The Impact of Ultraviolet Light on Survival and Behavior of the Human Bed Bug, *Cimex lectularius* Linnaeus

Research Thesis

Presented in Partial Fulfillment of the Requirements for graduation *with research distinction in Entomology* in the undergraduate colleges of The Ohio State University

by

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April 2013

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Abstract

The dramatic resurgence of bed bugs in the United States poses significant problems for individuals, public health officials, and the pest control industry. Currently no individual control measure, chemical or otherwise, has proven to be one hundred percent effective in the removal of bed bugs from domestic structures. Alternative methods of control are needed to aid in a broader removal strategy. Ultraviolet light as a control tactic for arthropods is a new concept only now being explored. UV light is known to damage DNA; however, its various effects on arthropods have not been well documented. This study examines the impact of ultraviolet light on bed bug survival and behavior, and is a first step in determining the potential of ultraviolet light as a control measure. Two developmental stages of *Cimex lectularius*, the egg and the first nymphal instar, were exposed to ultraviolet light for periods of 1, 2, 5, or 10 sec at a distance of 4 cm. A dose response curve was created by calculating mortality following an interval of 2 weeks. Behavioral observations were also conducted to assess the effects of UV exposure on the host-seeking abilities of first instar nymphs. Nymphs were exposed for periods of 5 or 10 sec then released to search for a human host from a distance of 34 cm. The success rates and search times were recorded. Results of both experiments indicate UV light negatively impacts bed bug survival and host-seeking abilities.

Introduction

Recently the United States has seen a massive rise in bed bug infestations (CDC and EPA, 2010), and the bed bug (Hemiptera: Cimicidae) is quickly becoming one of the most important urban pests in the nation. Unfortunately, bed bugs are also one of the most challenging urban pests in terms of control. Bed bugs are resilient and difficult to remove due to their cryptic behavior and general physiology. They are active nocturnally when hosts are sleeping and unaware but then seek harborage in hidden and protected areas during the day (Bonnefoy, et al., 2008). Flattened bodies allow them to squeeze into cracks and crevices (Krinsky, 2009), making removal by physical or chemical control methods difficult.

Bed bug saliva contains anesthetic agents, making the initial bite painless (Delaunay, et al., 2011) and unlikely to awaken a sleeping host. Other compounds such as anticoagulants, vasodilators, and proteolytic enzymes are also present in saliva and contribute to the allergic reaction experienced by many individuals (Delaunay, et al., 2011). Human response to bed bug bites varies from asymptomatic to serious allergic reactions, which include rare cases of anaphylaxis. Typically, however, bites result in nothing more than small raised bumps, which can be red, itchy, and irritating. There is also a risk of secondary skin infections induced by excessive scratching at bite sites (CDC, 2013). Presently there are no studies that suggest bed bugs are capable of transmitting any human pathogens via their saliva, despite the fact that they have been shown to harbor a variety of infectious agents known to affect humans (Delaunay, et al., 2011). Chronic infestations of bed bugs may result in blood loss significant enough to induce anemia, especially in individuals already weakened by other factors. The presence of bed bugs in a home may also lead to sleeplessness and anxiety (Krinsky, 2009).

Bed bugs are often classified as a nuisance pest rather than a public health hazard because they do not transmit disease (CDC, 2013). However, this does not change the fact that bed bugs are responsible for a variety of health concerns, both physical and emotional, and must be considered a serious pest problem. Taking this into consideration, the CDC recently released a joint statement with the EPA declaring bed bugs a significant public health problem (CDC and EPA, 2010).

Eradicating bed bugs from a premises is extremely challenging. In previous years, DDT was highly effective in eliminating bed bugs and provided long lasting protection against future infestations. However, bed bugs began developing resistance to DDT, and following a government ban in 1972, the pest control industry began to rely increasingly on pesticides such as pyrethroids (Potter, 2008). Over-reliance on these compounds has been linked to increased pesticide resistance in bed bug populations (CDC and EPA, 2010; Haynes, et al., 2007). Since pesticide applications are only partially effective in removing bed bugs from a structure, they are typically combined with other control methods such as heat or steam treatments (Bonnefoy, et al., 2008). Other physical controls such as vacuuming or laundering items at high temperatures are also common strategies (Krinsky, 2009). Bed bug removal is a complicated and expensive process that almost always requires multiple treatments to ensure complete eradication from a structure (Potter, 2008). As no single tactic is one hundred percent effective in eliminating infestations on its own, a combination of control methods must be used in an Integrated Pest Management approach (Bonnefoy et al., 2008). Due to the individual inadequacies of current control methods, the pest control industry has been forced to seek out novel treatments that might augment overall control strategies. The purpose of this study is to examine whether ultraviolet

light is capable of killing bed bugs. If successful, UV light could potentially be developed into a new, supplemental treatment for bed bug infestations.

UV light is well known for its ability to damage DNA (Rastogi, et al., 2010). Researchers have taken this knowledge and applied it to the destruction of potentially harmful microbes (Lutz, et al., 2010). However, very little research has been done on the effects of UV light on arthropod pests. One study demonstrated definite mortality in American house dust mite eggs (*Dermatophagoides farinae*) following exposure to UV-C light (Needham, et al., 2006). Dr. G.R. Needham (personal communication) also detailed another ongoing project that suggests UV-C is effective in killing the egg and larval stages of the cat flea (*Ctenocephalides felis)*.

This research project examines the effects of UV-C light on bed bug eggs and first instar nymphs. These two stages were chosen as reasonable starting points based on their anticipated vulnerability to UV light due to smaller body mass. Eggs are typically the most resilient stage, with pesticides having little to no effect on them. If UV light could be used to kill bed bug eggs, it would offer consumers a safe, non-toxic way to treat sensitive areas like mattresses. UV treatment could prove to be an inexpensive addition to current control tactics.

We hypothesize that UV-C light will cause mortality in bed bug eggs and first instar nymphs. We predict that eggs will be the more vulnerable of the two stages due to the high amount of embryonic development occurring at this stage. We also predict that longer exposure times will result in higher mortality. In regards to host-seeking behavior, we hypothesize that individuals surviving treatment will have a decreased ability to locate and/or reach a human host. We predict that longer exposure times will result in increasingly greater damage to host-seeking abilities.

Materials and Methods

Insect:

The study arthropod, *Cimex lectularius* Linnaeus, Harlan strain, was obtained from several colonies originally established by Harold Harlan, formerly of the National Pest Management Association. These colonies were maintained in the Ohio State University Insectary at an average temperature of 25ºC and relative humidity of 20%. Blood-feedings from a human host were conducted at varying intervals according to colony needs.

Exposure Apparatus:

Initial tests were conducted to optimize UV-C exposure while housing bed bugs individually in titer plate wells. A 35-watt UV-C bulb housing was removed from an Oreck Halo (model number UV-ST 3290) vacuum cleaner. However, removing the bulb from the vacuum cleaner eliminated the possibility of using a switch to turn the light on and off. Initial tests involved holding the housing by hand and turning the light on and off by plugging in then quickly unplugging the light from the socket. This created obvious problems with exposure consistency both in regards to distance from the subjects and exposure times. There was also a concern that turning the light on and off could alter the bulbs strength from one exposure to the next.

To avoid these complications, an apparatus was constructed by the Ohio State's Biological Sciences Shop to hold the bulb housing at a consistent distance of 4 cm from test subjects. The apparatus also featured a sliding plate that could be moved aside to begin exposures. This allowed the light to remain on, while offering greater control over the timing of exposures. Using this apparatus and a 96 well titer plate, it was determined that direct exposure

to the bulb would only be possible in rows 3 and 5 or similarly rows 8 and 10 if the opposite end of the plate was used. Therefore, individuals were only placed in these rows for exposure. The temperature change during exposure was also measured to rule out heat as a variable. It was determined that, during the maximum exposure time of 10 sec, the temperature beneath the bulb increased by approximately 3°C. This raised the overall temperature from 21° to 24°C. This temperature range falls well below 45°C, the thermal death point for bed bugs. We concluded that heat generated by the light would have no significant effects on bed bug survival during the short test intervals used in this experiment.

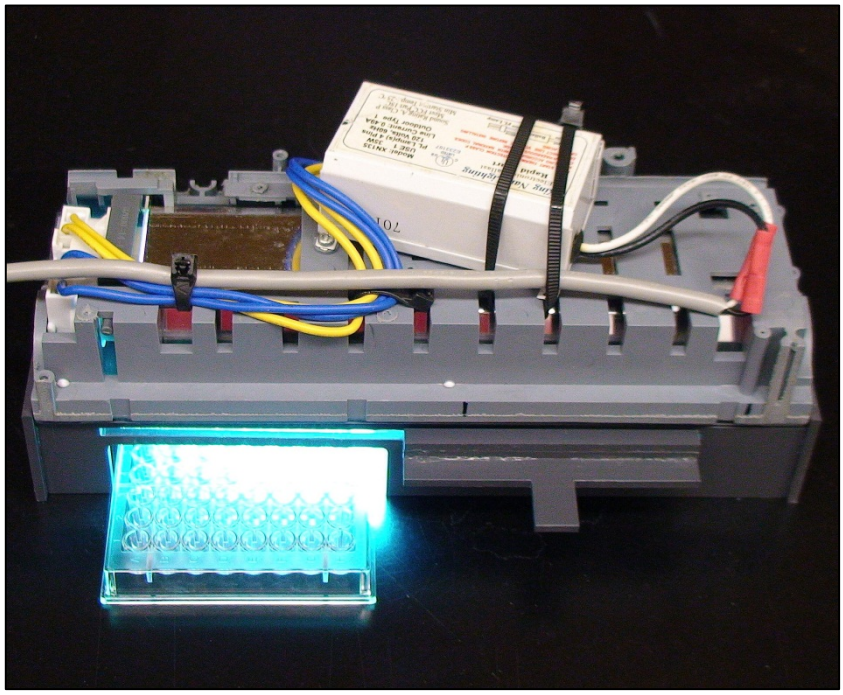

Figure 1: *Bulb housing inserted into apparatus. In this image the sliding plate has been moved aside, allowing exposure of the titer plate to UV light.*

<u>Experiment 1:</u> Determining the effects of UV light on bed bug eggs

Adult bed bugs were collected at random from lab maintained colonies and housed in petri dishes in groups of 7 with a 6:1 female to male ratio. When offspring were required, bed

bugs were blood-fed on a human volunteer host and allowed to reproduce. Each petri dish contained accordion folded filter paper to offer harborage and a substrate on which females could lay eggs. Equal numbers of eggs were chosen at random as either control or test subjects from a total sample size of 640 eggs. On the day of treatment, control groups were harvested from the same general pool of eggs as treatment groups. This ensured they would reflect the same genetic composition and be subject to the same environmental conditions of temperature and humidity as treated groups. To minimize damage due to handling, eggs were harvested by carefully cutting the filter paper around them. The eggs themselves were never touched, and the glue holding them to the substrate was not disturbed. The paper holding an individual egg was then placed in the bottom of a titer plate well with the egg facing up. The titer plate was positioned beneath the apparatus and the sliding plate moved aside to expose test subjects to UV light. Exposure periods were 1, 2, 5, or 10 sec. Eggs were observed following exposure, and final percentage mortality calculated after 14 days.

***Experiment 2:* Determining the effects of UV light on bed bug first instar nymphs**

As described above, adult bed bugs were fed and allowed to reproduce. Eggs were left on the substrate until nymphs emerged. Equal numbers of nymphs were chosen at random to be used as either control or test subjects from a total sample size of 896 nymphs. Again, control groups were harvested on the day of treatment from the same general pool of nymphs as treatment groups. To minimize damage due to handling, nymphs were harvested using a small, delicate paint brush and placed directly into titer plate wells. Exposure protocol was carried out as mentioned above. Nymphs were exposed to UV light for periods of 1, 2, 5, or 10 sec. Each nymph was then provided a small piece of filter paper to use for harborage within the well. In

initial test experiments, nymphs without substrate to stabilize themselves upon were observed wasting a great deal of energy trying to find purchase on the slick plastic surface. After several days of this behavior, they would eventually retract their legs and appear to be dead. Nymphs given filter paper, however, were able to move and behave normally. Thus, to minimize excess exertion as a variable, substrate was provided to all subjects. Nymphs were observed following treatment, and final mortality was calculated after 14 days.

Figure 2: *Images of mortality following treatment. Clockwise beginning from the top: a first instar nymph, a nymph in the process of emerging, an egg that failed to hatch.*

<u>Experiment 3:</u> Determining the effects of UV light on the host-seeking behavior of first instar nymphs

A sample size of 84 nymphs was harvested using the same methods detailed above. Nymphs were randomly assigned to treatment or control groups. On the day of treatment, control groups were harvested from the same general pool of eggs as treatment groups. (As

before, the control groups contained equal numbers to exposure groups.) Using the same exposure protocol as the previous experiments, 30 individuals were exposed to UV light for a period of 10 sec. Due to time constraints, only 12 individuals were exposed at 5 sec. Following an interval of 14 days, the host-seeking behavior of individuals that survived exposure was observed. During the experiment several measures were taken to better mimic real world conditions. Bed bugs are typically nocturnal feeders, thus tests were conducted in the dark during evening hours and lit by a red light, which is invisible to bed bugs. Subjects were also given mattress material as a more natural substrate to crawl across. A rectangular piece of mattress ticking with an area of 19 x 34 cm was spread across a porcelain tray. A human host placed an arm on the tray to span one short side of the material. Individual bed bugs were placed on the far edge of the material at a distance of 34 cm. (Only the conductor of the experiment was present in the room while behavior work was conducted. These conditions were maintained to eliminate the chance of bed bugs being attracted to and/or confused by multiple sources of carbon dioxide and body heat.) Subjects were given a maximum time of 15 minutes to make contact with the host. If individuals failed to reach the host during this time, the attempt was counted as a failure. Success rates and search times were recorded.

Results

Experiment 1: Determining the effects of UV light on bed bug eggs

Two, 5, and 10 sec treatment groups demonstrated higher levels of mortality than control groups. There was no appreciable difference between the 1 sec treatment group and its corresponding control group.

	Exposure Time			
	10 sec	5 sec	2 sec	1 sec
Treatment Mortality (%)	95*	100	35	21
Control Mortality (%)	0	3	6	18*

Table 1: *Egg stage mortality in treatment and corresponding control groups (n=640, 40 replicates with 16 individuals each)*

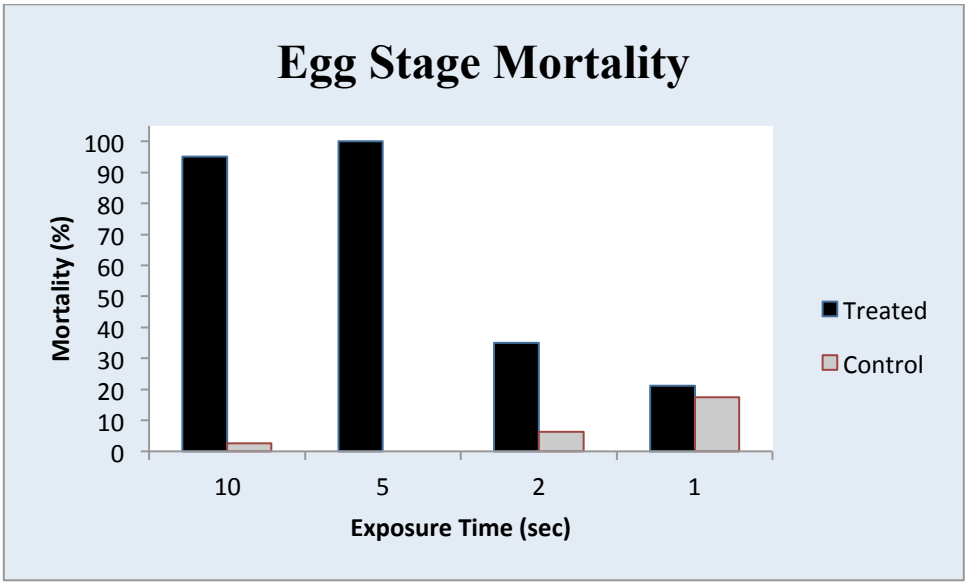

Figure 3: *Egg stage mortality in treatment and corresponding control groups after 14 days*

*See Discussion

***Experiment 2:* Determining the effects of UV light on bed bug first instar nymphs**

The 5 and 10 sec treatment groups demonstrated a higher level of mortality than control groups. 1 and 2 sec exposure groups experienced very little to no mortality, and there was no appreciable difference from their respective control groups.

	Exposure Time			
	10 sec	5 sec	2 sec	1 sec
Treatment Mortality (%)	62	24	3	0
Control Mortality (%)	0	1	1	0

Table 2: *Nymphal stage mortality in treatment and corresponding control groups (n=896, 56 replicates of 16 individuals each)*

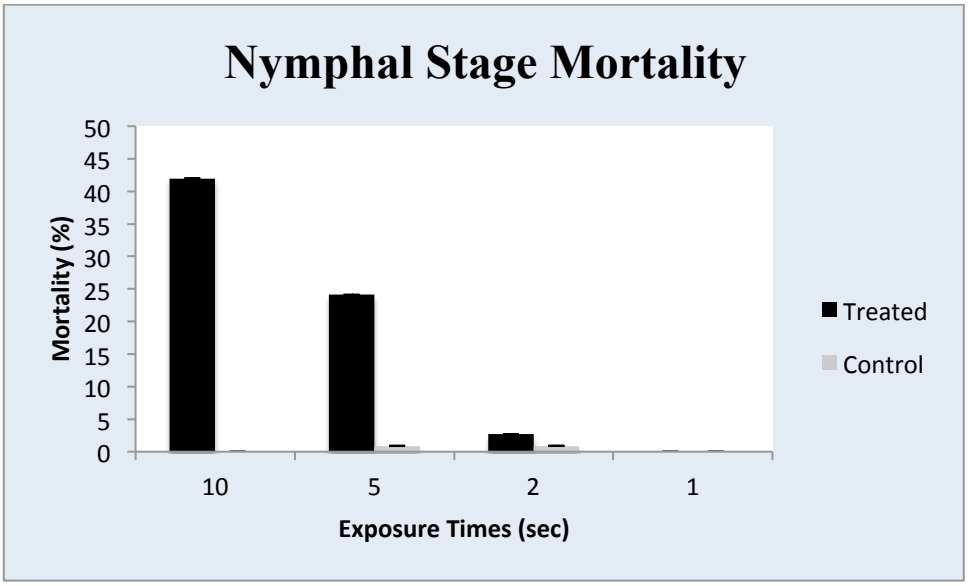

Figure 4: *Nymphal stage mortality in treatment and corresponding control groups after 14 days*

<u>Experiment 3:</u> Determining the effects of UV light on the host-seeking behavior of first instar nymphs

Host seeking success rate:

Exposure to 10 sec of UV light decreased the host-seeking success rate of first instar nymphs. Only 13% of individuals were successful in reaching the host during the 15 min of allotted time. In contrast, the control group was able to make contact with the host 90% of the time. Exposure to 5 sec of UV light also decreased the success rate of nymphs. Only 33% of treated individuals were successful in reaching a host, while 100% of individuals in the control group were successful.

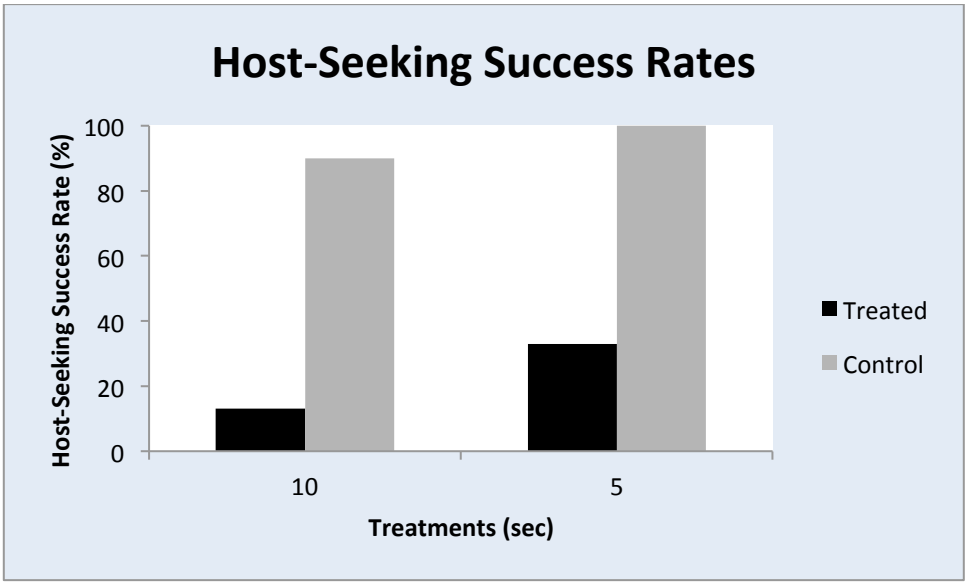

Figure 5: *Host-seeking success rates in treatment and corresponding control groups*

Time Required to Reach a Host:

The average time required to locate a host was also affected by UV exposure. The 10 sec treatment group required a significantly longer time compared to the control ($t\ (58)$=7.87,

$P < 0.0001$, one-tailed, $Mean_{treatment} = 873$ sec, Std =102 , $Mean_{control} = 527$ sec, Std =218 , unsuccessful individuals were assigned the maximum time of 900 sec). The 5 sec treatment group also required a significantly longer time to reach a host in comparison to its control (($t(22) = 4.36$, $P = .000126$, one-tailed, $Mean_{treatment} = 725$ sec, Std =273 , $Mean_{control} = 359$ sec, Std = 102, unsuccessful individuals were assigned the maximum time of 900 sec).

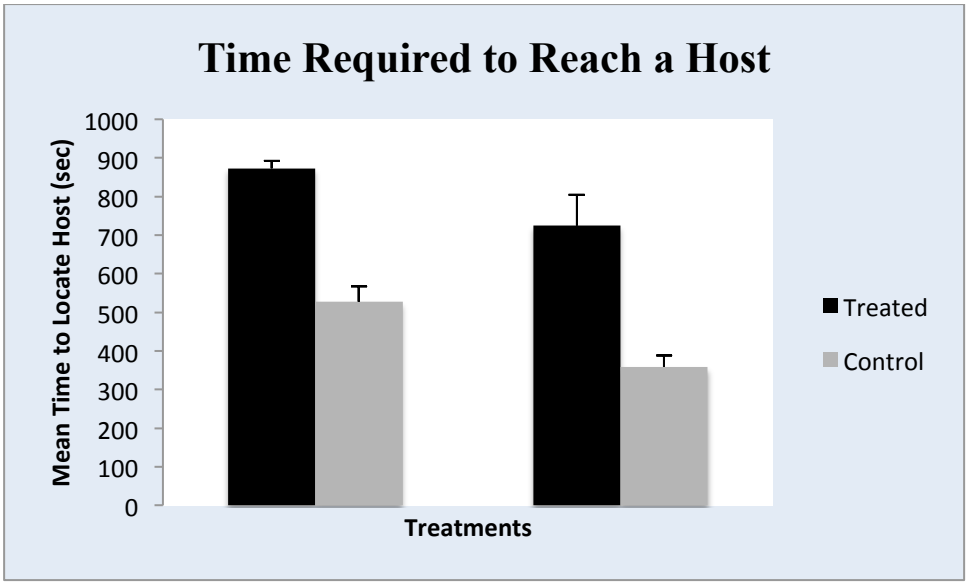

Figure 6: *Mean time required to reach a host in treatment and corresponding control groups (Unsuccessful individuals were assigned the maximum time of 900 sec.)*

Discussion

The damaging effects of ultraviolet light on DNA have been documented (Rastogi, et al., 2010); however, its impact on arthropod survival and behavior has gone largely unexplored. Based on current research demonstrating significant damage to flea eggs and larvae (G.R. Needham, Personal Communication), this study investigated whether similar effects could be observed with bed bugs. We hypothesized that UV-C light would cause mortality in bed bug eggs and first instar nymphs, and that increased exposure would result in higher mortality

compared to shorter exposures. We also hypothesized that host-seeking abilities in individuals surviving treatment would be negatively impacted. We predicted that longer exposure times would have a greater negative impact on host-seeking abilities than shorter exposure times. The results of our study supported both hypotheses.

Egg stage mortality was significantly higher in groups exposed to 2, 5, or 10 sec of UV light. 5 and 10 sec exposures were particularly devastating to hatch rate, with almost no subjects surviving. One sec exposure appeared to have no effect on mortality. It should be noted that mortality within the 1 sec control group was extremely high compared to other controls. There was no indication as to what may have caused this increase. However, since control deaths were elevated, it does cast some doubt on the accuracy of treatment results for the 1 sec exposure. Mortality in first instar nymphs was somewhat less dramatic, with only the 10 and 5 sec exposures showing significant effects. The 10 sec exposure was the only treatment to cause mortality higher than 50%. This followed our prediction that eggs would be the more vulnerable of the two stages. Whether this is due to the amount of development occurring, body mass, or some other factor is still unknown.

Our prediction that longer exposure times would induce higher mortality was supported by results in the nymphs. Mortality consistently dropped as lower exposure times were used. However, the 10 sec group within the egg category did not follow the trend, with mortality falling slightly below that of the 5 sec group. A few eggs successfully hatched in the 10 sec group the day after exposure, indicating the eggs were older than the other test subjects. We believe treatment was less effective because these eggs were further along in their development.

Behavioral observations supported the hypotheses that host-seeking abilities would be adversely impacted by exposure to UV light. Both the 5 and 10 sec exposures significantly decreased the host-seeking success rate in first instar nymphs. There was also a significant difference in the time required to reach a host, with exposure groups performing worse than their respective controls.

The prediction that a longer exposure time would result in a greater decrease in host-seeking success was also supported. The 5 sec exposure group had a significantly higher success rate than the 10 sec group. It should be noted that this experiment does not distinguish between causes leading to the inability to find a host, such as damage to sensory receptors or physical damage resulting in lack of energy or decreased capacity for movement. Further experiments would be required to distinguish between the causes for failure or increased host-seeking time. Future experiments would also benefit from performing tests on adults and other instars, as well as an increased sample size for host-seeking behavior.

This project strongly suggests that UV light is effective, both in killing bed bugs, and in impairing their ability to reach a host. Perhaps the most encouraging aspect of this study was the dramatic effect UV light had on the egg stage. While eggs have proven to be most resistant to current control tactics, they are highly vulnerable to UV treatment. This knowledge could be valuable to the pest control industry as they continue to develop novel tactics in bed bug control. Particularly appealing is the fact that UV light is a non-chemical control that, with proper shielding, could be used by consumers without the aid of professionals. Whether or not UV light could be harnessed into a practical control method is yet to be seen, but this study offers initial proof of concept that it is possible to kill bed bugs using only light.

Acknowledgements

I would like to thank Dr. Glen Needham and Mr. George Keeney for their advice and assistance throughout this project. Further thanks go to Zeynep Benderlioglu for her assistance in statistical analysis. Finally, I would also like to thank the members of the Ohio State Biological Sciences Shop for creating the bulb apparatus used in the experiments.

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