Effectiveness of Alcohol as a Do-it-Yourself Treatment to Combat the Bed Bug, *Cimex lectularius*

A Senior Research Thesis

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ABSTRACT

In the past decade, few other insects have made a comeback as great as the bed bug, *Cimex lectularius* (Hemiptera: Cimicidae). The stigma that generally surrounds bed bugs leaves many victims in a state of mental trauma and unrest, and bed bug infestations can be seen as a taint that many people do not admit to possessing. Furthermore, bed bugs are the most expensive household pest to treat professionally. This has led to increased use of do-it-yourself (DIY) products. Many Ohioans routinely use rubbing alcohol as a primary insecticide alternative.

The purpose of our study was to evaluate the effectiveness of commonly available rubbing alcohol (isopropyl) products (50%, 70%, and 91%) and 100% ethanol against bed bugs. Control bed bugs were treated with equivalent volumes of each alcohol product. Untreated bed bugs served as an internal control.

Three experiments were conducted using the EPM bed bug strain. In the first experiment adults had been treated with a direct topical application and observed after 24h. Nymphs were sprayed directly during the second experiment and mortality rate was observed. Eggs were also sprayed directly, however egg hatch rate was noted instead of mortality rate. Topical application to adult bed bugs affected fewer than 15% of bugs and none were dead after 24 h. Direct spray application to mixed-stage groups of nymphs resulted in a rate-dependent mortality with the heaviest rate leading to 76.7-100% mortality after 7 days among the alcohol treatments.

Spray data for eggs showed that between 63-100% of eggs hatched overall 3 weeks after applications were made. Overall topical application of alcohol to bed bug adults resulted in minimal mortality; however, high rates of alcohol is effective for killing bed bug nymphs when directly sprayed. Limited efficacy was observed for eggs since even at the highest rates, 63% or

more hatched within 3 weeks after application. Note that the rates applied in this study represent very high application volumes and may not be practical or safe.

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CHAPTER 1: INTRODUCTION

1.1 Bed Bug Biology

The bed bug, *Cimex lectularius,* is a parasite which typically feeds on blood during the night while the host is asleep (Alvaro et al. 2007). Adults are reddish brown, oval, dorso-ventrally flattened, and about 5mm in length (Ter Porteen and Prose 2005). Bed bugs feed by leaving their refuge in order to find a host. Bed bug saliva contains anesthetics and anticoagulants (Delaunay et al. 2011). These substances help to make the bite painless and allow the blood to flow more freely. Adults tend to be fully engorged on blood after ten to twenty minutes of feeding (Reinhardt and Siva-Jothy 2006). Females must feed in order to produce eggs (Reinhardt and Siva-Jothy 2006). While is it not necessary for males to feed as often. Adults can go twelve months without a blood meal, and up to two years without blood at cooler temperatures (Ter Porteen and Prose 2005). Nymphs however require blood meals more frequently and it is needed in order for them to molt to the next nymphal stage (Ter Porteen and Prose 2005). There are five nymphal stages, and nymphs grow over the course of six weeks (Ter Porteen and Prose 2005).

Bed bugs reproduce by traumatic insemination (Reinhardt and Siva-Jothy 2006). This is reproduction by which the male genitalia pierce the female's abdomen and inserts the sperm directly into the female body cavity. (Stutt and Siva-Jothy 2001). The female can then lay up to 4 eggs per day and may lay up to approximately 200 eggs over her lifetime (Ter Porteen and Prose 2005). The time taken for eggs to become fully formed adults typically takes about six weeks (Ter Porteen and Prose 2005).

Bed bugs do not have social structures as other insects such as bees, but they do tend to aggregate due to pheromones that is released (Gries et al. 2015). They have mysterious habits, and tend to find harborage along the mattress and box spring, furniture, crack and various other locations (Potter et al. 2006).

1.2 Studies of Bed Bugs Insecticide Treatments and Other Control Methods

Many studies have been conducted to test the efficacy of various insecticides on the bed bugs. Research has also been conducted to test for bed bug resistance against certain products. Major insecticide classes such as pyrethroids have become less effective against bed bugs as these insects increase their resistance (Pereira et al. 2009). In a field study by Moore and Miller (2009), the insecticides were able to reduce bed bug numbers to an extent, but were unable to eradicate the problem. Which means that additional methods are necessary to further eliminate the population. Laboratory studies also done by Moore and Miller (2006) led to similar conclusions, that pyrethroids were unable to prevent bed bugs from finding a host. Another study done by Romero et al. (2007) also showed high levels of resistance to pyrethroid insecticides.

Other methods of treatment may be necessary, as certain insecticidal treatments are unable to stand alone. Heat treatments, which causes some mortality, may be used in addition to pesticide applications. (Pereira et al. 2009). A study done by Wang (2012) found that carbon dioxide fumigation was highly effective against bed bugs, and can be considered as a useful alternative to other fumigation methods. Another study also done by Wang (2011) shows that certain bed bug monitors are effective tools against bed bugs and could be used to detect infestation and evaluate population levels.

CHAPTER 2: DO-IT-YOURSELF BED BUG TREATMENTS

2.1 Introduction

Urban insect infestations have become a somewhat familiar aspect of life today, but none can compare to the bed bug, *Cimex lectularius* (Hemiptera: Cimicidae). These historically significant insects were nearly eradicated in the U.S. by the 1950s, but they currently are making a rapid comeback as one of the leading pest concerns. In Ohio, the number of bed bug cases has grown exponentially during the last few years. This has caused many Ohio cities to be ranked among the most bed bug infested cities in the nation according to Orkin (Abejuela-Matt 2014).

Bed bugs are considered a public health issue due to their adverse effects on humans. These health effects include allergic reactions, secondary bacterial infections, and blood loss induced anemia in severe bed bug infestations (Center for Disease Control 2013). Due to the many concerns that the public holds about bed bugs, treatment options are being explored by scientists and citizens alike, in order to pinpoint a few of the best possible methods.

Bed bug eradication approaches can be separated into two general categories, professional and non-professional (do-it-yourself [DIY]). There are many reasons why individuals turn to DIY insecticidal products. First, professional chemical treatments for bed bugs are usually very expensive and can be well out of the price range of many people. Second, bed bugs are now showing resistance to some pyrethroid pesticides (Zhu et al 2013), so there is no guarantee that costly pesticide treatments will eradicate the problem. Third, there is also a highly negative stigma surrounding bed bugs, which can cause mental trauma and also dissuade people from reporting a problem (Goddard and De Shazo 2012). Due to the uncertainty that the infestation will be eradicated, the risk of a costly transaction, and the social stigma associated with bed bug infestations, many people are discouraged from using professional treatment options. These factors contribute to an increasing number of people looking for an alternative method. Thus, DIY options have become an important aspect of the bed bug control process for the average person.

There are many DIY chemical methods in the market today, many of which have no research to support their efficacy. Individuals also turn to other methods such as bug bombs in attempts to treat their infestation. However, recent research has shown that bug bombs, a common DIY product, have little to no effect on bed bugs despite their product claims (Jones and Bryant 2012). Other DIY chemical treatment options include natural products that are formulated as oils and also aerosol sprays. Many residents and health officials in Ohio believe that rubbing alcohol is an effective combatant against bed bugs, but there has been no research to support this claim. The ineffectiveness of DIY insecticidal products is a cause for concern, considering the fact that many people choose these as primary methods of control. In extreme cases, misapplication and over-application of DIY chemicals for bed bug control can result in injury or death to affected individuals and their families.

The current investigation will be undertaken to evaluate the efficacy of isopropyl alcohol against bed bugs. This will enhance our understanding of the various DIY products, specifically isopropyl alcohol and ethanol. Since these products are easily obtainable in various concentrations, providing information that validates whether or not they are effective will be beneficial to the general public. If it is found that alcohol does in fact significantly affect bed bugs, the results can suggest which concentration(s) will be most effective. This would then provide a basis for the claim that alcohol is effective. The results of these tests will be key to increasing the general public's knowledge of DIY products and hopefully divert them away from using ineffective DIY products.

2.2 Objectives and Hypothesis

There are two objectives of this experimental study. The first is to test the efficacy of commercially available rubbing alcohol at 50%, 70%, 91%, and 100% concentrations against bed bugs. The second objective is to test whether or not the volume if liquids affects bed bug mortality. Based on assumptions of the nature of higher concentrations and higher volumes. Two hypothesis were made. The first hypothesis is that the higher the concentration of alcohol, the more bed bugs that will be affected. The second hypothesis is that the larger the volume of liquid, the more bed bugs that will be affected.

2.3 Materials and Methods

2.3.1 Experimental Setup: Rubbing alcohol at different concentrations were purchased at local stores. These include 50%, 70%, 91%, and 100% alcohol concentrations. Ethanol was used as a substitute for 100% rubbing alcohol (neither is commercially available). Distilled water was used as a control treatment in order to test whether or not bugs are solely affected due to "drowning." The non-treatment control was done to test against bugs dying due to factors other than alcohol or water applications. This could include bugs dying due to being fed with old or contaminated blood. Each concentration of alcohol will be applied to the bugs in volumes of 2, 4, and 6 µl. Controls will consist of distilled water applied at each of the three volumes. The negative control group of twenty bugs will not receive any liquid application.

Bed bugs of all life stages were tested, including adults, the five nymphal stages, and eggs. The first topical phase of testing was done with the adult bed bugs. Twenty adult bed bugs were used for each treatment, and 320 adults were used in total. A replicated was comprised of a single bed bug. Each bug was positioned in an individual well plate. An ice pack was used to cool down all of the insects and slow their movement in order for precise topical application to each bug's dorsum. For the first set of bugs, 50% alcohol at 2µl was applied using a pipette. This process is repeated for the remaining volumes and concentrations of alcohol.

The second set of experiments involved the use of bed bug nymphs. Unlike adults, nymphs are too small to allow for precise dorsal application. Hence, spray applications were made to the bugs in a confined area. The area of the bed bugs was calculated by measuring adult bed bugs using the Auto Montage digital imaging system. Further calculations were made to determine the volume/area of the bug. Also found was the volume that needed to be applied to the petri dish in correspondence with the 2, 4, and 6 µl applications used in the adult trials. This volume was determined to be 1, 2, and 3 ml sprayed respectively in correspondence with the topical treatments. The amount of liquid released with each pump of the spray was determined by weighing the volume of each spray. After preliminary testing, it was found that seven pumps of the spray bottle equals 1.1 ml. This meant that the three volumes will be applied in sets of 7, 14, and 21 pumps of the spray to be consistent with previous applications. Five spray bottles were used to contain each of the five concentrations of the liquid being tested. For each set of applications, ten nymphs were used in each replicate. There were three replicates to a set resulting in a grand total of 480 nymphs. Each group of 10 nymphs contained a random selection of bugs ranging from the first to the fifth stage. These groups were sprayed according to the corresponding volume and liquid type.

The third set of experiments involved the use of bed bug eggs. Groups of 75 females were fed and then paired with 5 males in order to produce viable eggs. Filter paper was placed in the petri dish along with the females in order for their eggs to be affixed to the paper. Clusters of eggs were evaluated using a microscope and non-viable eggs were removed. Eggs were then grouped, with each group containing 10 eggs per petri dish. Each petri dish was sprayed until all liquid types and volume concentrations were used as described for the tests with nymphs. These included the water control groups and the non-water negative control group. Eggs were monitored for three weeks to evaluate hatch rate.

After the initial evaluation results were completed, it was determined that a fourth and final testing phase needed to be done. For this phase of testing, the adult trials were re-done using spray applications instead of the topical application. This allowed for consistency and comparisons to be made between application types. Fifth instar nymphs were fed and allowed to molt into new adults. Methods for this adult testing were the same for the nymph spray testing. Groups of ten adults made up a replicate, with three replications to a set. The groups were sprayed according to the corresponding volume and type.

2.3.2 Observation Methods: In order to have a consistent method for determining the state of bed bug health after application, guidelines for observation were created. Bed bug responses were categorized into four groups: healthy, ataxic, moribund, and dead. Healthy bugs were those that were alert and responded to stimuli. Ataxic describes bugs that did not respond quickly to stimuli and had uncoordinated or sluggish movement. Bed bugs classified as moribund do not have any locomotion, and they responded to stimuli only with twitching of their appendages. Bugs that are dead have absolutely no response to stimuli. Final observation analysis was collected after 1 day for the topical treatments, after 7 days for the nymph and adult spray treatment, and after 21 days for the egg treatment

2.4 Results

Different experimental phases resulted in various bed bug mortality rates among the life stages. The first phase which consisted of topical application to adults, resulted in very little adult mortality. 5% of bed bugs were affected at the following rates: 6μl of 50%, 4μl of 70%, 4μl of 91%, and 6μl of 91% isopropyl alcohol. The highest rate of mortality was 15% affected at 6μl of 70% isopropyl alcohol. All other treatments resulted in no adult bed bug mortality.

For the second nymphal phase of the experiments there is increased mortality for many of the treatments. In figure 1 the mortality of the bed bugs increases as the application rates go from light to heavy. The exception to this is the 50% isopropyl alcohol application (Figure 1). For 50% isopropyl alcohol, the light rate results in about twenty percent mortality, the medium application rate results in about eighty percent mortality, and the heavy application rate results in about seventy-five percent mortality (Figure 1). The highest mortality rate during this phase was one hundred percent mortality after the heavy 91%, and heavy 100% isopropyl alcohol and ethanol applications (Figure 1). The medium application rates for each of the treatments resulted in mortality rates of between sixty-seven and eighty-five percent mortality (Figure 1). The light application rates had a range of eighteen to thirty-nine percent mortality (Figure 1).

Low mortality rates were observed in the egg treatment phase. In figure two which represents the hatch rate of eggs, it is observed that the hatch rate is above eighty percent for most of the treatments. The heavy 100% ethanol treatment has the highest mortality, or lowest hatch rate of about sixty-eight percent (Figure 2). A one hundred percent hatch rate is observed in the light 100% and medium 100% ethanol treatments. It is also observed in the light water treatment.

The adult spray treatments had higher mortality rates than the adult topical treatments. According to figure three, mortality increased as the volume of each treatment increased from light to heavy. The highest mortality rate is approximately seventy-four percent in the heavy 70% isopropyl alcohol treatment (Figure 3). For the alcohol treatments, the light and medium volumes had a mortality rate range of zero to seventeen percent (Figure 3). For each of these treatments the heavy application rate produced the highest mortality.

2.5 Discussion

After analyzing these results, it can be stated that certain treatments provide evidence to support both hypotheses. To begin this discussion with the first phase of topical treatments, it is observed that this experimental treatment phase produced the lowest mortality rates of the four treatments. This is an indication that this treatment method is likely to be less effective than spray treatments. It was observed many times during application, that the drops of alcohol would roll off of the bed bug before it was thoroughly saturated. However, the topical application was chosen as a treatment method due the ability to apply precise measurements of material onto the bed bug.

Spray treatments were conducted in order to simulate real world application methods, and because precise topical treatments would be near impossible to conduct on live nymphs and eggs. An apparent trend can be observed in both the nymph and adult spray treatments. This trend is that mortality increases as volume of the treatment increases. The 70%, 91%, and 100% alcohol treatments are recognized to follow this pattern for the nymph tests (Figure 1). All four of the alcohol treatments are observed to follow this pattern in the adult spray tests (Figure 3). Larger volumes of alcohol applied, lead to more saturation of not only the bed bug but also the petri dish where the bed bugs were contained. This implies that heavier volumes result in longer bed bug exposure to the material, and it increases the likely hood that the bed bug will be affected.

Another trend is observed for both the nymph and adult spray application. This trend is that higher concentrations of alcohol resulted in higher mortality of the bed bugs. However, this trend is less distinct for the nymph treatment and the results were variable. One thing that can be noted, is that mortality rate in relation to the concentration of alcohol, tended to peak at either 70% or 91% isopropyl alcohol. In the nymph application, mortality peaked at 91% isopropyl alcohol in the light volume rate (Figure 1). The medium volume treatment did not have a clear peak, as it decreased at 91% and increased again at 100% alcohol (Figure 1). However, the high volume treatment peaked at 70% and continued to maintain its high mortality rate throughout the 91% and 100% alcohol treatments (Figure 1).

This trend has more distinct results in the adult spray treatment. Here we can clearly see that mortality rate peaked at 70% isopropyl alcohol concentrations (Figure 3). This is observed in the medium volume treatment where mortality peaked at 70% concentration and maintains this mortality until it tapers off at the 100% ethanol concentration (Figure 3). The heavy volume treatment mortality rate dramatically increases at 70% and then proceeds to taper off at 91% onward (Figure 3). The only exception to this trend during the adult spray treatment was the light volume treatment, which peaked at 91% concentration and decreased at 100% (Figure 3).

There is a possible explanation for the observed peak in mortality rates at lower concentrations, instead of at the highest which is 100% ethanol. This theory can be explained by the fact that alcohol evaporates faster than water. Therefore, as the concentration of alcohol increases and the less diluted it becomes, the faster it evaporates. This implies that either 70% or 91% isopropyl alcohol will be concentrated enough to affect the bed bug, while also being diluted enough to not evaporate before it can affect the bed bug. One explanation for the variable results in the nymph test is that due to the size range and biology of nymphs, they were likely to be most affected at either 70% or 91% depending on the nymphal stage. This is because the nymph tests consisted of a random mixture of nymphs of all stages, some of which were significantly larger than others. However, the adult size is fairly consistent, and their exoskeletons are more hardened than the nymphs, which may have lead to more consistent results.

Egg spray treatments had high hatch rates, which means that mortality was low. The majority of hatch rates fell in the eighty to one hundred percent range (Figure 2). These results were expected as bed bug eggs are highly resistant and there is a lack of effective insecticides against them (Campbell and Miller 2015). There is no particular trend that can be observed in the results for this treatment. However, we do see that hatch rates in the controls were variable and they were similar to hatch rates in other treatments (Figure 2). This implies that there are other reasons why some of the eggs did not hatch. This could be due to variability in the viability of some eggs.

Although there was some mortality seen, this do-it-yourself method of bed bug treatment is not very effective. Treatment against adults and eggs had lower mortality rates, and nymph treatments, which showed the most potential, also had variable results. This shows that alcohol treatments are not foolproof and will not eradicate the problem entirely. In addition to its ineffectiveness, there is also little practicality in this method. The results showed that the heavy rates usually had higher mortality, but these high volumes lead to heavy saturation of the testing petri dish. When these heavy volumes were converted to real world application rates, it was found that they were too high to have a safe and practical use in a building. Dousing furniture at these rates could pose a potential fire hazard. Furthermore, directly handling such large volumes of alcohol at high concentrations pose health risks in the form of alcohol poisoning through skin absorption (Leeper et al. 2000).

2.6 Conclusion and directions for future study

To conclude, this study has some evidence to show that alcohol efficacy increases as the volume of application increases, and as the concentration of alcohol increases to a certain extent. It can be stated that these treatments are non practical, similarly to other do-it-yourself treatments such as bug bombs. (Jones and Bryant 2012). From this study we can see that alcohol has some potential to be a fairly reliable treatment method against bed bugs, as there were significant mortality rates observed. However, the volume of alcohol needed for it to be effective proves to be a limitation. There is one study that can be done in the future to hopefully overcome this. This is to figure out what aspect of alcohol composition affects bedbugs, whether it be certain elements that cause dehydration or other factors. Alcohol has been used by the general public to get rid of pest problems for a long time, so a possible reformulation to make it more effective could be a potential direction for future research with the *C. lectularius.*

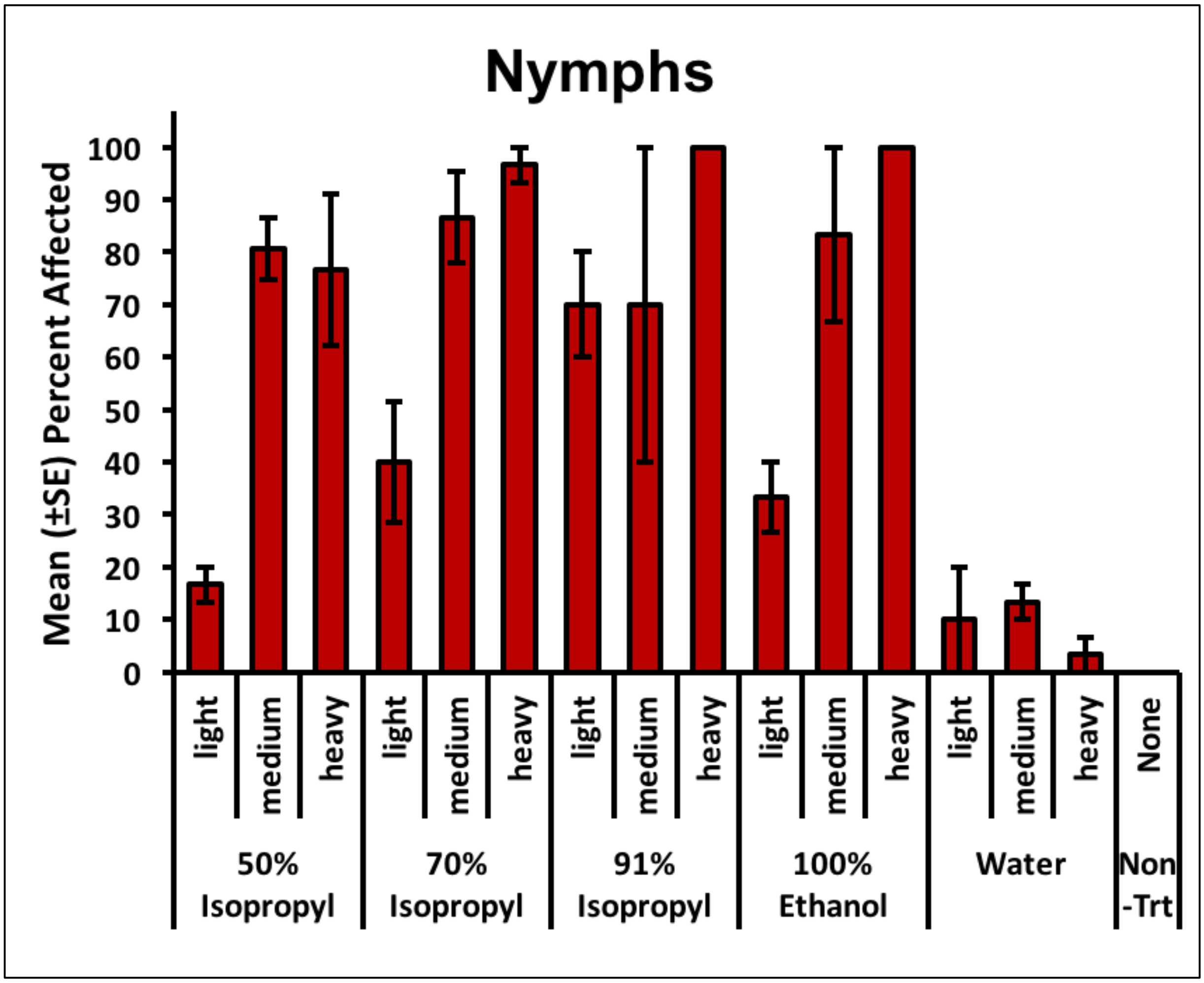

Fig. 1. Percent (mean ± SE) of affected (moribund and dead) bed bug nymphs 7 days after being sprayed with various alcohol products at light, medium and heavy rates (19, 39, and 58 µl/cm^2).

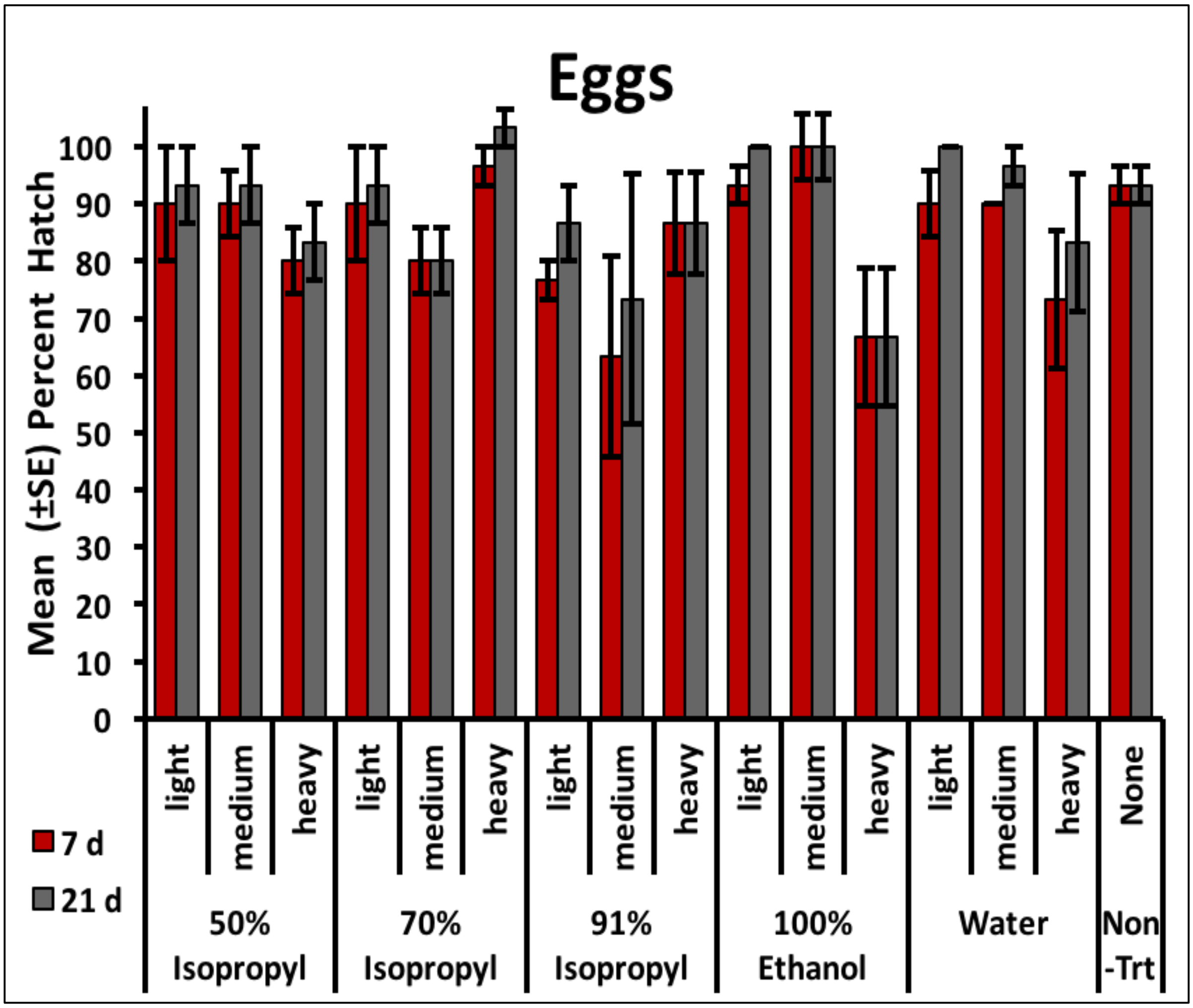

Fig. 2. Percent (mean ± SE) of bed bug eggs that hatched 7 and 21 days after being sprayed with various alcohol products at light, medium and heavy rates (19, 39, and 58 µl/cm^2).

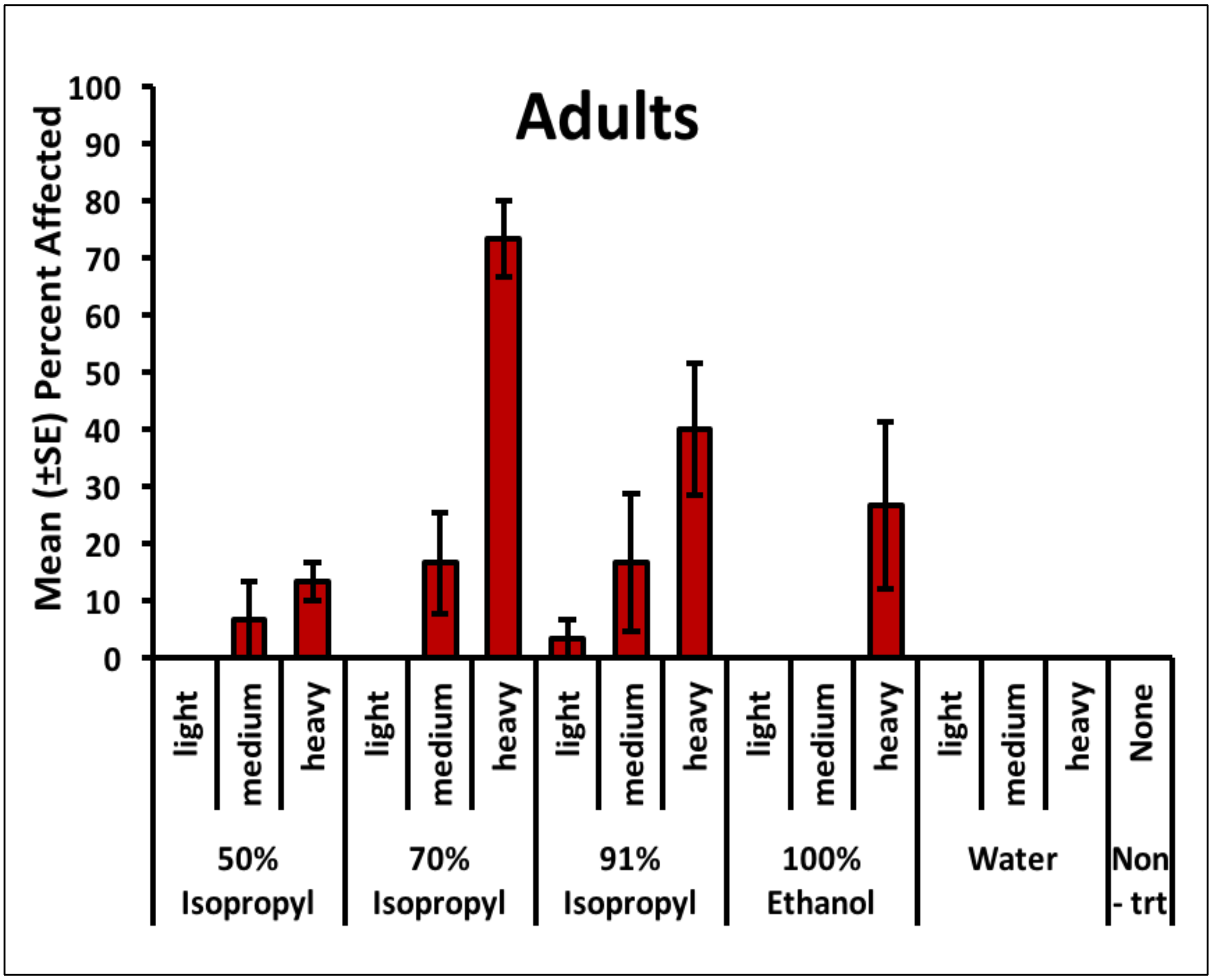

Fig. 3. Percent (mean ± SE) of affected (moribund and dead) bed bug adults 7 days after being sprayed with various alcohol products.

List of References

Abejuela-Matt, V. L. 2014. Bedbugs Biting Back? A Multifactorial Consideration of Bedbug Resurgence. J. Pat. Cent. Res. and Rev. 1: 93-98.

Campbell, B. E., and D. M. Miller. 2015. Insecticide Resistance in Eggs and First Instars of the Bed Bug, Cimex lectularius (Hemiptera: Cimicidae). Insects. 6: 122-132.

Center for Disease Control and Prevention. 2013. Bed Bug FAQs. U.S. Dep of Health and Human Services. http://www.cdc.gov/parasites/bedbugs/faqs.html

Fletcher, M. G., and R. C. Axtell. 1993. Susceptibility of the bedbug, *Cimex lectularius*, to selected insecticides and various treated surfaces. Med. and Vet Entomol. 7: 69-72.

Goddard, J., and R. De Shazo. 2012. Psychological Effects of Bed Bug Attacks (Cimex lectularius L.). The Amer. J. of Med. 125: 101-103.

Gries, R., S. Fraser, R. Britton, M. Holmes, H. Zhai, J. Draper, and G. Gries. 2015. Bed Bug Aggregation Pheromone Finally Identified. Angewandte Chemie. 54: 1135-1138.

Jones, S. C., and J. Bryant. 2012. Ineffectiveness of over-the-counter total-release foggers against the bed bug (Heteroptera: Cimicidae). J. Econ. Entomol. 105: 957-963.

Leeper, S. C., A. L. Almatari, J. D. Ingram, and K. E. Ferslew. 2000. Topical absorption of isopropyl alcohol induced cardiac and neurologic deficits in an adult female with intact skin. Vet. Hum. Toxicol. 42: 15-17.

Moore, D. J., and D. M. Miller. 2009. Field evaluations of insecticide treatment regimens for control of the common bed bug, Cimex lectularius. PestManagSci. 65: 332-338.

Moore, D. J., and D. M. Miller. 2006. Laboratory evaluations of insecticide product efficacy for control of *Cimex lectularious*. J. Econ. Entomol. 99: 2080-2086.

Pereira, R. M., P. G. Koehler, M. Pfiester and W. Walker. 2009. Lethal effects of heat and use of localized heat treatment for control of bed bug infestations. J. Econ. Entomol. 102: 1182-1188.

Potter, M. F., A. Romero, K. F. Haynes, and W. Wickemeyer. 2006. Battling bed bugs in apartments. Pest Control Technol. 34: 44D52.

Reinhardt, K., and M. T. Siva-Jothy. 2007. Biology of the Bed Bugs (Cimicidae). Annu. Rev. Entomol. 52: 351–74.

Romero, A., M. F. Potter, D. A. Potter, and K. F. Haynes. 2007. Insecticide resistance in the bed bug: a factor in the pest's sudden resurgence? J. Med. Entomol. 44: 175- 178.

Stutt, A. D., and M. T. Siva-Jothy. 2001. Traumatic insemination and sexual conflict in the bed bug Cimex lectularius. PNAS. 98: 5683-5687.

Ter Poorten, M. C., and N. S. Prose. 2005. The return of the common bed bug. Pediatr. Dermatol. 22:183–187.

Wang, C., L. Lihua, and X. Ming. 2012. Carbon dioxide fumigation for controlling bed bugs. J. Med. Entomol. 49: 1076-1083.

Wang, C., W. Tsai., R. Cooper, and J. White. 2011. Effectiveness of bed bug monitors for detecting and trapping bed bugs in apartments. J. Econ. Entomol. 104: 274-278.

Zhu, F., H. Gujar, J. R. Gordon, K. F. Haynes, M. F. Potter, and S. R. Palli. 2013. Bed bugs evolved unique adaptive strategy to resist pyrethroid insecticides. Sci. Rep. 3: 1456.