychological disorders such as emotional distress have been documented in relation to bed bug infestations (Goddard 2009).

Fear of bed bugs may result in signs of delusory parasitosis, an emotional disorder in which people believe that insects are causing them discomfort (Webb 1993).

Bed bugs are considered to be pests of significant public health importance (CDC/EPA 2010); however, there is no evidence that bed bugs transmit disease (Goddard 2009). No research has been published demonstrating the vector competence of bed bugs according to Koch's four postulates which associates a particular organism with a certain disease (Kreager et al. 1990), although mechanical transmission of certain pathogens is possible (Feldlaufer et al. 2010, Jupp et al. 1983, Jupp and Lyons 1987, Silverman et al. 2001). Lowe and Romney (2011) reported finding methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE) from bed bugs in Vancouver, BC, Canada.

Bed Bug Control Complications

Bed bug infestations are increasing worldwide (Paul and Bates 2000, Krueger 2000, Hwang et al. 2005) because of international travel, international migration, altered pest management practices, and increased resistance of bed bugs to certain pesticides (Romero, 2007). Bed bug cryptic behavior requires alternative control strategies. The limited number of safe, effective, and economically practical bed bug control tactics lead to control complications. Recently, researchers and pest management professionals have been concerned with bed bug resistance against pyrethroids (Busvine 1959, Romero 2007). Insect resistance to contact and residual treatments have resulted in multiple and overdosed treatments that caused human health problems and environmental concerns (Hayes and Laws 1991, White and Leesch 1995, MMWR 2011). Many countries have restricted the use of chlorinated hydrocarbon, organophosphate, and carbamate insecticides, resulting in fewer chemical control options. These problems emphasize the need for the development of selective control alternatives against bed bugs.

Lack of public awareness plays a significant role in bed bugs being a difficult target to control; however, the possibility of obtaining and transporting bed bugs can be reduced with increased public awareness. Bed bug infestations usually persist months before becoming noticed or reported (Cooper 2006; Wang 2010). It is often not possible to eradicate the population using a stand-alone chemical treatment after a bed bug colony has been established.

A result of the difficulty in controlling bed bugs is the high cost of treatment (Miller 2009). Bed bugs impact humans economically due to financial loss associated with control costs, legal cases, and other expenses encountered due to repeated attempts of controlling the pest (Doggett 2004, Gangloff-Kaufmann 2006). Results from a 2011 summer survey conducted by the University of Kentucky and the National Pest Management Association with approximately 415 pest control companies found that 80% of respondents indicated the need for multiple treatments to control bed bug infestations (Potter et al. 2011). Long term management costs can be reduced by detecting initial bed bug infestations as soon as possible, and verifying the complete elimination of the infestation (Cooper 2006).

Volatile Insecticides

Recently published research has been conducted on the lethal effects of volatile chemicals against many insects including: pest cockroaches, mosquitoes, house flies, and fruit flies (Ngoh et al. 1998, Scharf et al. 2006, Chaskopoulou et al. 2009). High vapor pressure, low boiling point, and low molecular weight allow many insecticidal active ingredients to volatilize . Unlike other formulations such as liquid sprays and dusts, volatile insecticides possess fumigant-like qualities that penetrate inaccessible areas and leave the bed bug without the option of avoiding residual pesticides. Volatile insecticides are usually detrimental to all insect life stages (Chaskopoulou et al. 2009, Lehnert et al. 2011, Stanbridge 2011). Different volatile compounds have different modes of action, and can attract, repel, or kill insects (Ngoh et al. 1998). Gas

exchange, energy conservation, temperature, oxygen, and carbon dioxide contents are important factors affecting volatile insecticide effectiveness (Bond et al. 1967, Calderon and Leesch1983, Pereira et al. 2009, Lehnert et al. 2011, Stanbridge 2011).

There is extensive research on using volatile insecticide treatments against important pests such as flies, lice, cockroaches, mosquitoes, and stored food product pests (Chaskopoulou 2007, Chaskopoulou et al. 2009, Scharf et al. 2006, Nguyen et al. 2007, Philips et al. 2010, Bagavan et al. 2011), but quite limited against bed bugs. Safe volatile treatment methods have low human toxicity, residue, and persistence in the environment (Meikle and Stewart 1962, Chaskopoulou et al. 2009). Widely used fumigants such as methyl bromide and phosphine are either being phased out or restricted due to insect resistance, mammalian toxicity, or persistence in the environment. This leads to interest in alternative pest control options that entail cost effective concentrations and effective application systems (Bagavan et al. 2011).

Fumigants

A fumigant is a chemical that can exist in the gaseous state in sufficient concentration to be lethal to a given pest organism (Bond 1984). Historically, fumigants such as from burning sulfur and cyanide were effective against bed bugs despite the insect's cryptic behavior. However, these extremely toxic chemicals were eventually replaced by DDT in 1945 (Potter 2011). Phosphine, sulfuryl fluoride, and methyl bromide are currently the only fumigants registered for use in the United States, each having unique modes of action and molecular properties. Fumigant action and toxicity are effected by the chemical and physical properties of the gas, length of insect treatment, gas containment inside treatment area, temperature, and the life stage of pest. (Stanbridge 2011). Gases leaking into non-target areas, evacuation of structures, and evacuation of adjacent structures to the site being fumigated are all potential disadvantages to fumigation treatments.

Fumigation is the fastest method to completely eradicate established bed bug populations because vapors can reach areas that more conventional insecticide treatment such as residuals and dusts cannot access. Fumigants are toxic to all life stages of insects and can result in 100% control after a single treatment. Fumigation treatments can be more cost effective than other treatment options. Fumigants are often chosen in scenarios where the pest threshold for a product or sensitive living environment requires zero pest tolerance. The main disadvantage to fumigation treatments is potential leakage into non-target areas, resulting in regulatory agency mandated evacuation of structures and adjacent sites. Fumigation as a stand-alone treatment does not provide residual killing activity to prevent re-infestation. Labor, damage, and liability costs are encountered more often in fumigations than other conventional control methods (Miller 2009, Stanbridge 2011).

Comparison of Volatile Insecticides and Fumigants

Use of both fumigation and volatile insecticide treatments requires personal protective equipment and technician training. Volatile and fumigation treatment concerns include: aeration procedures, insecticide exposure limits to workers, and re-entry thresholds Liability issues for the pest control company may become an important factor to consider.

Volatile compounds do not penetrate through materials as readily as fumigants such as methyl bromide and sulfuryl fluoride (Scheffrahn 1993); therefore, fumigation tents and licenses are not required when using volatile insecticides. Treatment methods that use volatile insecticides usually are less labor intensive than control with fumigants, and may be relatively inexpensive because the extensive preparation involved in tenting is avoided.

Intense labor, overhead costs and required liability coverage result in many pest control companies to subcontract their fumigation work to others that specialize in tent fumigation. State and environmental evacuation regulations differ between volatile and fumigation treatments

(Hottel et al. 2011). Unlike fumigants, some volatile natural insecticidal compounds have low toxicity to humans and animals; thus, some products can be exempt of EPA regulations (Isman 2006).

Factors That Influence Insecticide Vapor Toxicity

Insects are exposed to volatile toxins through respiration. Theoretically, increased respiration will expose insects to more toxins. Many insects are not as active at lower temperatures because of decreased respiration and metabolism (Bond 1975). The respiratory rate and the amount of treated air taken in by insects determine the insect susceptibility following insecticide vapor exposure (Cotton 1932, Sun 1947); however, other investigations determined variations in toxic effects which were dependent upon the fumigant being tested (Lindgren 1935).

Multiple aspects of insecticides must be considered in making recommendations to optimize their use. Many studies have determined temperature to affect the lethal activity of various insecticides (Wadleigh et al. 1991, Scott 1995, Satpute et al. 2007, Boina 2009). Temperature effects on insecticide efficacy is generally similar within a given insecticide class (Boina 2009). Many studies have determined the toxicity of organophosphate insecticides to insects be positively correlated with temperature (Scott 1995, Satpute et al. 2007), whereas investigations of insect toxicity of pyrethroid insecticides determined a negative temperature/toxicity correlation (Scott 1995, Musser and Shelton 2005, Satpute et al. 2007). However, some studies have determined toxicity/temperature correlation to vary within the same insecticide class (Scott 1995). The insect species, the temperature range studied, the insecticide mode of action, the application procedure, and the amount of insecticide that has been contacted or been taken up by the target organism can affect the relationship between temperature and toxicity (Sparks et al. 1982, Toth and Sparks 1990).

Essential Oils

Essential oils were used as insecticides in their natural state within plant tissues until water distillation methods were discovered (Franzios et al. 1997). Higher plants naturally produce as a means of defense and protection against insect predators and micro-organisms. Essential oils are mixtures of many plant components that are liquid at room temperature when distilled (Enan 2001). Essential oils are used regularly in the food and beverage industry as flavoring ingredients, odorants in fragrances, and insect control (ICPS 2008). Essential oils and their constituents have been evaluated as alternative methods of insect control due to their potentially low toxicity, abundant source of bioactive chemicals, and minimal environmental impact.

Essential oils can contain more than sixty individual components (Senatore 1996) including monoterpenes, which represeent the majority of components in essential oils. Monoterpenes dissolve in fats and oils, and easily pass through the insect's cuticle upon contact (Tisserand and Balacs 1995). The terpenoid structure influences insect toxicity of essential oil constituents and the insecticidal activity is species-dependent (Isman 1999). Monoterpenoids have been widely researched due to their insecticidal, repellent and/or anti-feedant properties (Tunc et al., 2000, Papachristos and Stamopoulos, 2002)

Primary Research Objectives

The primary objectives of the present research were to (1) Determine the effects of three temperatures (17, 28, and 36°C) on the toxicity of the organophosphate dichlorvos, the essential oil secondary components trans-anethole, eucalyptol, linalool, and anise seed oil to bed bugs; (2) Optimize an application system to rapidly release dichlorvos vapors from resin strips; (3) Evaluate the efficacy of localized heating of dichlorvos impregnated resin strips.

DDVP

Mattson et al. (1955) claimed that an organic phosphorus compound of remarkable insecticidal powers had been discovered after testing insecticide vapor toxicities to flies. A particular organic phosphorous compound caused high fly mortality initially; however, fly survival increased as air was pushed over the 0,0-dimethyl 2,2,2-trichloro-l-hydroxyethyl phosphonate (I) treated material. They determined that the compound being tested had an extremely lethal impurity, and the organophosphorous 2, 2-dilchlorovinyl dimethyl phosphate (dichlorvos) was isolated from the organic pesticide trichlorfon (Mattson et al. 1955).

Dichlorvos used in pest control is commonly referred to as DDVP, an abbreviation for its chemical name 2, 2-dichlorovinyl dimethyl phosphate (Brooks & Schoof, 1964). Technical grade dichlorvos is a dense, colorless, synthetic organic chemical that has a sweetish odor, is slightly soluble in water, and readily evaporates into the air. Dichlorvos was registered for insecticidal use in the United States under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) in 1948 (EPA 1987b). Dichlorvos is manufactured commercially by a chloral and trimethyl phosphite chemical reaction and by heating trichlorfon, which quickly converts to dichlorvos above a pH of 6 (CremLyn 1978, ATSDR 1997, Sittig 1980).

Large scale production of dichlorvos began in the late 1950s (IARC 1979). In the United States, estimates for the amount of dichlorvos manufactured during 1971, 1974, and 1981 were over 1.8 million kg annually (WHO 1989). The United States produced an estimated 907,000 kg of dichlorvos in 1985 (EPA 1988).

Dichlorvos usage in 1989 of about 408,000 kg was less than previous years, which led to a decrease in production. Dichlorvos production continued to decrease throughout the 1990s due to changes in use patterns, restrictions, and cancellations of registrations (WHO 1989; EPA 1993). The EPA (1991) determined that dichlorvos had the potential to cause cancer, leading to the

recommendation to cancel most dichlorvos use in 1995. Dichlorvos used as a pesticide was banned in homes for the first time in 2006, except in specialized areas (EPA 2006). Currently, dichlorvos is classified as a probable human carcinogen based on effects that have been observed in rats and mice (EPA 1993, 1994).

In the past, dichlorvos products have been formulated as dusts, granules, emulsifiable concentrates, wettable powders, flea collars, baits, and impregnated resin strips (WHO 1989, EPA 1990, Agrochemicals Handbook 1991). Current dichlorvos formulations include aerosols, emulsifiable concentrations, and impregnated resin strips (EPA 2006). AMVAC Chemical Corporation in Los Angeles, CA is the only current dichlorvos manufacturer in the United States. Dichlorvos has been sold under many trade names including Vapona®, Atgard®, Nuvan®, and Task® (USEPA 2006). Dichlorvos has been studied mostly for use against flies, mosquitoes (Brooks & Schoof, 1964, Matthysee 1973; Chaskopoulou 2009), and general household pests (Wright 1971). Nuvan Prostrips® contain 18.6% of the active ingredient dichlorvos and is currently registered for household pest control use including cockroaches, silverfish, and bed bugs. Recently, DDVP formulated resin strips have been combined with heat to produce more efficient insect control (Lehnert et al. 2011.) Studies on the toxic effects of dichlorvos to bed bugs provide a standard of comparison for other volatile compounds which may have lower mammalian toxicity.

Significance of Research

There is no published research on volatile compounds with low mammalian toxicity used against bed bugs. Volatile insecticides such as dichlorvos have shown positive results and acute toxicity against many insect pests (Ngoh et al. 1998, Scharf et al. 2006, Chaskopoulou et al. 2009). Investigation of insecticide substitutes for highly toxic pesticides could result in decreased human exposure to toxins without adverse environmental effects. Bed bug resistance to

insecticides has resulted in multiple and overdosed control treatments (Hayes and Laws 1991; White and Leesch 1995, MMWR 2011). There have been limited efficacy studies using treatments other than contact and residual insecticides. Temperature variation can increase insecticide toxicity; thus, resulting in reduced active ingredient concentrations. This research provides information directly pertaining to volatile pesticides that are both registered and un-registered for pest control use.

CHAPTER 2
EFFECT OF TEMPERATURE ON TOXICITY OF VOLATILE COMPOUNDS TO BED BUGS, *CIMEX LECTULARIUS* L.

Introduction

Bed bug, *Cimex lectularius* L. (Heteroptera: Cimicidae) infestations are increasing worldwide (Paul and Bates 2000, Krueger 2000, Doggett et al. 2004, Hwang et al. 2005) causing human discomfort and economic loss (Doggett et al. 2004, Goddard 2009). Human suffering from severe bed bug infestations can result in health consequences including death from excessive insecticide exposure (Hayes and Laws 1991, White and Leesch 1995, CDC 2007). Thus, there is a great need for development of selective control alternatives against bed bugs. Because bed bugs can hide in very small cracks and crevices, volatile compounds may be able to reach and kill insects that could otherwise avoid contact with other formulations such as liquid sprays and dusts.

Dichlorvos, also known as DDVP, is a cholinesterase-inhibiting organophosphate that targets the nervous system. The chemical structure, molecular weight, and boiling point result in dichlorvos being readily volatilized (WHO 1989). Dichlorvos is classified as a potential human carcinogen (ASTDR 1997, WHO 1989), and its household use is restricted to unoccupied areas. As a result, less toxic volatile pesticides as bed bug control alternatives should be investigated.

Increased public concern about human health and environmental effects caused by traditional fumigants such as phosphine, sulfuryl fluoride, and methyl bromide has encouraged the investigation of natural volatile compounds that have insecticidal properties (Isman 1999). Essential oils used for insect control are possible alternatives to traditional insecticides because of their low toxicity to humans and animals, as well as their low persistence in the environment. Essential oils are secondary plant substances that contain many volatile, low-molecular-weight terpenes and phenolics. Essential oils and their constituents exert insecticidal effects or reduce

and disrupt insect growth at several life stages (Regnault-Roger et al. 2012). They have been used in the past and are still used as fragrances for perfumes and flavorings for food items (Isman 2006).

In the United States, all pesticides must be registered by the EPA under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). However, minimum risk pesticides, which include several essential oils, are exempt from the registration requirements (EPA PR Notice 2000-6) as long as conditions are met. Insecticides that are exempt from registration can be placed on the market faster than traditional insecticides (Isman 2000). Further, the constituents of plant volatile oils have long been known to affect the behavioral responses of pests; their monoterpenoid components appear to be the most useful as insecticides or anti-feedants (Veal, 1996).

Multiple aspects of insecticides must be considered while making recommendations to optimize their use. Many studies have determined that temperature affected the lethal activity of various insecticides (Wadleigh et al. 1991, Scott 1995, Satpute et al. 2007). Temperature effects on insecticide efficacy to insects are generally similar within a given insecticidal class (Scott 1995). Studies have determined the toxicity of organophosphate insecticides to insects to be positively correlated with temperature, and the toxicity of type II pyrethroid insecticides to insects to be negatively correlated with temperature. However, some studies have determined temperature-toxicity correlation to vary within the same insecticide class (Scott 1995, Musser and Shelton 2005, Satpute et al. 2007). Factors other than insecticide class have been found to affect the temperature-toxicity relationship of insecticides including: insect species, temperature range, insecticide mode of action, application procedure, and amount of insecticidal exposure (Sparks et al. 1982, Toth and Sparks 1990).

Although much literature is available on the lethal effects of insecticides to bed bugs, recent studies on the effects of naturally occurring volatile compounds against bed bugs have not been published. Further, most studies that assessed insecticide toxicity to bed bugs did not consider environmental factors such as temperature variation. Therefore, the objective of this study was to determine the *in vitro* effects of temperature on the toxicity and insecticidal efficacy of the organophosphate dichlorvos, the essential oil anise seed, and secondary essential oil compounds (trans-anethole, linalool, and eucalyptol) to bed bugs.

Materials and Method

Insects

Bed bugs (*Cimex lectularius* L.) obtained from an insecticide susceptible laboratory-reared strain (Fort Dix Strain originally from Dr. Harold Harlan, Armed Forces Pest Management Board, Silver Springs, MD) were used in the experiment. Bed bugs were maintained in the laboratory at 26 ± 2°C, and 55 ± 5% RH. Bed bugs were given access to blood once or twice each week on live domesticated chickens, *Gallus gallus domesticus* (Fig. 2-1). Bed bug colony maintenance on live chickens was done according to UF/IACUC approval E876. Bioassays were performed 5-14 days post-adult emergence. The newly eclosed adults used in this study were fed to repletion 2-4 days before exposure to insecticides.

Chemicals

Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate, >99.7%) was obtained from American Vanguard Corporation (AMVAC), Los Angeles, CA) in liquid form. A concentrated 0.1% (v/v) dichlorvos stock solution was first prepared in analytical grade acetone and subsequently used to prepare the following solution (v/v) concentrations: 0.05%, 0.01%, 0.005%, and 0.001%. When considering the densities of the dichlorvos and acetone, the above concentrations correspond to concentrations of 1242, 621, 124, 62.1, and 12.42 ug dichlorvos/ mL of solution. Essential oil

components (trans-anethole, linalool, eucalyptol), and the essential oil anise seed were obtained from Sigma-Aldrich (St. Louis, MO). These concentrates (>97% purity) were serially diluted with analytical grade acetone to appropriate concentrations.

Each dilution was vortexed (Vortex Genie 2, Scientific Industries Inc., Bohemia, NY) for 10 s to completely mix. Controls received a volume of acetone identical with the highest insecticide volume that was tested. Between uses, bioassay jars were washed in a dish washer and baked ~12 h at 90° C in a drying oven to eliminate any chemical residue.

Bioassay

Groups of 5-10 insects were exposed to vapors from insecticide solutions and acetone controls in 125 mL wide-mouth septa-jars with PTFE Teflon septa screw caps (Thermo Fisher-Scientific, Rockwood, TN). PTFE Teflon screw caps were chosen to prevent the volatile compounds from being absorbed into the septa. For the dichlorvos tests, a thin layer of gypsum plaster (Dap Products Inc., Baltimore, MD) was applied with a paintbrush on the jar's glass bottom to provide a substrate for bed bugs to rest at least two days prior to the tests. A circular piece of filter paper was placed in the jar's bottom to provide a substrate for the bed bugs to rest during the essential oil volatile tests. Bed bugs were placed in jars 2 - 4 hours before treatment to acclimatize. An insecticide solution or acetone aliquot of 2 µl was injected through the septa directly onto inner bottle wall ~ 4 cm from the bottom of Teflon septa using a 50-µl gas-tight syringe (model 1700, 22s GA, SN, NO. 2., Hamilton Company, Reno, NV) that was attached to a PB600 (Hamilton Company, Reno, NV) repeating dispenser (Fig. 2-2).

Air concentrations (µg [AI]/ mL air) were calculated given the jar volume of 125 mL and the solution injected. Following the insecticide treatment, the jars with bed bugs were maintained for 24 h at 17, 28, and 36°C. The temperature-controlled environments were monitored and the temperatures were recorded by electronic HOBO data loggers (Onset Computer Corp., Bourne,

MA). The jars were removed after 24 h, and the bed bugs were transferred to a clean Petri dish that was lined with untreated filter paper. Bed bugs that did not move after being touched with a wooden applicator were counted as dead, and those that moved but could not flip over while on their backs were counted as moribund. Seven replicates of the dichlorvos, trans-anethole, and linalool treatments were prepared. Four replicates of the eucalyptol treatments and three replicates of the anise seed oil treatments were prepared.

Data Analysis

These experiments were analyzed using a two-way analysis of variance (ANOVA) to test for effects of temperature and insecticide concentration on bed bug mortality. Percent mortality data was arcsine-transformed to meet the assumptions of normality. When significant effects were obtained in ANOVA, differences between treatment means were compared using Fisher's protected least significant differences (LSD) test (≤ 0.05; JMP Student Edition, Version 9.0 (SAS Institute, INC., Cary, NC).

Results

Bed bug mortality caused by dichlorvos vapor was significantly affected by concentration ($df = 5$, $F = 39.55$, $p < 0.0001$; Fig. 2-3), temperature ($df = 2$, $F = 26.17$, $p < 0.0001$; Fig. 2-4), and their interaction ($df = 10$, $F = 4.46$, $p < 0.0001$; Fig. 2-5). There was no acetone control mortality. Significant mortality (60%) was observed with 0.001 µg dichlorvos/ mL air at 17°C (Fig. 2-5). At higher temperatures (28 and 36°C), there was significantly lower bed bug mortality (Fig. 2-4) than the lowest temperature (17°C). Mortality decreased as temperature increased at every tested concentration (Fig. 2-5).

Bed bug mortality caused by anise seed oil vapor was also significantly affected by concentration ($df = 4$, $F = 21.41$, $p < 0.0001$), temperature ($df = 2$, $F = 24.73$, $p < 0.0001$), and their interaction ($df = 8$, $F = 3.70$, $p = 0.0041$). There was no acetone control mortality.

Significant mortality was observed with 3.9 µg anise seed oil/ mL air (Fig. 2-6). All anise seed oil concentrations that were tested at the highest temperature (36°C) resulted in 100% bed bug mortality. Greater bed bug mortality was observed at the highest temperature (36°C) than the lowest temperature (17°C) with similar anise seed oil concentrations (Fig. 2-7). Mortality increased as temperature increased at every anise seed oil concentration except the lowest (3.9 µg anise seed oil/ mL air), in which greater bed bug mortality was observed at 17°C than 28°C (Fig. 2-8).

Bed bug mortality caused by trans-anethole vapor was significantly affected by concentration ($df = 5$, $F = 64.24$, $p < 0.0001$; Fig. 2-9), but not by temperature ($df = 2$, $F = 0.87$, $p = 0.4215$). Significant concentration x temperature interaction effects ($df = 10$, $F = 2.09$, $p = 0.0317$) were observed (Fig. 2-10). The acetone control resulted in < 15% bed bug mortality. Significant mortality was observed at 1.6µg trans-anethole/ mL air and increased to 100% at 4.0µg trans-anethole/ mL air (Fig. 2-9).

Only linalool concentration significantly affected bed bug mortality ($df = 5$, $F = 4.53$, $P = 0.0016$). Temperature ($df = 2$, $F = 1.28$, $p = 0.2863$), and temperature x concentration interaction ($df = 10$, $F = 1.28$, $p = 0.2863$) did not significantly affect bed bug mortality. Acetone control mortality was < 5% of the total bed bugs tested at all temperatures. Mortality significantly different from control mortality was only observed at 0.1 and 11.7 µg linalool/mL air (Fig. 2-11).

Bed bug mortality caused by eucalyptol was significantly affected by concentration ($df = 5$, $F = 10.62$, $p < 0.0001$; Fig. 2-12), but not by temperature ($df = 2$, $F = 2.45$, $p = 0.0957$). Significant concentration x temperature interaction effects ($df = 10$, $F = 3.51$, $p = 0.0013$) were observed (Fig. 2-13). No acetone control mortality was observed at 17 and 36°C; however,

acetone control mortality was observed (<15%) at 28°C. Mortality significantly different from control mortality was only observed at 0.1 and 7.4µg eucalyptol/mL air (Fig. 2-12).

Discussion

Total eradication of all bed bug life stages is critical, and residual insecticides are currently the main option for bed bug control. Numerous studies have examined the toxic effects of insecticide interaction with temperature, but did not explore the lethal effects of exposing bed bugs to dichlorvos vapors or the insecticidal action of essential oil volatile compounds to bed bugs held at different temperatures. This series of bioassays demonstrated that temperature may affect the lethal action of insecticides used in practical bed bug control treatments.

In general, all experimental compounds showed toxicity to bed bugs; however, toxicity levels varied amongst the volatile compounds. The organophosphate insecticide dichlorvos exhibited a consistent negative temperature-dependent toxicity correlation. This was the first report that demonstrated a negative temperature-toxicity relationship of an organophosphate and differed from results of previous findings with other insect species that showed positive temperature-toxicity correlations with dichlorvos and other organophosphates (Lindgren 1935, Sun 1943, Eesa 1989). The toxicity of dichlorvos decreased substantially with increasing temperature, suggesting that this compound is less efficacious against bed bugs when insects are exposed to dichlorvos vapor at higher temperatures.

Increased dichlorvos vapor toxicity to bed bugs at lower temperatures could be the result of a negative feedback system. Increased temperature may trigger a more efficient bed bug physiological defensive response that degrades toxic materials. Low molecular weight compounds volatilize more quickly at higher temperatures, resulting in bed bugs being exposed to higher insecticide concentrations for shorter periods of time than at lower temperatures, and ultimately recovering.

Optimization of application is needed to provide optimal conditions for volatilization of dichlorvos without diminishing the effectiveness of the active ingredient against bed bugs. In exposure to vapors, the main access to the organism is airborne, and the volatile substance enters through the spiracles as part of the respiratory process (Mill 1985). The substances are transported to different tissues through the network of tracheas and tracheoles before reaching their site of action. The toxic effect of a substance depends on different toxicokinetic steps, but also on its physicochemical properties. Variability in bed bug mortality response to volatile compounds at different temperatures could be the result of the biological mechanisms involved in the metabolism of toxic substances.

From the anise seed oil (~95% anethole) and trans-anethole results, it is clear that the adults of *C. lectularius* are susceptible to the composition of this essential oil. Anise seed oil activity can be attributed, to a considerable degree, to the presence of anethole as the main component. However, in this case, the toxicity of the volatile compounds depends greatly on its volatility rate. At the same concentration (4.0µg/ mL air), trans-anethole killed 100% of the exposed bed bugs at 17, 28, and 36°C, while the average mortality of all three treatment temperatures resulted in the anise seed oil test caused 50% mortality. However, anise seed oil caused significantly higher bed bug mortality at 36°C than 17 and 28°C, while the effects of temperature did not significantly affect mortality of bed bugs exposed to trans-anethole. Observations during the anise seed oil treatment preparations and injections indicated that the viscosity of the oil was much higher than the other tested compounds. The 2 uL injections of anise seed oil solution onto the bioassay jar's inner wall persisted much longer than the other test compounds, suggesting that the high temperature allowed the anise seed oil to eventually volatilize, while the lower temperatures limited volatilization.

Some synergistic effect was observed between the solvent (acetone) and the alcohol linalool resulting in greater bed bug mortality with low linalool concentration than intermediate concentrations. At higher linalool concentrations, bed mortality may be caused by linalool alone, while mortality at lower concentrations may result from a synergistic effect between linalool and acetone. The potential synergistic effects of solvents were observed previously (Sims and Appel 2007). Eucalyptol may not be a future bed bug control option.

There are no studies regarding the vapor toxicity of essential oils or their components to bed bugs. Investigation of the mode of action of natural insecticidal products and insecticides is of practical importance for bed bug control because it may elucidate the most appropriate formulation, method of delivery, and resistance management. Quantifying the effect of temperature on the toxicity of insecticides against a target pest is essential in making informed selections of insecticides based on natural and manipulated environmental conditions. Insect control treatments using volatile compounds require home temperature control systems disabled so that the insecticide is contained in the structure only, resulting in the treatment area to approximate outdoor temperature in the absence of temperature control.

Based on our findings, dichlorvos should be most effective in controlling bed bugs when the outdoor temperature is lower (17°-28°C) than when it is higher (~36°C), as opposed to anise seed oil, which is presumably more effective at higher temperatures. Seasonal temperature fluctuations may impact the action of insecticides used in practical bed bug control treatments, varying the results of eradication efforts. Further, temperature modifications during bed bug treatments may alter the susceptibility of the insects to the insecticide.

Dimensions of the room being treated should also be taken into consideration. A larger chamber, such as a bedroom, presents challenges that are not encountered in a laboratory setting.

Further, the quality of the seal, the application system and insecticide formulation, the method of chemical distribution, temperature variations, and air circulation may play a crucial role in the effectiveness of volatile compounds against bed bugs in practical applications.

Future studies may elucidate the biochemical mechanisms driving the increased survivorship of bed bugs that are exposed to dichlorvos at higher temperatures and potential variations in detoxification of insecticides due to temperature differences. Future research should also explore the enhancement of potency of essential oils/components by adding synergists and stabilizers. The principal concern of novel chemical development is the safety to humans and the environment. Although biopesticides are less harmful than traditional fumigants in terms of general pesticide toxicities, it is important that essential oils, like other pesticides in the market, meet safety requirements for users, consumers, and the environment. However, the low cost, availability, and environmentally friendly status aid in both registration and public acceptance.

Figure 2-1. Container of bed bugs placed underneath the wing of live chicken to allow insects to blood feed. Photo courtesy of author.

Figure 2-2. Insecticide solution and acetone controls injected with syringe through the septa directly onto inner bottle wall. Photo courtesy of author.

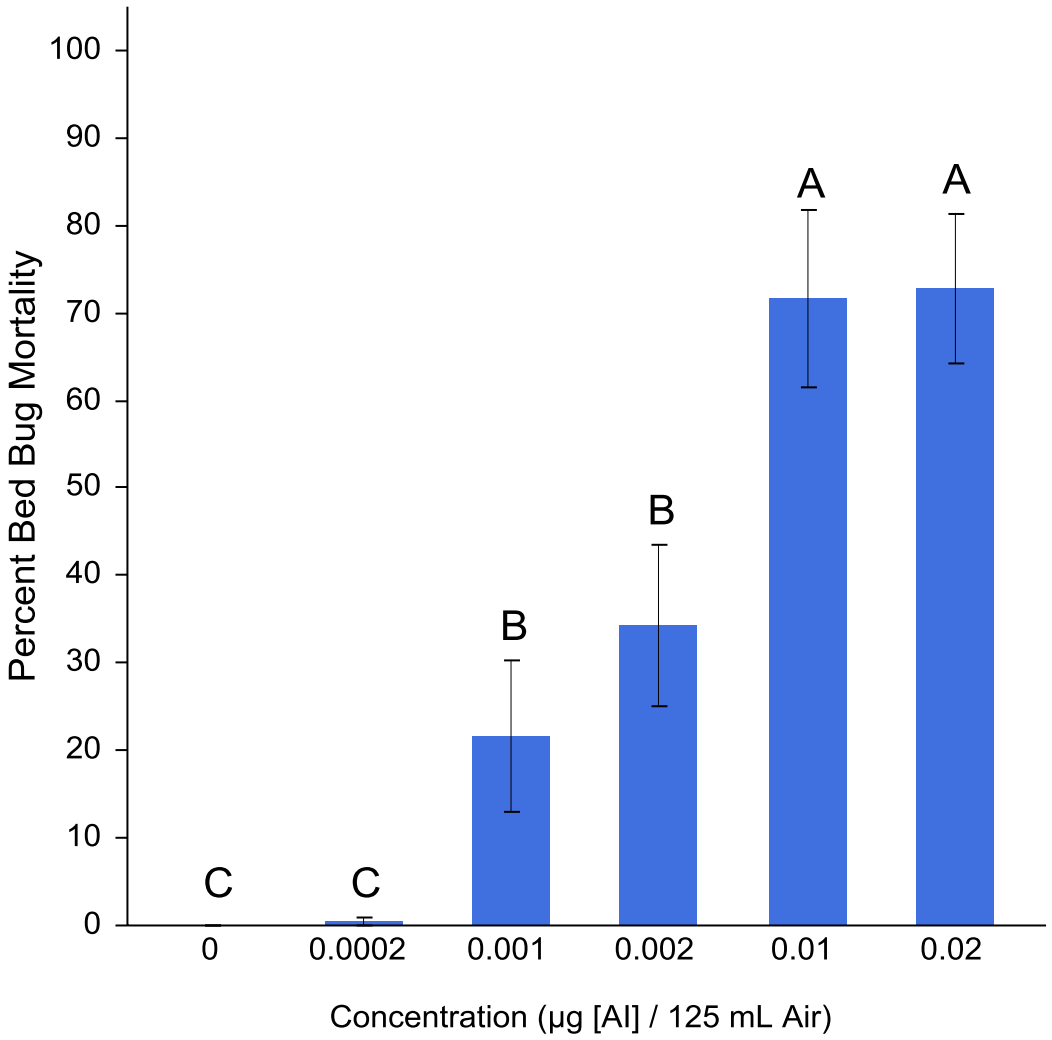

Figure 2-3. Effect of concentration on the toxicity to bed bugs exposed to dichlorvos. Mean ± SE percent bed bug mortality after 24 h exposure to dichlorvos concentrations. Levels not connected by the same letter are significantly different (analysis of variance followed by Student's t-test, $P > 0.05$).

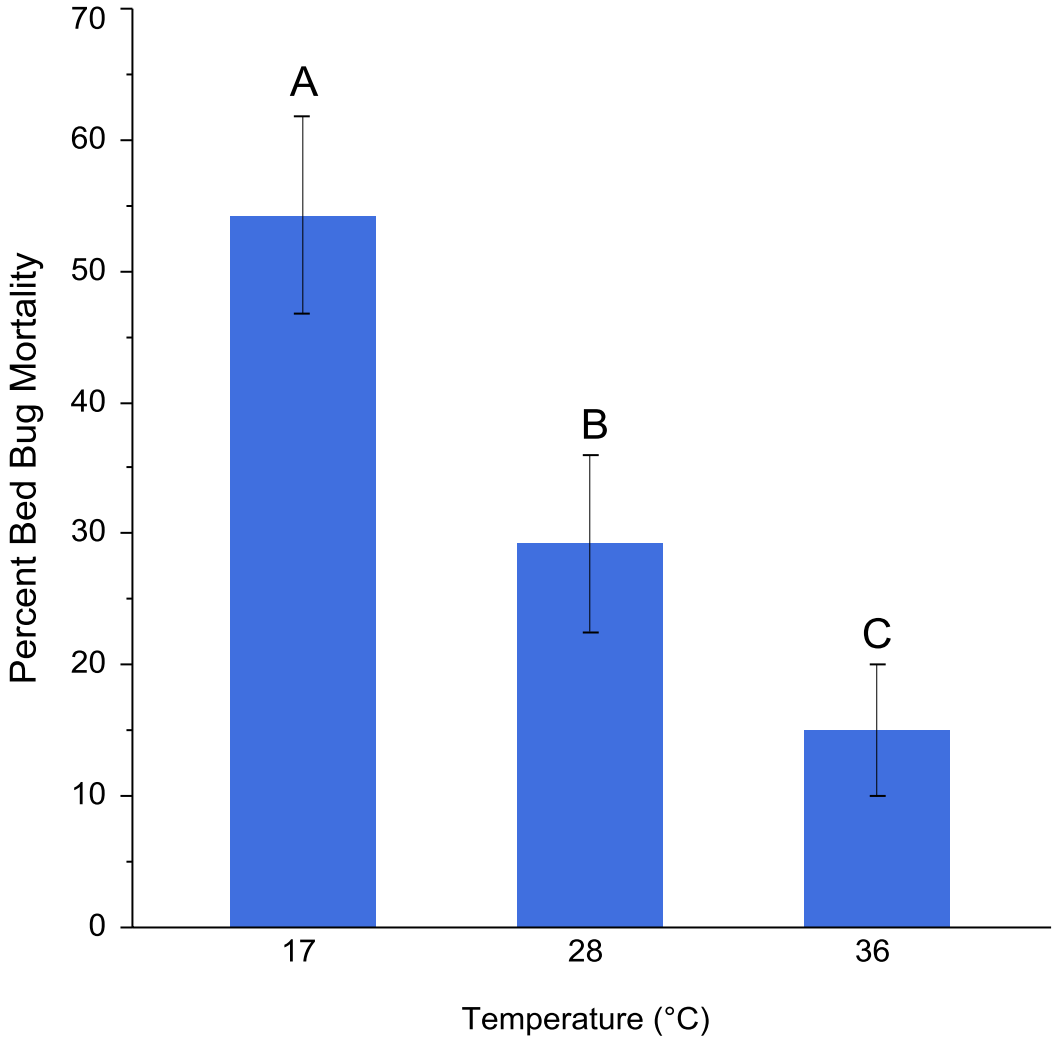

Figure 2-4. Effect of temperature on the toxicity to bed bugs exposed to dichlorvos. Mean ± SE percent bed bug mortality after 24 h exposure to dichlorvos concentrations at 17, 28, and 36°C. Levels not connected by the same letter are significantly different (analysis of variance followed by Student's t-test, $P > 0.05$).

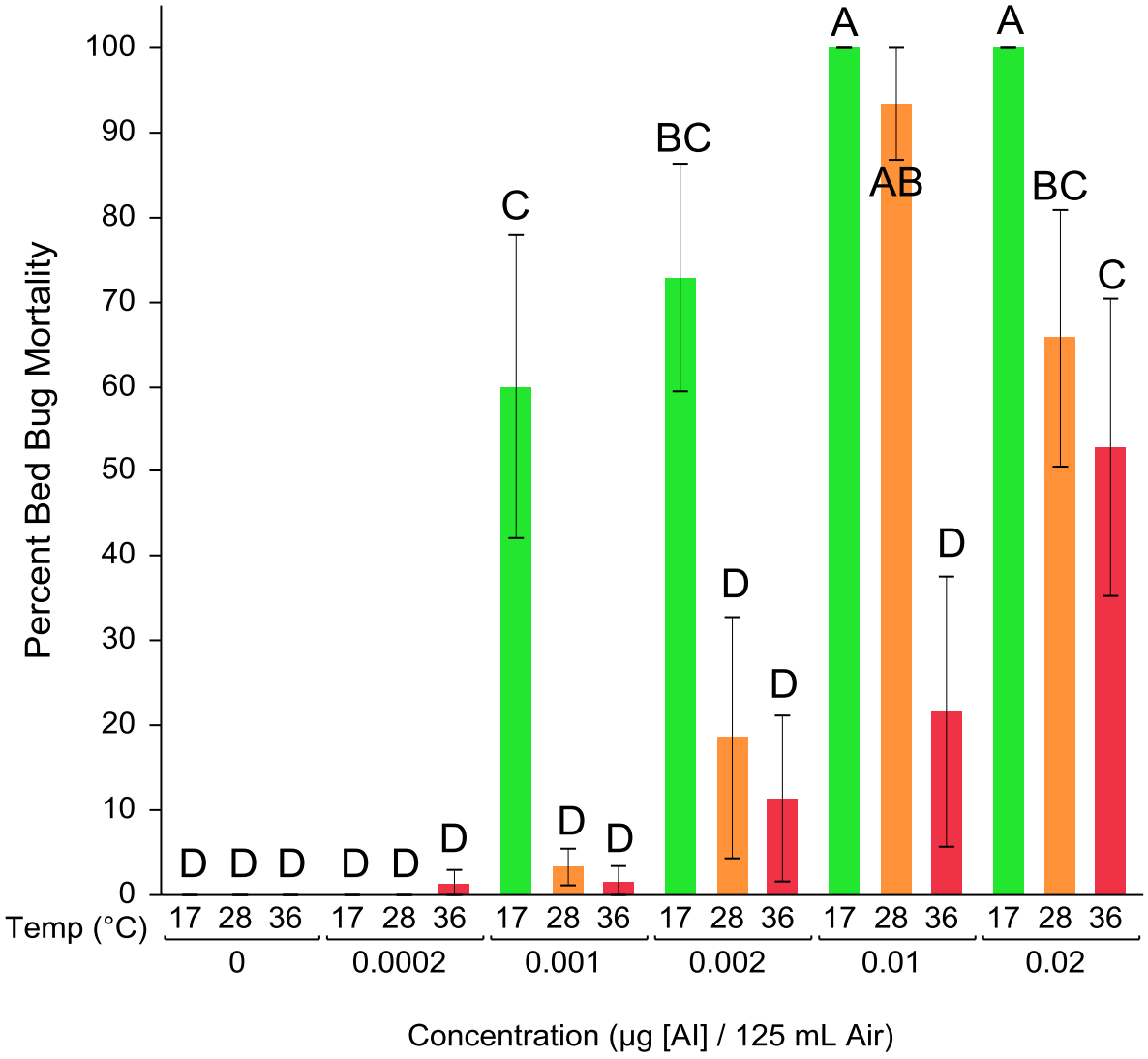

Figure 2-5. Effect of the interaction of temperature and concentration on the toxicity to bed bugs exposed to dichlorvos. Mean ± SE percent bed bug mortality after 24 h exposure to dichlorvos concentrations at 17, 28, and 36°C. Levels not connected by the same letter are significantly different (analysis of variance followed by Student's t-test, $P > 0.05$).

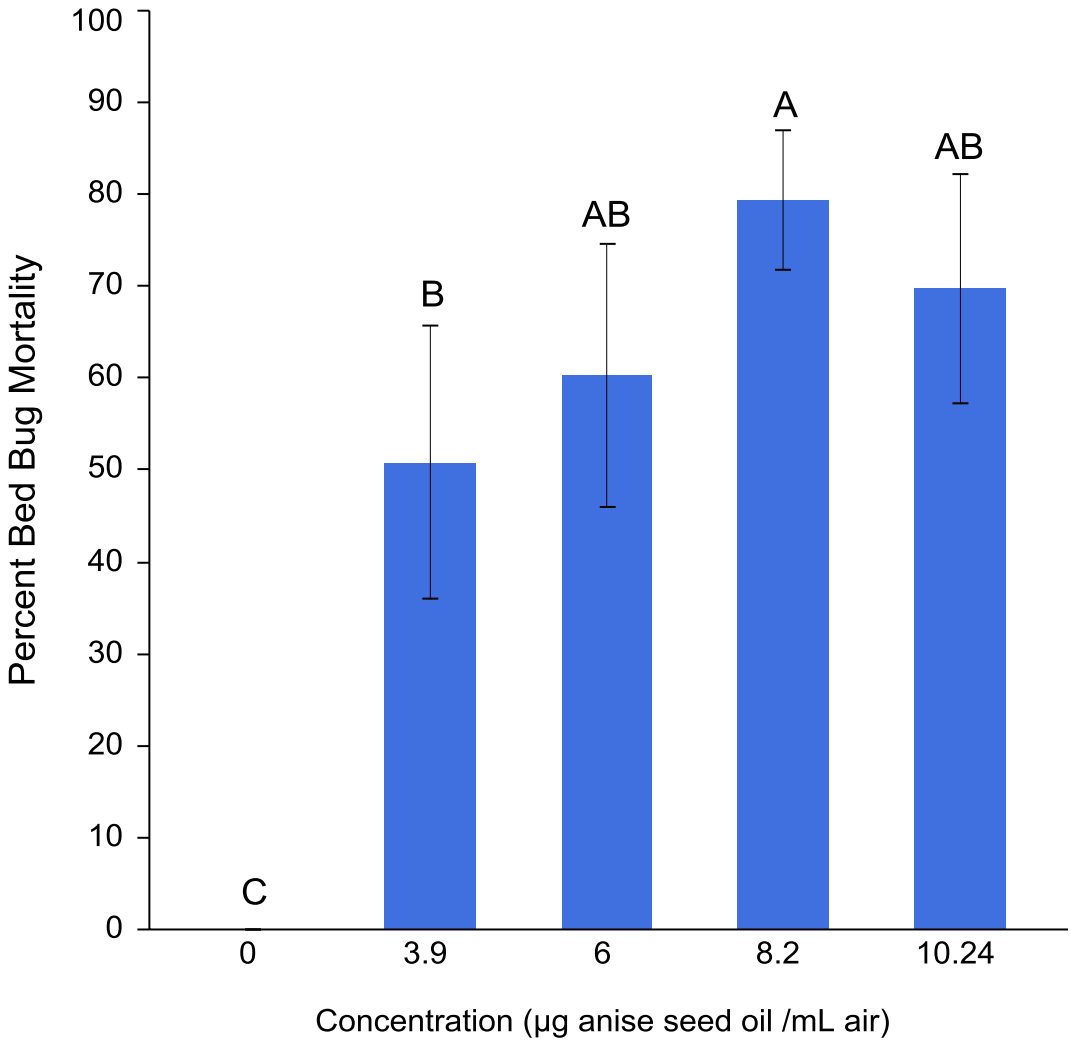

Figure 2-6. Effect of concentration on the toxicity to bed bugs exposed to anise seed oil. Mean ± SE percent bed bug mortality after 24 h exposure to anise seed oil concentrations. Levels not connected by the same letter are significantly different (analysis of variance followed by Student's t-test, $P > 0.05$).

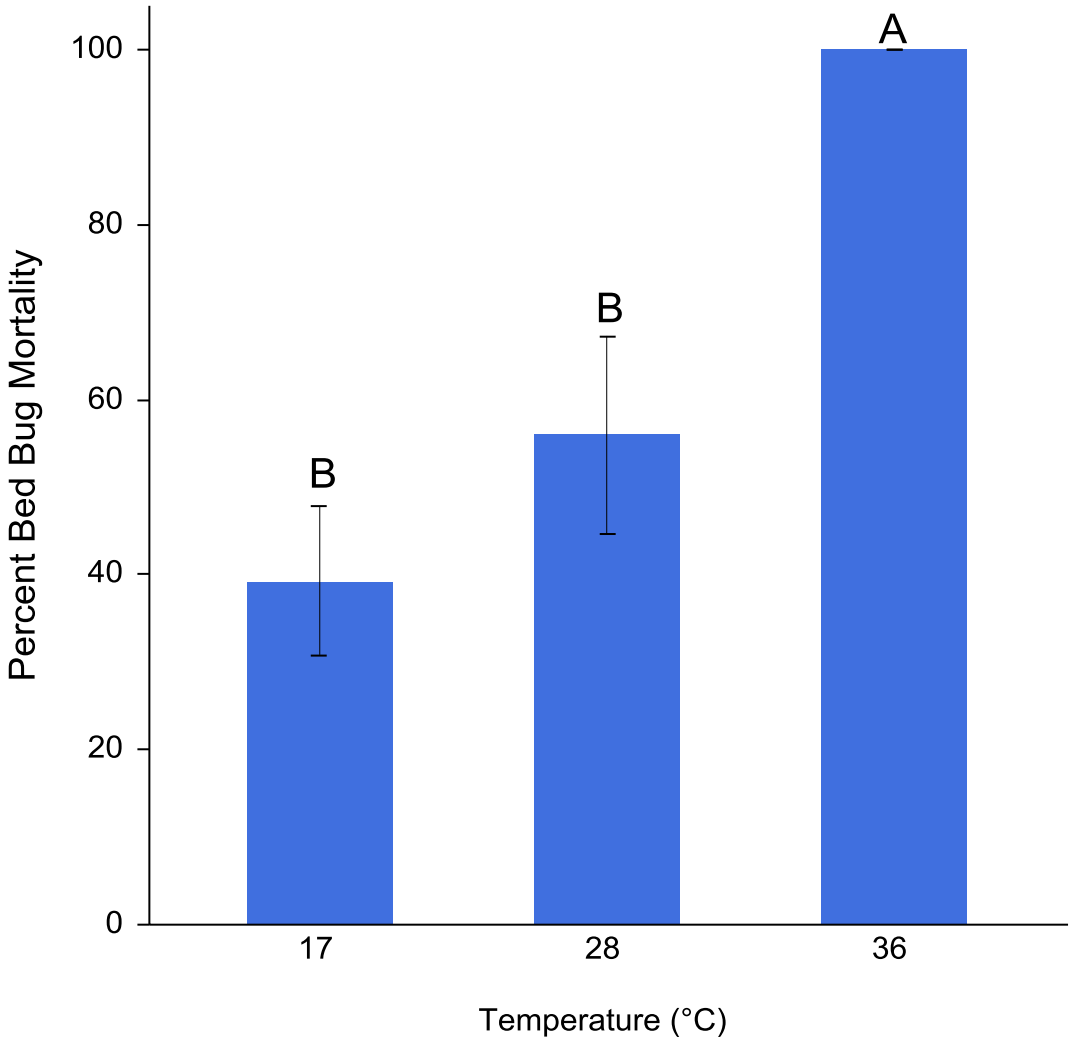

Figure 2-7. Effect of temperature on the toxicity to bed bugs exposed to anise seed oil. Mean ± SE percent bed bug mortality after 24 h exposure to anise seed oil concentrations at 17, 28, and 36°C. Levels not connected by the same letter are significantly different (analysis of variance followed by Student's t-test, $P > 0.05$).

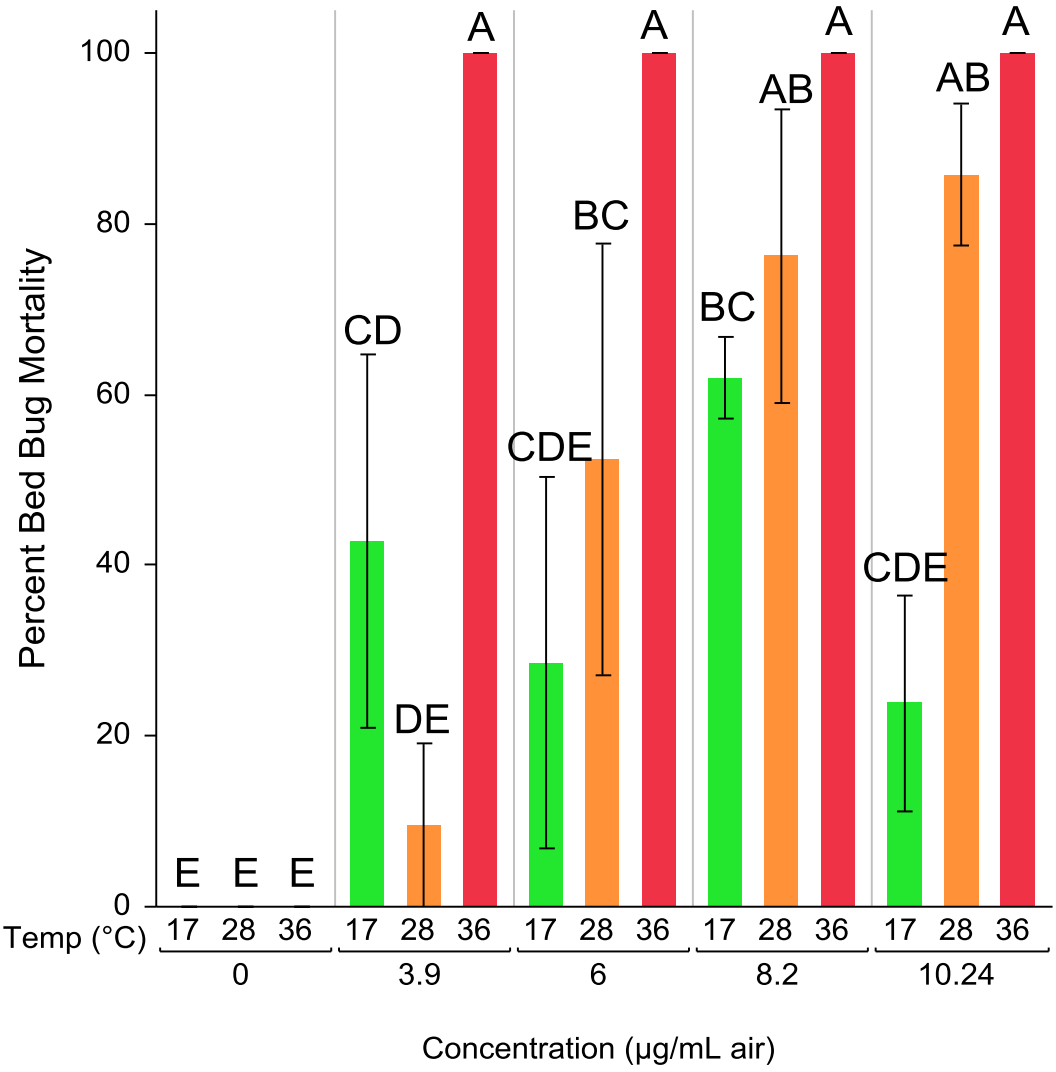

Figure 2-8. Effect of the interaction of temperature and concentration on the toxicity to bed bugs exposed to anise seed oil. Mean ± SE percent bed bug mortality after 24 h exposure to anise seed oil concentrations at 17, 28, and 36°C. Levels not connected by the same letter are significantly different (analysis of variance followed by Student's t-test, $P > 0.05$).

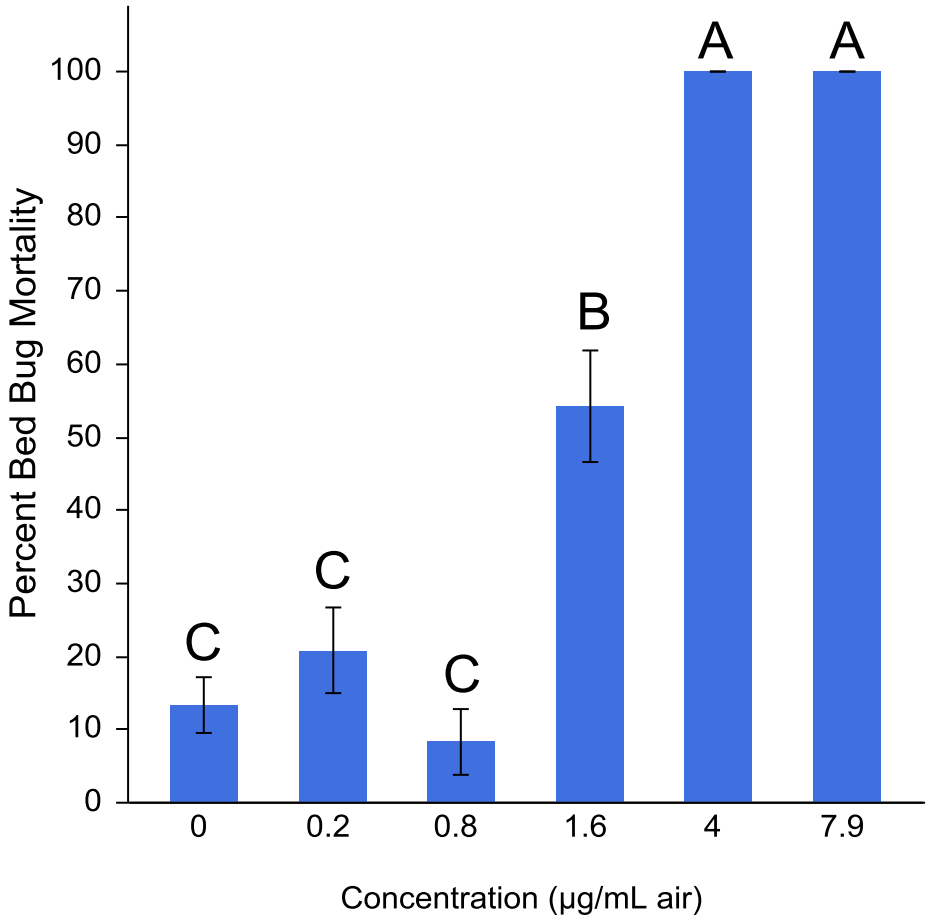

Figure 2-9. Effect of concentration on the toxicity to bed bugs exposed to trans-anethole. Mean ± SE percent bed bug mortality after 24 h exposure to trans-anethole concentrations. Levels not connected by the same letter are significantly different (analysis of variance followed by Student's t-test, $P > 0.05$).

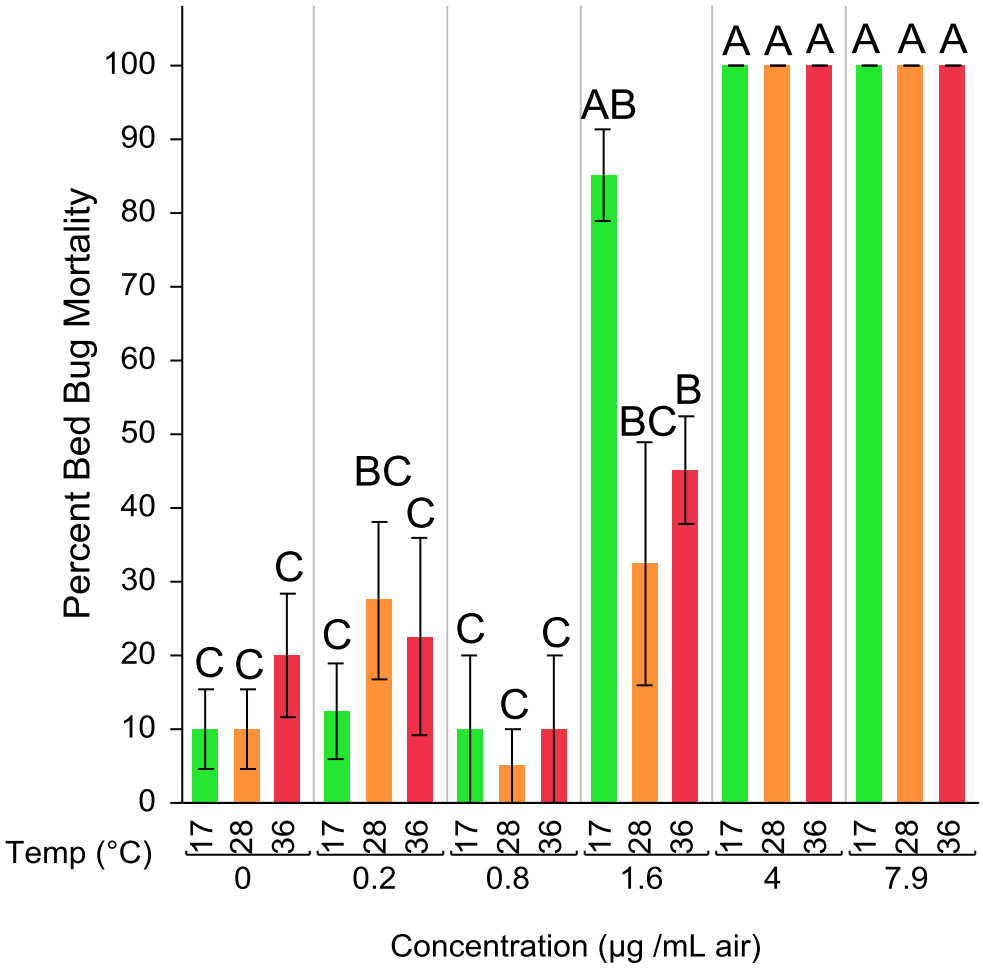

Figure 2-10. Effect of the interaction of temperature and concentration on the toxicity to bed bugs exposed to trans-anethole. Mean ± SE percent bed bug mortality after 24 h exposure to trans-anethole concentrations at 17, 28, and 36°C. Levels not connected by the same letter are significantly different (analysis of variance followed by Student's t-test, $P > 0.05$).

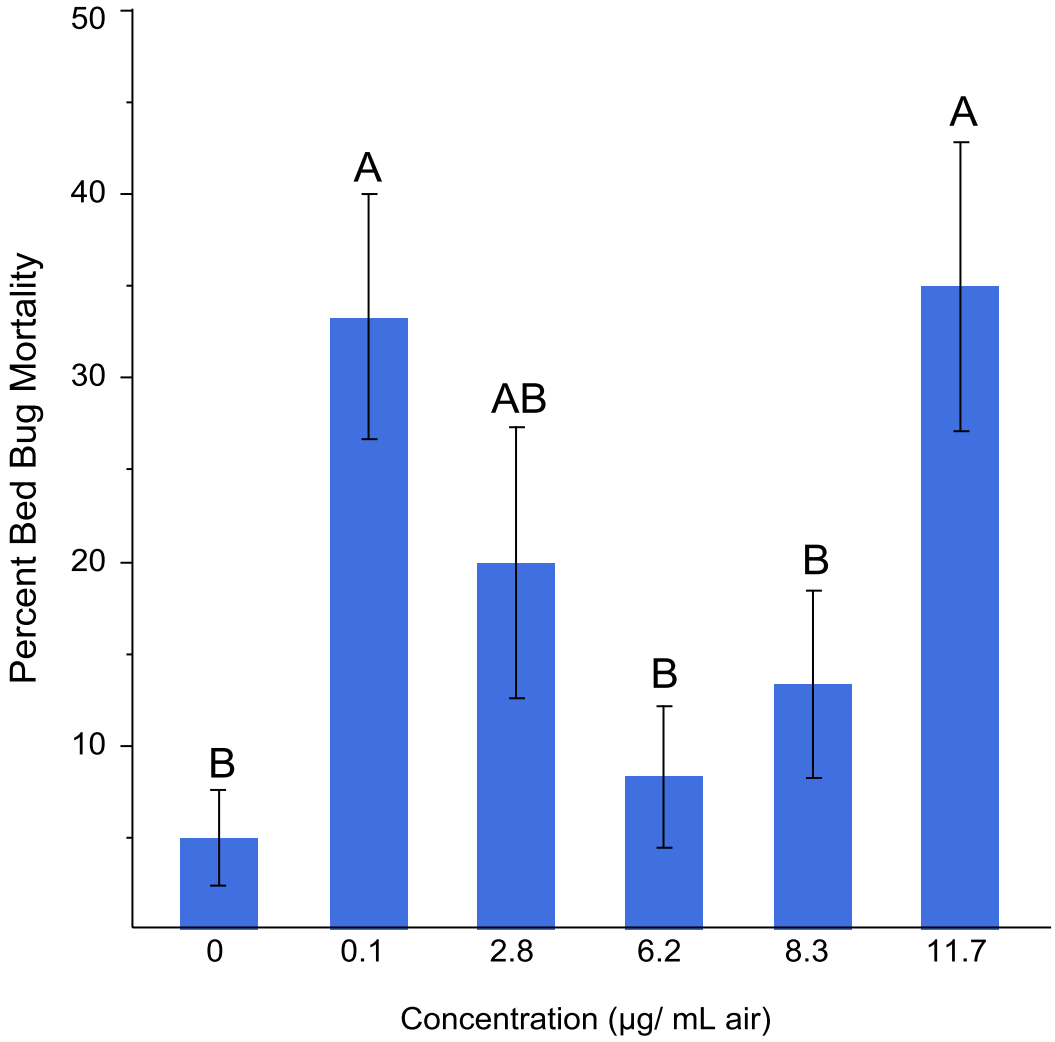

Figure 2-11. Effect of concentration on the toxicity to bed bugs exposed to linalool. Mean ± SE percent bed bug mortality after 24 h exposure to linalool concentrations. Levels not connected by the same letter are significantly different (analysis of variance followed by Student's t-test, $P > 0.05$).

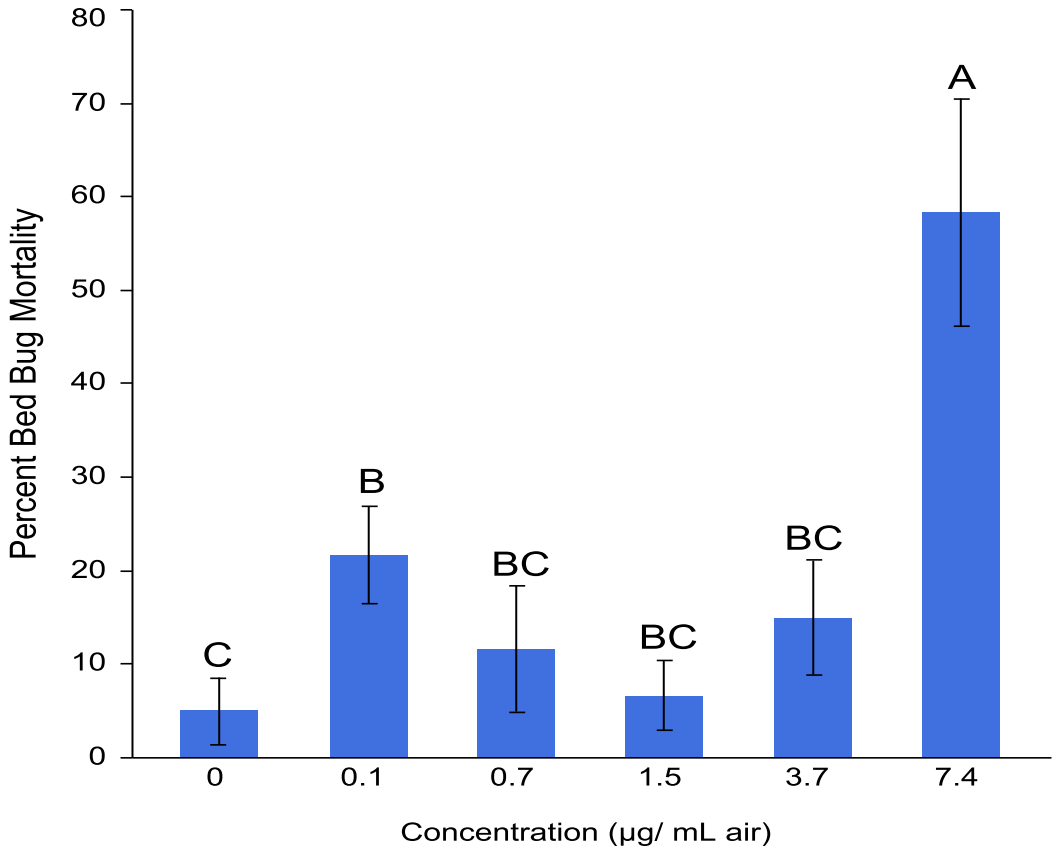

Figure 2-12. Effect of concentration on the toxicity to bed bugs exposed to eucalyptol. Mean ± SE percent bed bug mortality after 24 h exposure to eucalyptol concentrations. Levels not connected by the same letter are significantly different (analysis of variance followed Student's

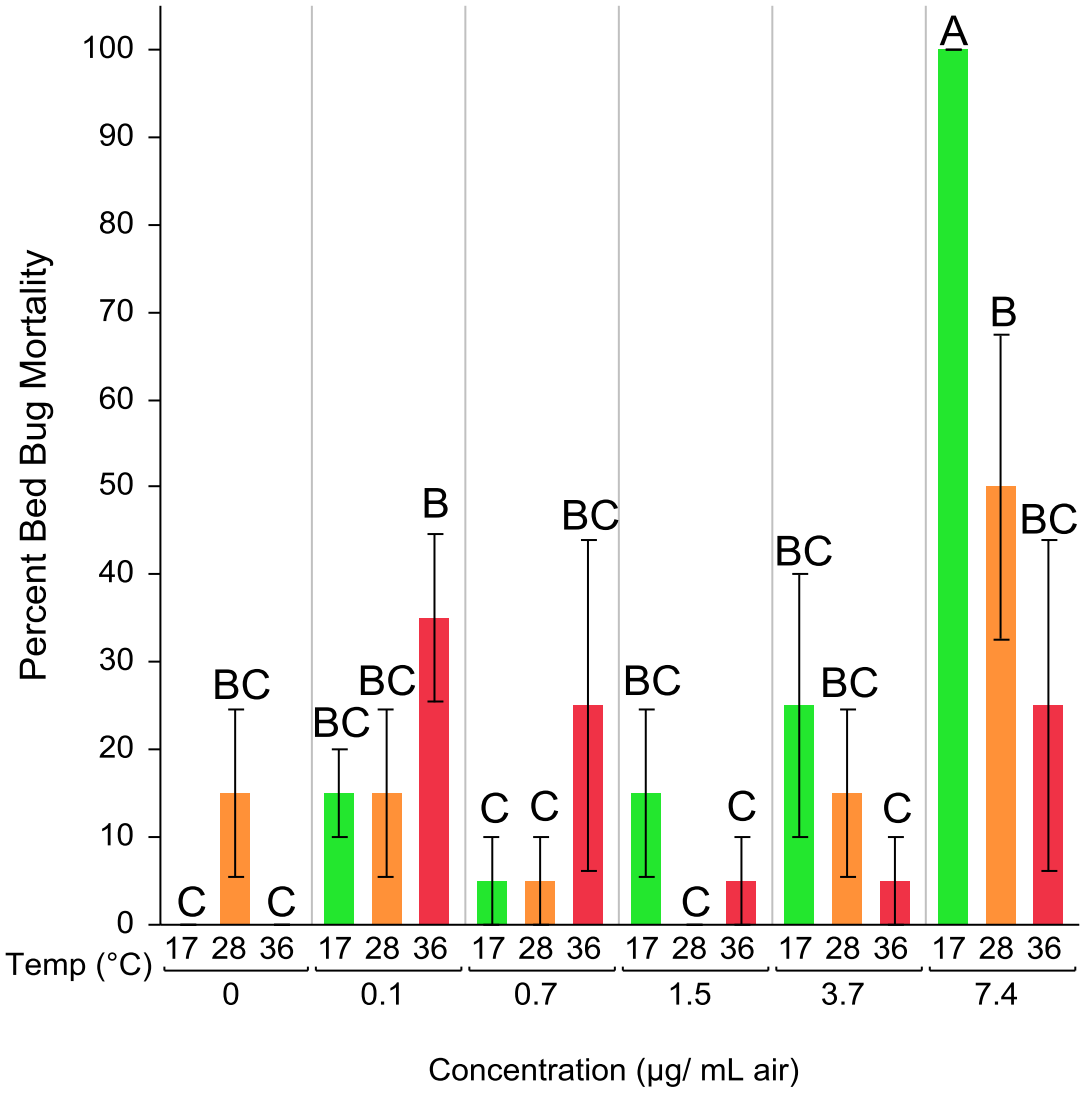

Figure 2-13. Effect of the interaction of temperature and concentration on the toxicity to bed bugs exposed to eucalyptol. Mean ± SE percent bed bug mortality after 24 h exposure to eucalyptol concentrations at 17, 28, and 36°C. Levels not connected by the same letter are significantly different (analysis of variance followed by Student's t-test, $P > 0.05$).

CHAPTER 3
ACCELERATED VAPOR RELEASE OF HEATED DICHLORVOS RESIN STRIPS FOR BED BUG CONTROL

Introduction

Control of bed bugs, *Cimex lectularius* L. (Heteroptera: Cimicidae), is limited to a number of safe, effective, and economically practical tactics, mostly those with pyrethroid active ingredients (Doggett and Russell 2008, Potter 2008). Insect resistance to contact and residual pyrethroid treatments has resulted in bed bug control complications (Hayes and Laws 1991, White and Leesch 1995, Romero et al. 2007) and high cost of treatment (Miller 2009). Bed bugs impact humans economically due to financial loss associated with control costs, legal cases, and other expenses encountered due to repeated attempts of controlling the pest (Doggett 2004, Gangloff-Kaufmann 2006). These problems emphasize the need for the development of selective, effective, and inexpensive control alternatives against bed bugs.

A potential agent for bed bug control is the organophosphate insecticide dichlorvos. Dichlorvos used in pest control is commonly referred to as DDVP, an abbreviation for its chemical name 2, 2-dichlorovinyl dimethyl phosphate (Brooks & Schoof, 1964). Dichlorvos has been studied mostly for use against flies, mosquitoes (Brooks & Schoof, 1964, Matthysee 1973; Chaskopoulou 2009), and general household pests (Wright 1971). In the past, dichlorvos products have been formulated as dusts, granules, emulsifiable concentrates, wettable powders, flea collars, baits, and impregnated resin strips (WHO 1989, EPA 1990, Agrochemicals Handbook 1991). Current dichlorvos formulations include aerosols, emulsifiable concentrations, and impregnated resin strips (EPA 2006). Dichlorvos has been sold under many trade names including Vapona®, Atgard®, Nuvan®, and Task® (USEPA 2006). Dichlorvos was banned in homes for the first time in 2006, except in specialized areas (EPA 2006). Currently, dichlorvos is classified as a probable human carcinogen based on effects that have been observed in rats and

mice (EPA 1993, 1994). AMVAC Chemical Corporation in Los Angeles, CA is the only current dichlorvos manufacturer in the United States. Nuvan Prostrips® contain 18.6% of the active ingredient dichlorvos and is currently registered for household pest control use including cockroaches, silverfish, and bed bugs. Because bed bugs have shown increased resistance to dichlorvos at higher temperatures (See Chapter 2), modifications to a previous study (Lehnert et al. 2011) using dichlorvos in heated rooms were investigated. The objectives of this investigation were to: (a) create an application system to rapidly release dichlorvos vapors from resin strips and (b) evaluate the efficacy of localized heating of dichlorvos impregnated resin strips as a potential cost-effective treatment for bed bugs requiring short exposure time.

Materials and Methods

Location

Volatile treatments were conducted in an unoccupied apartment (Fig. 3-1) within a quadriplex building (Fig. 3-2) located in Gainesville, FL. The apartment area was ~ 48 m^2 with an approximate volume of 118 m^3, and furnished with a bed, dresser, kitchen table, computer desk, and a couch to represent a natural environment.

Bed Bugs and Vial Placement

A preliminary experiment (no results shown) of four replicates was conducted in a sealed bedroom of the structure adjacent to the apartment used for the experiment described here. This preliminary experiment was conducted to investigate any variability between three bed bug strains known as KVS (collected from infestations around Kissimmee Valley, FL), Diamond Village (collected from student housing at the University of Florida, Gainesville, FL), and the pyrethroid-susceptible Harlan strain used in this report (Dr. Harold Harlan, Armed Forces Pest Management Board, Silver Springs, MD). The preliminary experiments were also conducted to

test for fan placement and settings. The heater and fan placement and settings were standardized after satisfactory results were achieved.

Bioassays were performed 5-14 days post-adult emergence. The new adults were fed to repletion 2-7 days before each replicate. Bed bugs (6) of mixed gender were carefully placed and held in 15 mL glass vials (Fisher Scientific Co., Pittsburgh, PA), with a piece of folded filter paper (30 x 30 mm) for harborage, ~ 24 h before they were used in each replicate. Vials were prepared one of three ways to determine differences in dichlorvos penetration (Fig. 3-3): 1) two layers of cotton cloth secured over the vial mouth with rubber bands and masking tape; 2) open; 3) sealed and placed in 125 mL wide mouth septa jar with the screw cap tightly secured. The third vial preparation method served as the untreated control. Open and covered vials were placed in the bedroom (3 positions: headboard, dresser, closet), the living room (2 positions: desk and dining room table), and the kitchen counter for a total of 6 vial locations (Fig. 3-4 a, b, c, d, e, f). Each of the two control vials were placed at the living room and the bedroom for the duration of the experiment. A total of 14 vials and 168 bed bugs were used for each replicate of the experiment.

Dichlorvos Strip Heaters

Two double slice bread toasters (Model 22605, Hamilton Beach, Southern Pines, NC) were converted to heat the dichlorvos strips and increase volatilization of the active ingredient (Fig. 3-5). The carriage-release mechanism was disabled so heat would be generated constantly. A cage was fabricated with wire mesh to hold the dichlorvos strips in the toast slots and between the heating coils. Two modified heaters were used per apartment. The heaters were placed on their sides a wooden platform ~30 cm above the finished floor. Desk fans (27 cm in diameter, Kaz incorporated, Southborough, MA) were placed directly behind the heater and set on high speed

to push volatiles out of the strip while preventing strips from overheating. The heaters were located in the corners of living room and the bedroom (Fig. 3-6, A and B).

Placement of Circulating Fans and Other Elements in Apartment

Box fans (51 cm in diameter, Lasko Products, West Chester, PA) were placed immediately behind the heater stand to drive dichlorvos vapor toward the center of either the living room or the bedroom (Fig. 3-6, A and B). A third box fan was placed so that it pushed dichlorvos vapor into the kitchen (Fig. 3-6 B). Oscillating fans (model 0029180, 60 cm in diameter, Utilitech, U.S.A.) were placed in the center of the living room and at the foot of the bed in the bedroom to circulate the air toward the ceiling and increase dichlorvos volatilization and distribution within the treated structure (Fig. 3-7, 3-8). All fans used for air circulation were turned on the lowest fan speed.

Dichlorvos Resin Strips and Treatment Applications

Nuvan ProStrips Plus (EPA Reg. No. 5481-554, American Vanguard Corporation, AMVAC, Los Angeles, CA) with 18.6% of dichlorvos were used in all replicates. Each strip (~65 g) contained approximately 12 g of dichlorvos.

Before each experiment, the air-handling system was disabled, windows and doors were closed, and any cracks or other large openings were sealed with painters tape. Apartments were treated with 1, 2, 3, or 4 strips that were placed in 2 modified heaters per apartment. For the 1 and 3 strip rates, a dichlorvos strip was cut in half, and each half was placed within each room in a separate modified heater. Treatment was done by heating strips for 6 h in closed apartment. At least three replicates were conducted for each treatment.

Resin strip weights were recorded, and bed bug mortality was counted by hour during the 6-h treatment. Bed bug mortality was visually checked without opening the covered vials and controls (Fig. 3-9). After the 6-h treatment, the heaters were turned off, the strips were re-

weighed, and the vials with bed bugs were removed from the treatment site. A final mortality determination was done 12-h after treatment initiation, or 6-h after the end of the treatment.. Bed bugs that were not able to right themselves were counted as dead.

Aeration

After the dichlorvos treatment was complete at 6 h, all windows and doors were opened and the oscillating fans were repositioned to pull air into the bedroom window and exhaust it through the front door for at least 48 h after each replicate (Fig. 3-8). The box fan at the kitchen entrance was repositioned to exhaust air from the kitchen into the living room area. The other box and desk fans were not repositioned.

Data Analysis

Mortality (%) data was arcsine transformed before analysis. The number of bed bugs killed (% mortality) and the average time for bed bug mortality(mean time to death) was analyzed by analysis of variance (ANOVA) with the main effects as treatment (# of strips/apartment), vial covering (open or cloth-covered), and location (vial placement inside the apartment). When significant effects were obtained in ANOVA, differences between treatment means were compared using Fisher's protected least significant differences (LSD) test (≤ 0.05; JMP Student Edition, Version 9.0 (SAS Institute, INC., Cary, NC).

Results

Mean Time to Death

Bed bug death after being exposed to Nuvan Prostrips was affected by the number of strips per apartment ($df=2$, $F=3.78$, $p=0.0267$), vial covering ($df=1$, $F=244.55$, $p<0.0001$), and location ($df=5$, $F=5.15$, $p=0.0004$). There was no control mortality. The average time to bed bug death (4-h) after exposure to 4 Nuvan Prostrips was significantly shorter than the average time to death for bed bugs that were exposed to 2 dichlorvos strips (4.9 h; Fig. 3-9). The mean time to bed bug

death was significantly shorter in open vials (2-h) than cloth-covered vials (6.6 h; Fig. 3-10) The bed bugs in vials placed in the apartment kitchen and closet required a significantly longer exposure time to die than bed bugs in vials that were placed on the desk, headboard, and dresser (Fig. 3-11). The mean time to bed bug death decreased in open vials as the number of strips used per apartment increased (Fig. 3-8). The mean time to bed bug death was significantly lower in open vials than cloth-covered vials for all treatments (Fig. 3-9).

6-h Bed Bug Mortality.

Bed bug mortality after being exposed to Nuvan Prostrips for 6 h was significantly affected by the number of strips per apartment ($df=3$, $F=19.87$, $p<0.0001$), vial covering ($df=1$, $F=280.18$, $p<0.0001$), and vial location ($df=5$, $F=4.71$, $p=0.0006$). The interaction of the number of strips and vial covering was significant ($df= 3$, $F=10.35$, $p<0.0001$; Fig. 3-15). The interaction of the number of strips and vial location was also significant ($df= 15$, $F=2.07$, $p=0.0165$).There was no control mortality. There were no significant differences in the mortality of bed bugs exposed to 3 or 4 strips; however, significance in mortality was observed between the remaining treatments (Fig. 3-12). Bed bugs exposed to 4 Nuvan Prostrips reached 82% mortality after 6-h, while 50% of bed bugs died after 6 h exposure to 1 strip. Mortality of bed bugs in open vials (96%) was significantly higher than those held in cloth-covered vials (44%) after 6 h exposure to dichlorvos (Fig. 3-13). Bed bugs that were exposed to dichlorvos in the closet and kitchen had significantly lower mortality than bed bugs exposed to dichlorvos at the headboard and dresser. Of all 6 locations, bed bugs in the kitchen had the least mortality (55%), which was significantly different than all other locations except the closet (64%). Bed bugs attached to the headboard in the bedroom had the greatest mortality (81%) of all locations after 6 h of exposure to Nuvan ProStrips (Fig. 3-14).

12-h Bed Bug Mortality.

Bed bug mortality at 12 h after the start of the experiment was significantly affected by the number of strips per apartment ($df=3$, $F=102.59$, $p<0.0001$), vial covering ($df=1$, $F=72.43$, $p<0.001$), and their interaction ($df=3$, $F=69.65$, $p=<0.0005$; Fig. 3-19). Total bed bug mortality (100%) was reached at 12 h when exposed to 2, 3, or 4 Nuvan ProStrips. Treatments using 1 Nuvan ProStrip resulted in 69% bed bug mortality (Fig. 3-17). Bed bugs held in open vials reached 99% mortality after 12 h while only 87% of bed bugs died when held in cloth covered vials (Fig. 3-18).

Discussion

The use of dichlorvos impregnated resin strips combined with heat is not new (Makara 1973, Pfeister et al. 2011). However, the application method used in this experiment localizes the increased temperature into a compact heater so that only the dichlorvos strips are heated. Although high temperatures increase insect metabolism and respiration, results from a previous study (See Chapter 2) showed a negative temperature-toxicity correlation between dichlorvos vapor and bed bug mortality, which led to this modification of a previous study reported (Lehnert et al. 2011). Because field applications of dichlorvos resin strips require that air handling systems be disabled, the ambient temperature inside the apartments were not monitored or manipulated to simulate real-world treatment conditions.

The combination of 2 Nuvan ProStrips (65g) hung on a polyvinyl chloride (PVC) stand ~15 cm from an oil-filled electric space heater + 1 box fan (~51 cm in diameter increased the efficacy of dichlorvos applications and volatilized DDVP 70 times faster than using the strips alone, resulting in 100% bed bug mortality after 36 h (Lehnert et al. et al. 2007). However, the results of the current investigation indicate a decrease in the dichlorvos exposure time needed to

eliminate bed bugs held in open and cloth-covered vials to 6 h with the addition of localized heating of Nuvan ProStrips and increased air circulation.

Use of compact heaters that rapidly release dichlorvos vapor from impregnated resin strips in combination with air circulation decreases the exposure and treatment time necessary for bed bug control. However, dichlorvos may be absorbing into the cloth, resulting in increased exposure that might not occur in practical bed bug treatments. Studies conducted after this experiment verified increased survival after 12 h mortality counts when bed bugs were removed from the apartment and placed in clean petri-dishes directly following the 6 h exposure period.

According to the Nuvan ProStrips label, four (65g) dichlorvos strips are necessary to eradicate bed bugs in the apartment used during this investigation (volume of 118 m^3). In this study, there was no difference in mortality after 12 h when bed bugs were exposed to 2, 3, or 4 strips, which is ½ to the full label dose. Future studies are needed to elucidate the dichlorvos air concentration needed per volume of a structure.

Bed bugs exposed to DDVP in the kitchen and bedroom closet resulted in either increased survivorship or slower death than bed bugs located in all other areas, suggesting unequal dichlorvos distribution in the treated structure. Practical dichlorvos vapor treatments will require modifications of the application method used in this investigation.

The fan placement and settings used in this experiment were chosen based on preliminary studies. The highest air circulation speed on the desk fans were required to minimize burning of the Nuvan ProStrips. When dichlorvos treatment was applied with the desk fans on the lowest setting, the temperature increased and melted the dichlorvos strip. The lowest fan speed was used on the box fans (Fig.) placed directly behind the heater stands. Preliminary tests with the box fans set at the highest speed resulted in decreased weight loss per strip. These observations

indicate that the heater temperature, the fans used to push dichlorvos out of the strip, and fans used to circulate air throughout the treatment area are critical to optimize the efficacy of Nuvan ProStrips for bed bug control. Variations in resin strip weight loss and bed bug mortality due to speed changes in the oscillating fan (located in living room and bedroom) that circulated dichlorvos-rich air toward the ceiling were not investigated; however, based on our observations, it is likely that slightly decreased air circulation will result in increased bed bug mortality.

The aeration time after dichlorvos treatment between replicates was at least 48 h. Two preliminary experiments with aeration time of approximately 8-12 h resulted in the bed room closet having the most rapid bed bug death. The faster kill in the bedroom closet could have been the result of persistent dichlorvos vapors. Future studies and aeration modifications combined with air sampling are required to ensure that this application method is safe for both technicians and homeowners re-entering the structure after treatment.

The results of this study indicate that the efficacy and time of treatment are both enhanced by using a method that rapidly releases dichlorvos strip vapor from a localized heating source rather than heating the entire structure. Future tests and improved application design are necessary to optimize treatment temperature and air circulation. Heating dichlorvos resin strips increases the active ingredient dose in air. Personal protective equipment (PPE) and appropriate technician training is necessary to prevent potential harmful effects to people, animals, and other potential non-targets (U.S. Environmental Protection Agency, 2006).

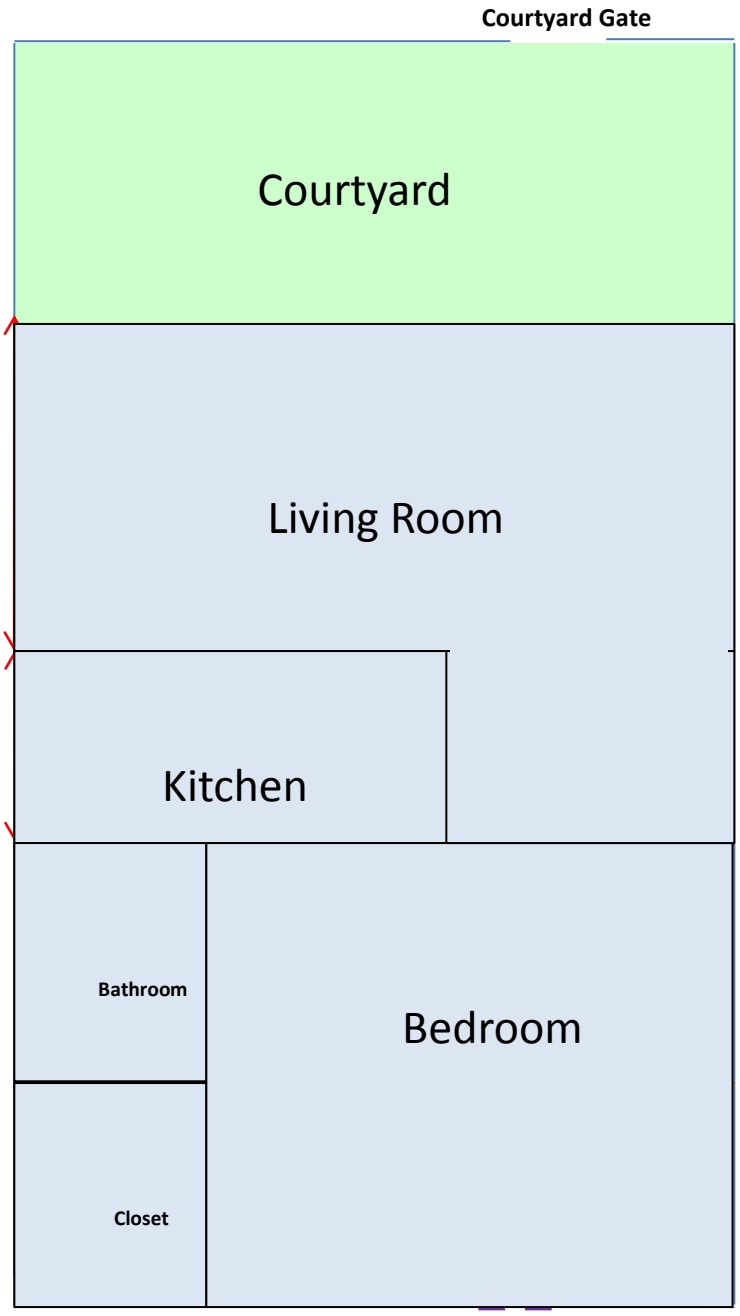

Figure 3-1. Floor plan of the apartment used in the experiment

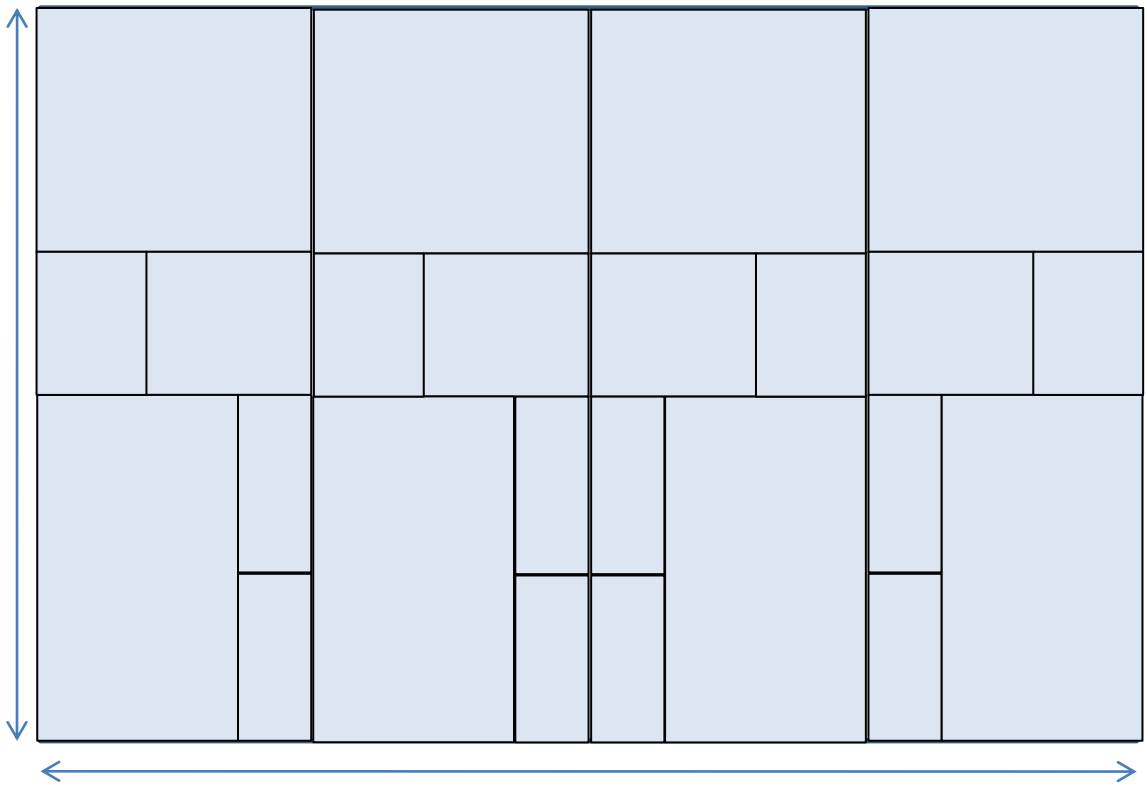

Figure 3-2. Floorplan of the group of four apartments in the quadriplex building that were used in the experiments. The layout of all four apartments mirror the layout in Fig. 3-1 above.

Figure 3-3. Vials that held bed bugs in the experiment were setup three different ways to test the penetration capabilities of dichlorvos impregnated resin strips. From left to right: 1) two layers of cotton cloth secured over the vial mouth with rubber bands and masking tape; 2) open; 3) sealed and placed in 125 mL wide mouth septa jar with the screw cap tightly secured. Photo courtesy of author.

Figure 3-4. 2 bread slice toaster modified to heat dichlorvos resin strips. The toaster is held on a wooden platform with a desk fan placed directly behind it to push volatiles out of strip while maintain temperature. Photo courtesy of author.

A

B

Figure 3-5. The modified heaters were placed in the corners of the B) living room and the A) bedroom with box fans positioned behind them to circulate dichlorvos vapors to the center of the rooms. Another box fan was positioned to circulate dichlorvos vapor into the B) kitchen. Photo courtesy of author.

Figure 3-6. Oscillating fans placed in the center of the living room and at the foot of the bed in the bedroom to circulate dichlorvos vapor towards the ceiling. Photo courtesy of author.

Figure 3-7. The oscillating fans were repositioned to exhaust dichlorvos vapors from structure and replace with outside air. Photo courtesy of author.

Figure 3-8. The apartment was entered every hour for 6 hours and bed bug mortality was visually checked without opening the cloth-covered vials. Photo courtesy of Mark Mitola.

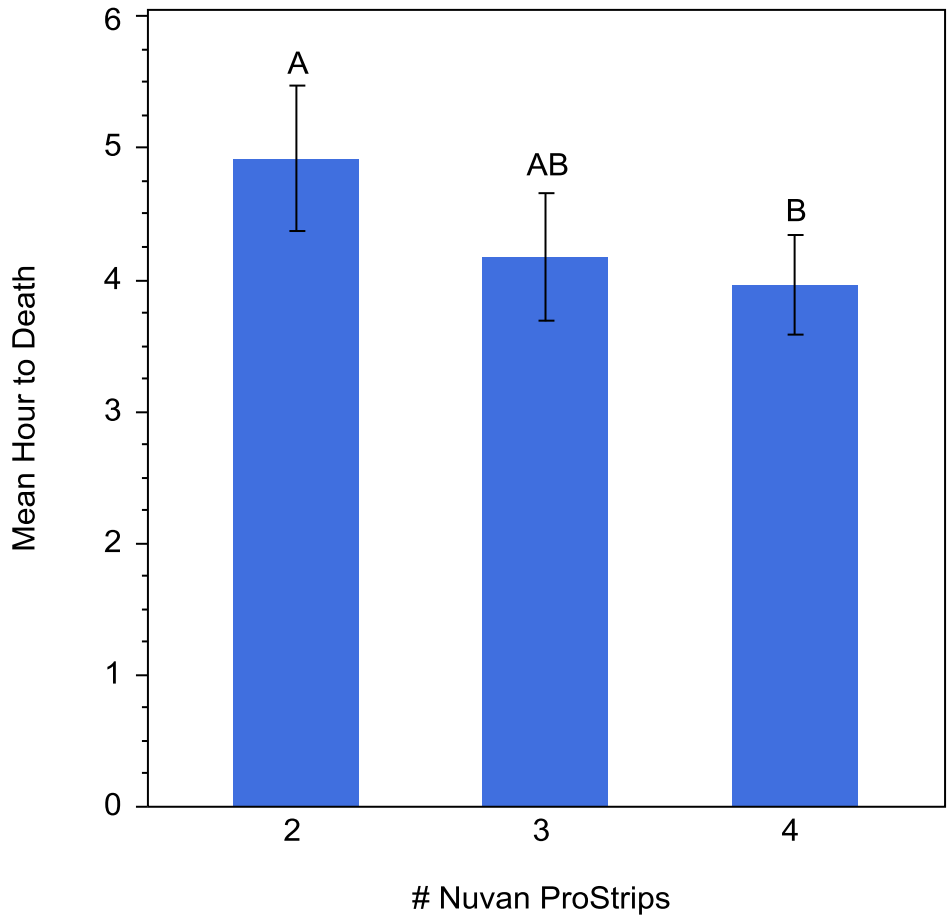

Figure 3-9. The mean time to death of an adult bed bug exposed to 2, 3, or 4 Nuvan ProStrips, placed in a 118 m^3 apartment.

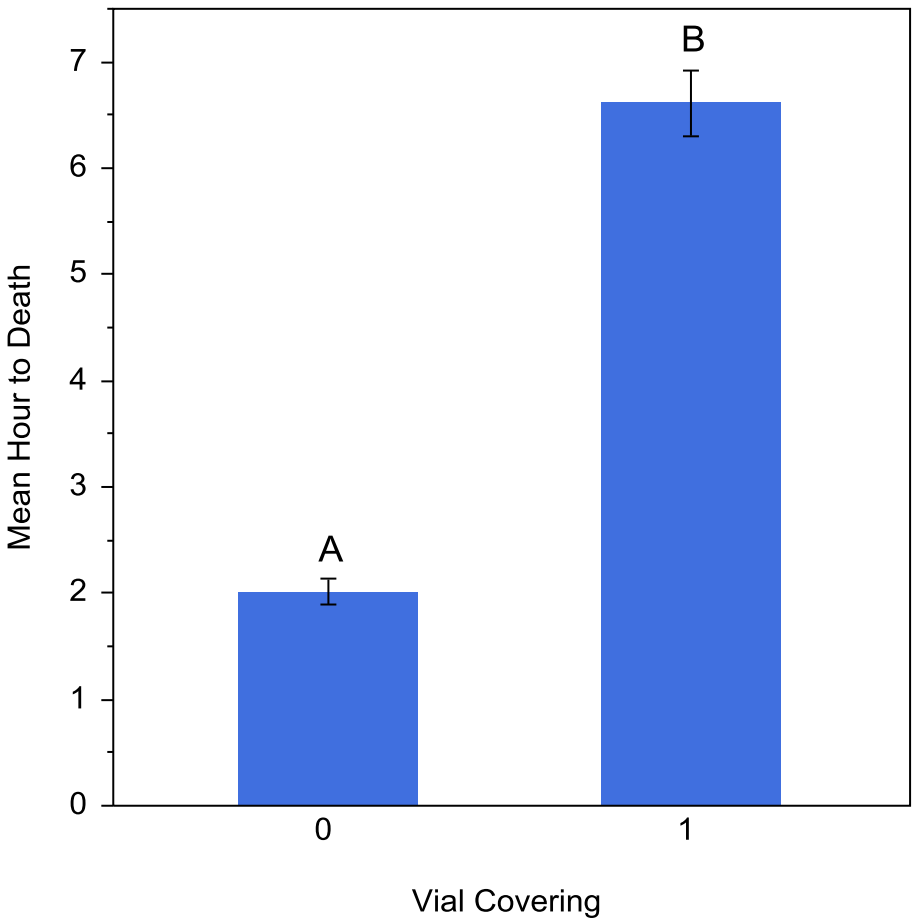

Figure 3-10. The mean time to death of adult bed bugs placed in open or cloth-covered vials and exposed to dichlorvos from heated Nuvan ProStrips in a 118 m^3 apartment.

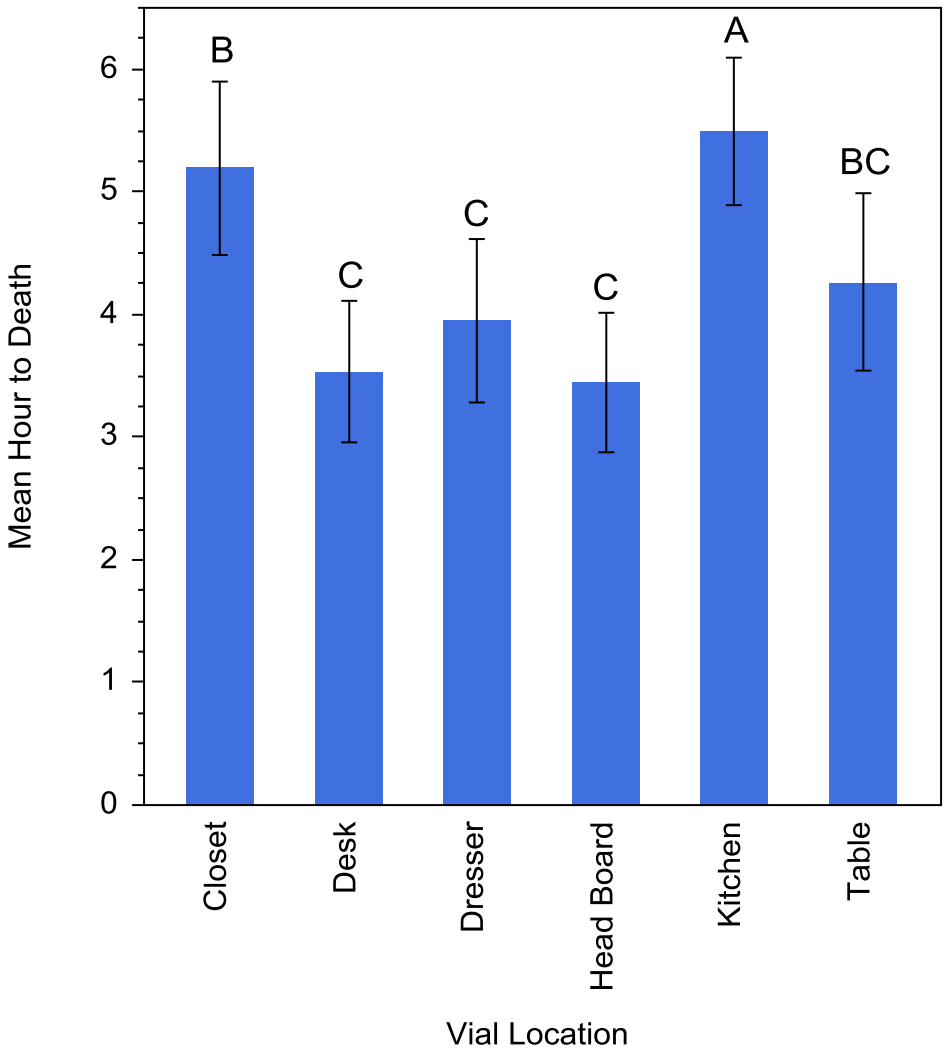

Figure 3-11. The mean time to death of adult bed bugs in vials placed in different locations and exposed to dichlorvos from heated Nuvan ProStrips in a 118 m^3 apartment.

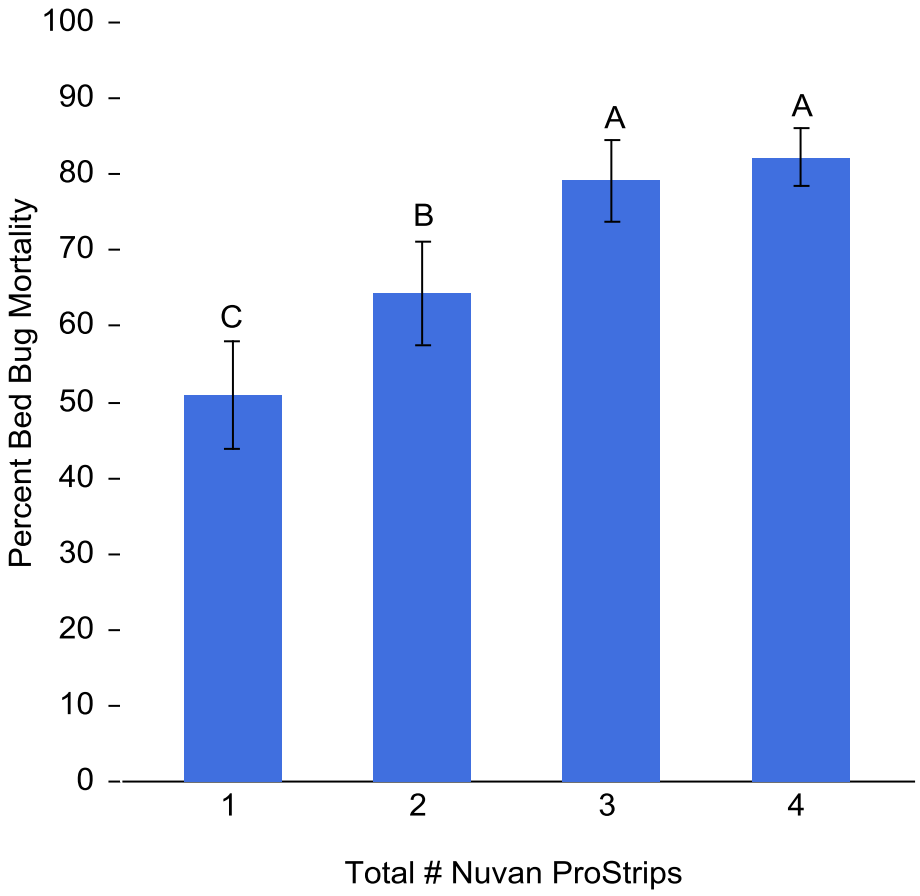

Figure 3-12. Bed bug adult mortality (%) after exposure to dichlorvos in apartments treated with 1, 2, 3, or 4 Nuvan ProStrips. Levels not connected by the same letter are significantly different (analysis of variance followed by Student's t-test, $P > 0.05$). Error bars represent ± SEM.

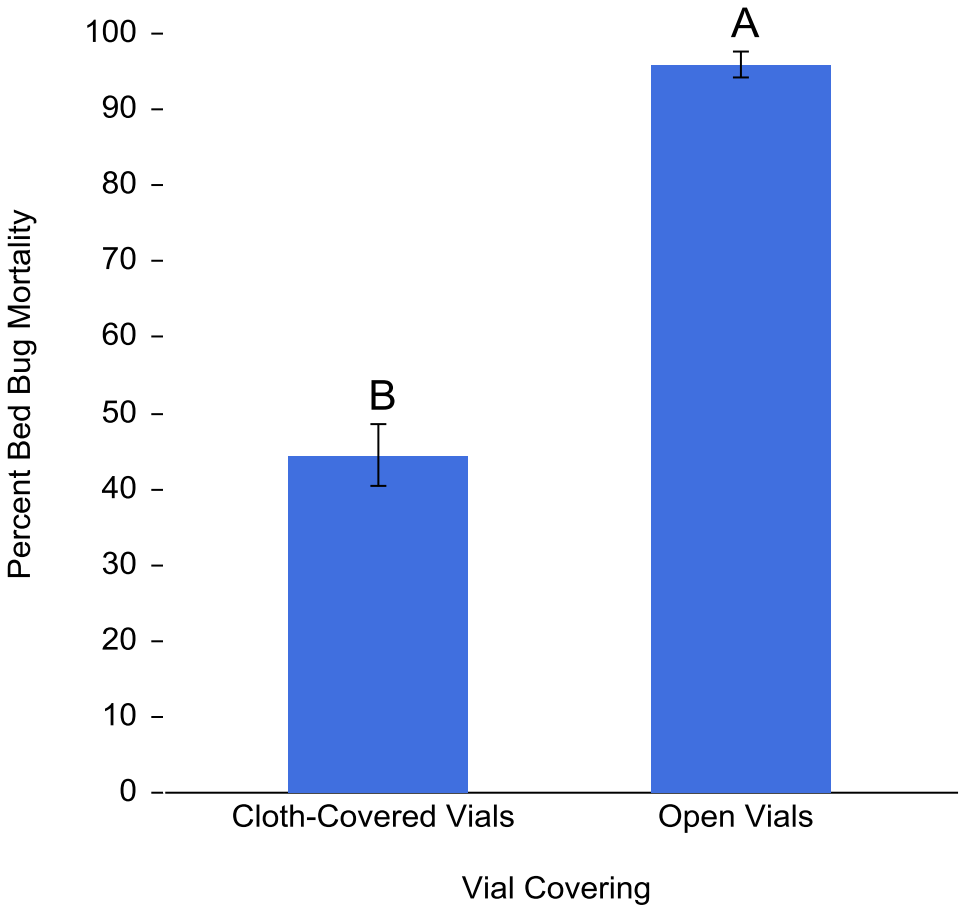

Figure 3-13. Percent mortality of bed bug adults exposed to Nuvan ProStrips for 6 h in open and cloth-covered vials. Levels not connected by the same letter are significantly different (analysis of variance followed by Student's t-test, $P > 0.05$). Error bars represent ± SEM.

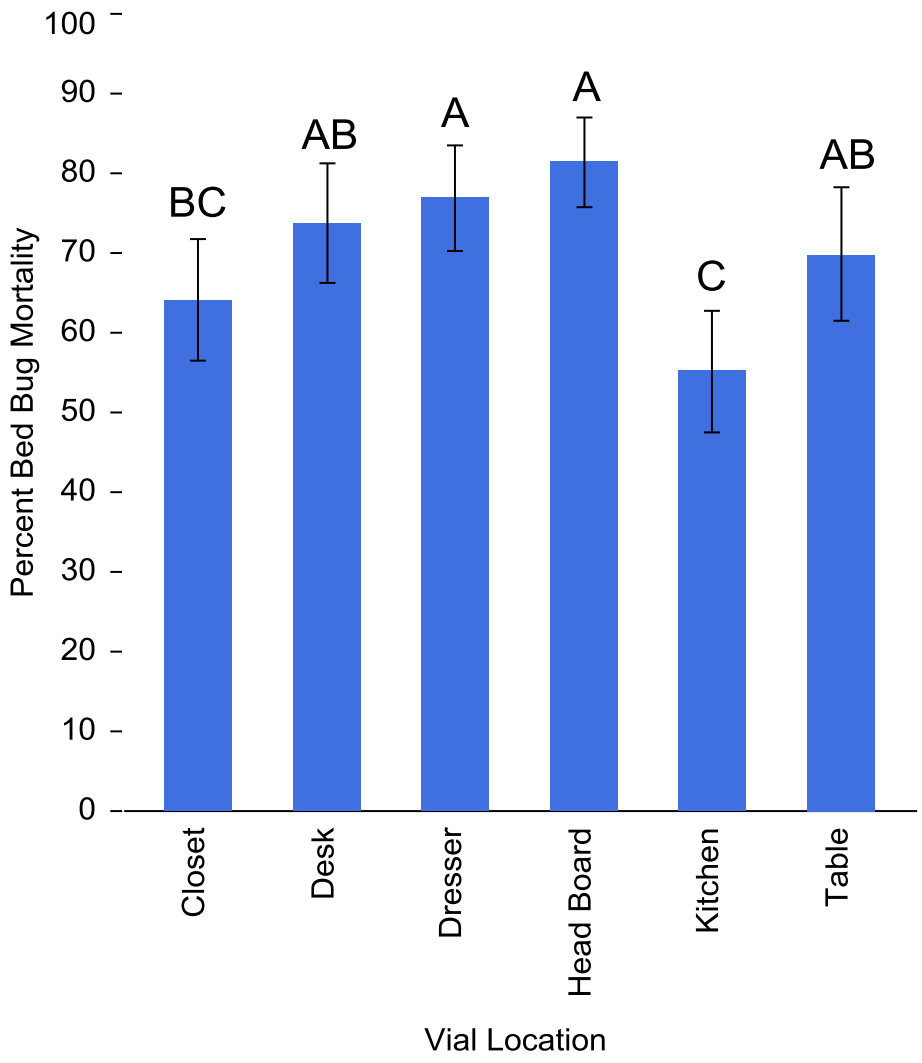

Figure 3-14. Percent mortality of adult bed bugs after 6 h of exposure to dichlorvos in different locations in Nuvan ProStrips-treated apartments. Levels not connected by the same letter are significantly different (analysis of variance followed by Student's t-test, $P > 0.05$). Error bars represent ± SEM.

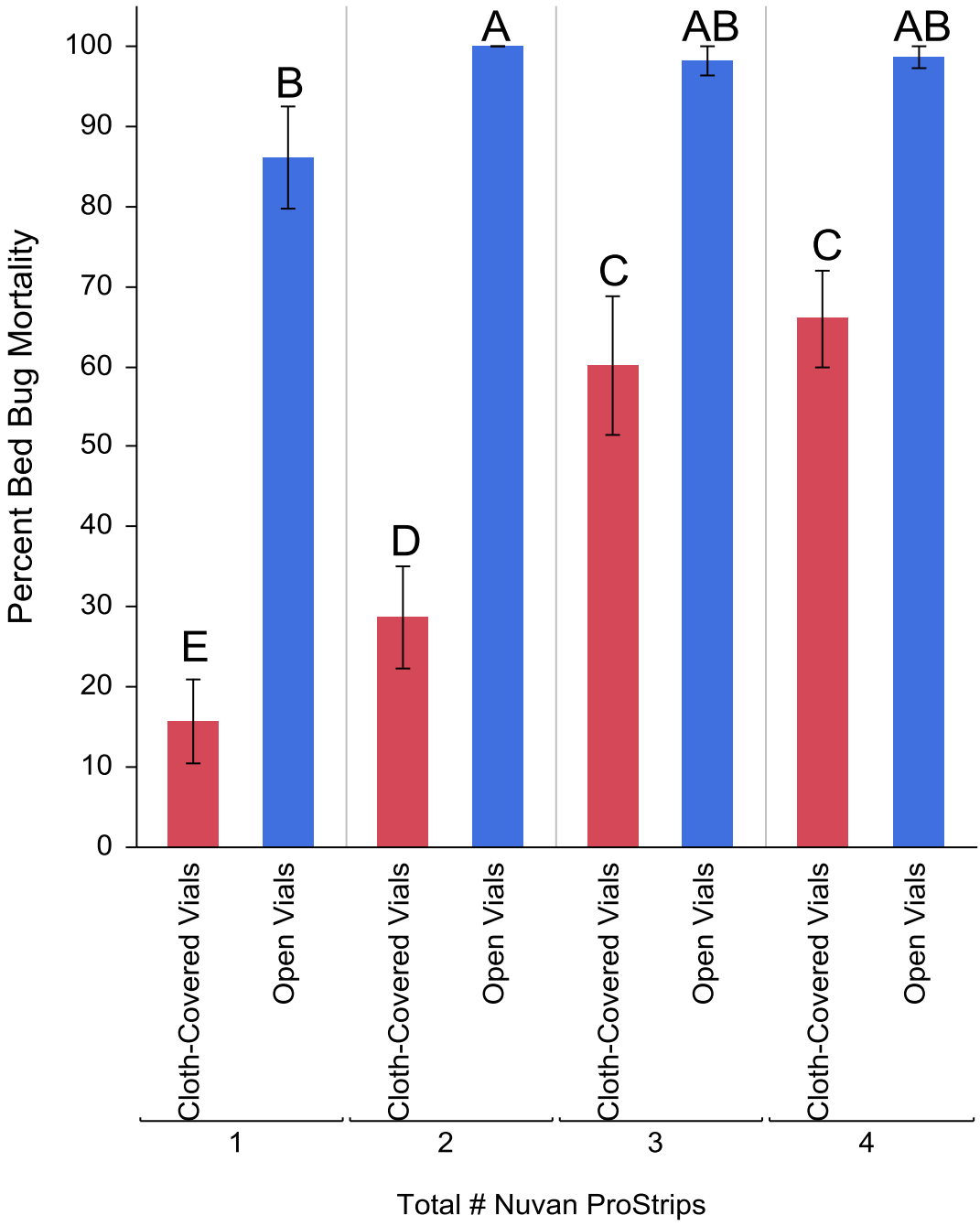

Figure 3-15. Percent mortality of adult bed bugs after 6 h of exposure to 1, 2, 3, or 4 Nuvan ProStrips in open and cloth-covered vials during apartment treatment. Levels not connected by the same letter are significantly different (analysis of variance followed by Student's t-test, $P > 0.05$). Error bars represent ± SEM.

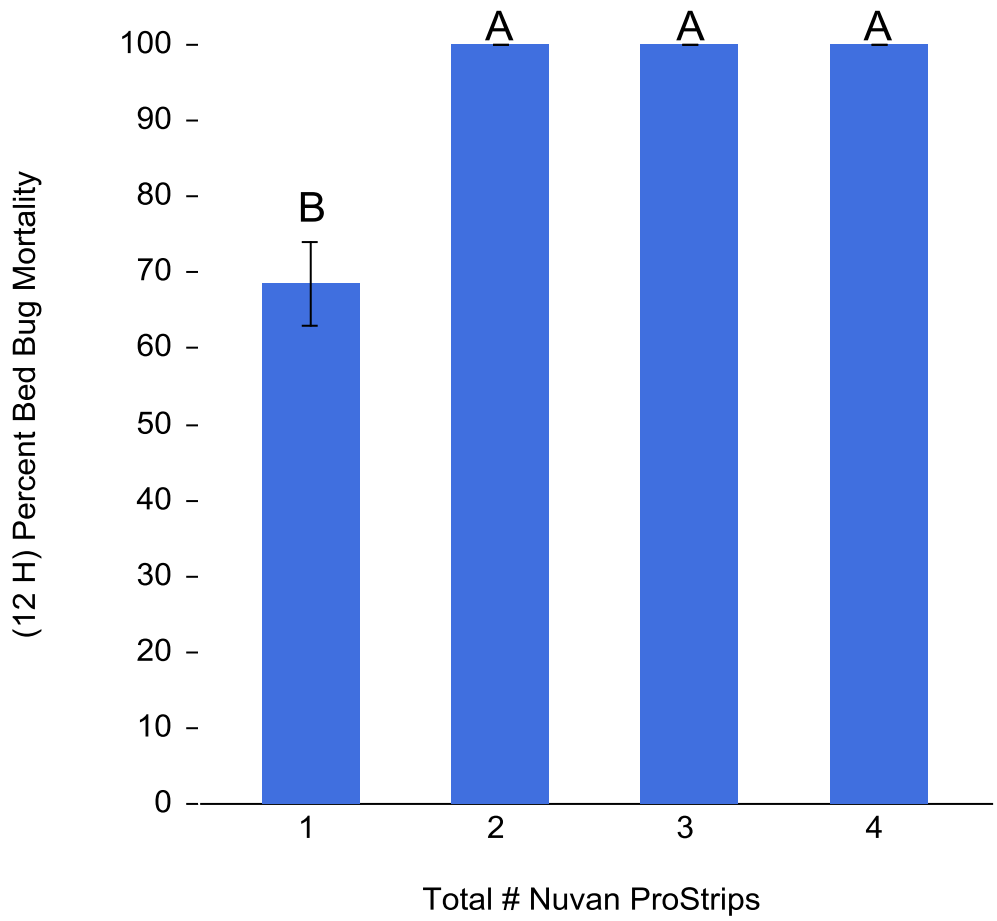

Figure 3-16. (12 h) Bed bug adult mortality (%) after exposure to dichlorvos in apartments treated with 1, 2, 3, or 4 Nuvan ProStrips. Levels not connected by the same letter are significantly different (analysis of variance followed by Student's t-test, $P > 0.05$). Error bars represent ± SEM.

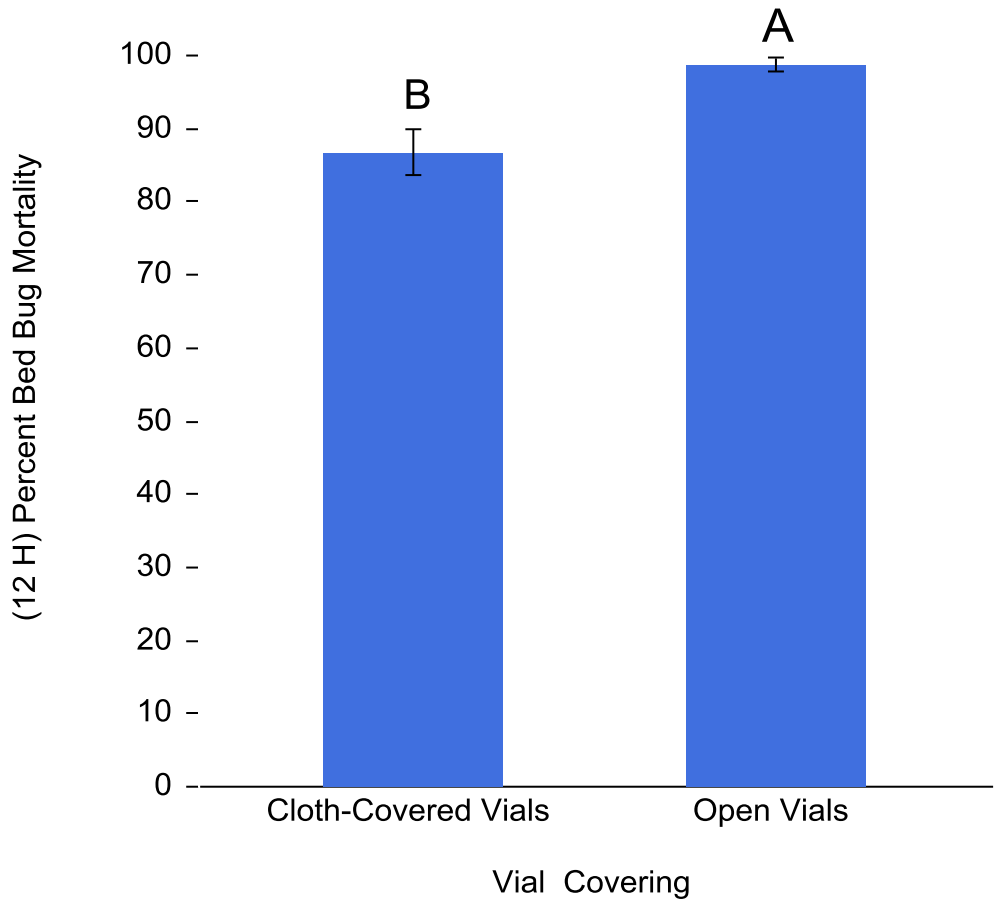

Figure 3-17. (12 h) Bed bug adult mortality (%) after exposure in open and cloth-covered vials to dichlorvos in apartments treated with Nuvan ProStrips. Levels not connected by the same letter are significantly different (analysis of variance followed by Student's t-test, $P > 0.05$). Error bars represent ± SEM.

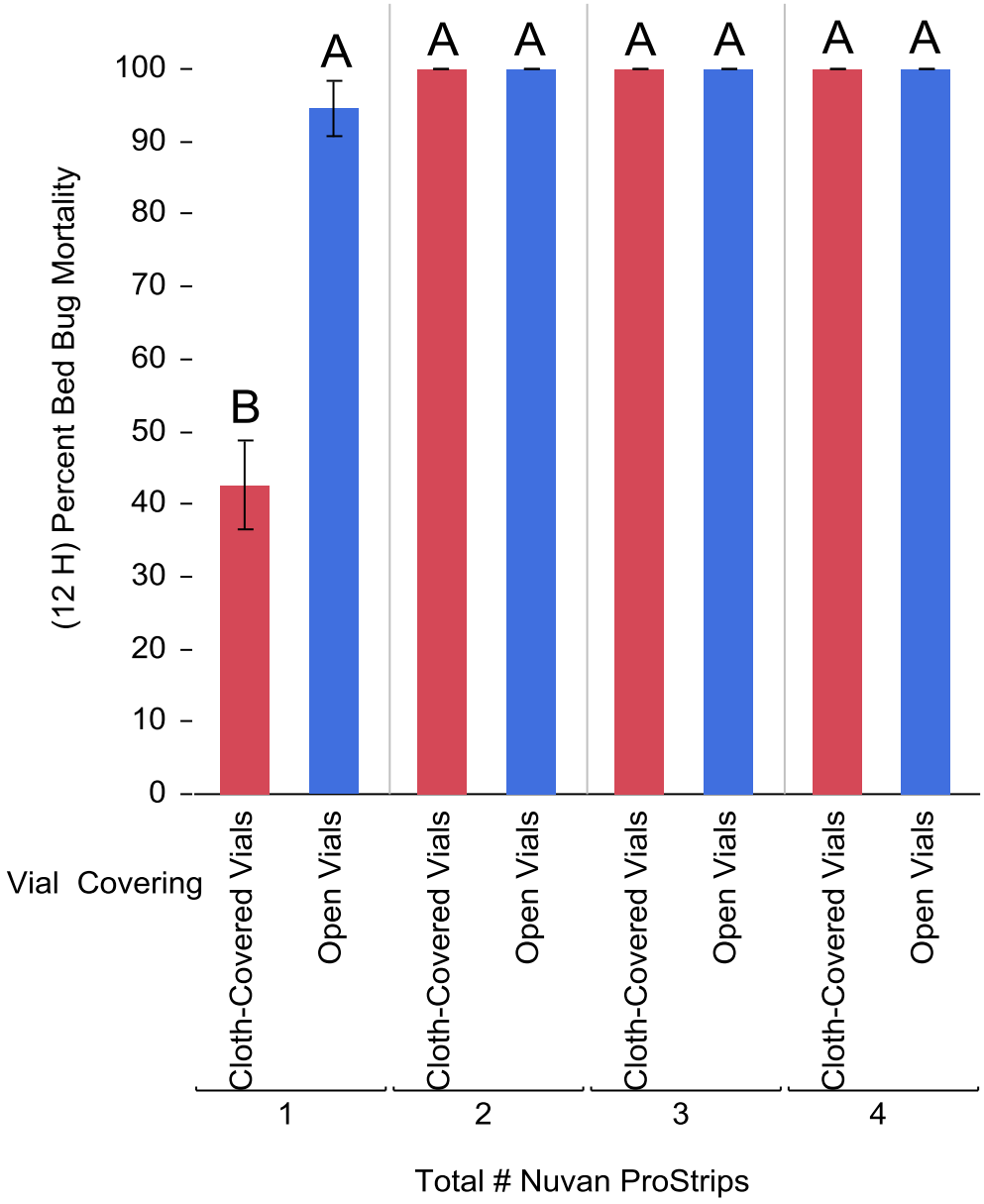

Figure 3-18. (12 h) Bed bug adult mortality (%) after exposure in open and cloth-covered vials to dichlorvos in apartments treated with 1, 2, 3, AND 4 Nuvan ProStrips. Letters represent the interaction of # of strips and vial covering. Levels not connected by the same letter are significantly different (analysis of variance followed by Student's t-test, $P > 0.05$). Error bars represent ± SEM.

CHAPTER 4
CONCLUSIONS AND FUTURE DIRECTIONS

Within the last 12 years, the bed bug has been a major factor in the pest control industry and has become a key domestic pest. The lack of research after bed bugs nearly disappeared after 1960 has limited our knowledge of its biology and control. The bed bug's resistance to pyrethroids may be a key factor in its resurgence. Although many residual insecticide products are available for bed bug control, most are pyrethroids and have not proven satisfactory in both laboratory and field settings. Therefore, this thesis attempts to provide insight on novel bed bug control alternatives.

In Chapter 2, I show that different temperatures combined with certain insecticide have significant effects on bed bug mortality when used as volatile treatments. My results show a negative temperature correlation between the concentration of the organophosphate dichlorvos and bed bug mortality. This was the first report of a negative temperature coefficient of an organophosphate. Because dichlorvos has historically proven to be an important and reliable insecticide compound, information pertaining to environmental factors that may affect its efficacy contributes meaningful knowledge to pest control industry and researchers.

I also show the effects of temperature on the toxicity of several essential oil components and 1 essential oil. Anise seed oil had a positive temperature-toxicity correlation to adult bed bugs. This was the first report on the effects of temperature on the toxicity of natural volatile compounds against bed bugs. The drive for environmentally friendly products results in the need for testing their efficacy. Although every insecticide reported in this thesis was toxic to bed bugs, future studies should evaluate the toxicity of essential oils to bed bugs in greater detail than what was done in this thesis. My results demonstrate that the toxic effects of volatile plant compounds may be used in a cooperative role in future bed bug control strategies.

In Chapter 3, I showed a novel method to rapidly release dichlorvos vapor from Nuvan ProStrips. This bed bug control method resulted in 100% bed bug mortality after 12 hours using ½, ¾, and the full label dose rate. The exposure time needed to kill bed bugs was 30 hours shorter than results in previous studies. The decrease in application time could result in increased profit for the pest control industry. Future studies are needed to evaluate the necessary aeration time and optimal aeration method required to reach acceptable air-contamination levels.

Results from my studies suggest that dichlorvos absorbs into materials after contact. For example, studies conducted after the experiment using Nuvan ProStrips indicated a greater bed bug survival rate when the insects were placed in clean petri dishes after treatment. Future investigations must be done to assess the rate of absorption of dichlorvos into materials when rapidly releasing its vapors by heating Nuvan ProStrips.

I hope that results from this thesis will encourage the investigation of novel bed bug control techniques used in conjunction with manipulation of environmental factors. Alternative bed bug control strategies are critical to addressing the current pyrethroid-resistance problems and lack of effective residual insecticides. Of particular urgency is the development of control methods that are both effective and environmentally friendly. The time taken for products to be registered by regulatory agencies allows greater opportunity for bed bug insecticide resistance, resurgence, and dispersal to occur. The incorporation of effective products in bed bug management that are exempt from registration could provide immediate relief that could not be accomplished with more conventional pesticides and insecticide formulations.

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