ABSTRACT

The common bed bug, *Cimex lectularius*, has long claimed a protist place in the homes, beds, furniture, and luggage of humans. With recent spikes in bed bug populations, especially in areas with poor demographics, and few effective ways to prevent infestations, it is an opportune time to study these prolific pests. Bed bugs utilize multiple receptors to detect heat, gas emission, and scent. With special focus on olfaction, trials are being conducted which test the efficacy of various essential oils and their mixtures in repelling bed bugs. A special focus on the behavior of the bugs was observed and recorded for these purposes. Experimentation consisted of 5 trial types. Each one utilized a test strip soaked in a test solution and a control strip soaked in a control solution. Trials varied in time conducted (from seconds to 24 hours or more) as well as in many other factors such as the container in which the trials were conducted, number of bugs used, and scent solution and control used. Variations allowed for the collection of many types of data and the application of results obtained in previous trials to help set parameters for subsequent ones. Applications of this experimentation are numerous.

INTRODUCTION

*Cimex lectularius* (Fig. 1), while suspicious in appearance, is a menace in the homes, apartments, public shelters, and luggage of people around the world. Bed bugs are a resilient pest and their rate of infestation continues to propagate throughout the world. What is more concerning still is the resilience of bed bugs to multiple forms of treatment. Namely, insectsicides are becoming less effective and treatment, while effective in killing large numbers of the parasites, cannot prevent reinfection and are therefore rendered obsolete in areas with high infestation rates (Raab et al., 2016). For this has been attributed in large part to increased travel and ineffective treatments. Bed bugs are small 2-5.4 mm obligate blood feeders known for their lack of wings and traumatic insemination (a means of reproduction in which the male bypasses the genital opening and pierces the female's abdomen for insemination) (Anderson et al., 2009). Their keen sensory receptors which allow them to detect the heat, carbon dioxide, and scent of their preferred host, humans, is what makes bed bugs such a consistent and costly problem. With such a prolific host and diverse locations to infest, *C. lectularius* continues to propagate throughout the world. What is more concerning still is the resilience of bed bugs to multiple forms of treatment. Namely, insecticides are becoming less effective and treatment, while effective in killing large numbers of the parasites, cannot prevent reinfection and are therefore rendered obsolete in areas with high infestation rates (Raab et al., 2016). For this has been attributed in large part to increased travel and ineffective treatments. Bed bugs are small 2-5.4 mm obligate blood feeders known for their lack of wings and traumatic insemination (a means of reproduction in which the male bypasses the genital opening and pierces the female's abdomen for insemination) (Anderson et al., 2009). Their keen sensory receptors which allow them to detect the heat, carbon dioxide, and scent of their preferred host, humans, is what makes bed bugs such a consistent and costly problem. With such a prolific host and diverse locations to infest, *C. lectularius* continues to propagate throughout the world.

MATERIALS AND METHODS

**Type 1**

Type 1 trials emphasized the repulsive or attractive nature of essential oils with a focus on the immediate behavior of an active bug. Of the numerous essential oils we have available, we were able to test three: peppermint oil, eucalyptus oil, and lavender oil. 100% denatured ethanol (EtOH) or mineral oil were used as the control. All solutions, including the controls, were made in a 4 oz. glass jar. Test solutions consisted of 0.5% of the essential oil and 99% of either EtOH or mineral oil. For the control, either 100% EtOH or 100% mineral oil were used. Initially a large cotton ball was divided in half; in one half we added 0.5mL of test solution, and to the other half we added 0.5mL control solution. A bed bug was retrieved from the colony and placed in the middle of a small white, plastic sizing tray (20cm x 26cm). As the bed bug was crawling in the tray, either the test ball or the control ball was placed about 3cm in front of the bed bug (Fig. 3). We randomized the order of the 10 attempts; five with the test solution and five with the control solution. In later replicates of Type 1 trials, we switched from cotton balls to cotton swabs that were dipped in either the test or control solution. Responses were recorded with +, - or 0 based on behavior. + Indicates a positive response, - indicates a negative response, and 0 indicates failed attempts or no response.

**Type 2**

Type 2 trials focus on the more long-term effects of the essential oils and whether bed bugs will transverse an essential oil ‘barrier’. The trial employed the use of the same test and control solutions as Type 1 but used different methods for setting up the trial and monitoring bed bug response. A 3cm wide strip of paper towel was used to bisect two large trays. One strip was saturated with 0.5mL of the test solution and the other with 0.5mL of the control solution (Figs. 4-5). Five bed bugs were then placed and retrieved at the far end of each tray. Responses were recorded upon starting the trial and after 20 minutes by monitoring the time it took for the bed bugs to begin crossing the paper towel strip.

**Type 3**

Type 3 trials, similar to Type 2 trials, explore the more long-term behavior of bed bugs and use the same essential oil and pheromone combination solution. The odorant co-introductions, spread of existing matrilines, and high rates of pyrethroid repellency against bloodsucking insects. *Physiological Entomology* 110: 382-392.

**Type 4**

In Type 4 trials we began looking into even longer trial times with a focus on how prolonged exposure to an essential oil and pheromone solution impacts the behavior of bed bugs. Type 4 trials were conducted by mixing bed bug alarm pheromone with hexanol to use as the test solution and using straight hexanol as the control solution. To begin the trial, two large trays were set up next to one another and carefully placed on glass cups. The entire setup was contained within a large metal tray. Both the test and control cups were prepared. Five bed bugs were gathered from the colony and placed on the stack of paper towels in each tray. A cotton swab was then broken in two with one end dipped in the pheromone and the other end dipped in the test solution. The swabs were placed at the center of the far end of each tray. The trays were then covered by a clear plastic sheet and labeled “control” and “test” respectively. A picture was taken at the end of 15 minutes and again after 24 hours. To quantify the results, the distance from the cotton swab (in cm) was recorded for each bed bug using a meter rule for scale.

**Type 5**

In Type 5 trials we extended the total trial time further and began to look at whether the bugs would crawl inside of an open box containing the essential oils and pheromone. A number of new variables were introduced in these trials. Two largeplexiglas containers housed the control and test. Within each container was a small cardboard box that was carefully taped to cover any small holes or folds that a bed bug might crawl into (Fig. 10). This was done to aid in gathering the final results. The test solution utilized a mixture of 6% peppermint oil, 18% hexanol and 6% octenol, and a mineral oil base. The control solution contained the mineral oil base. Ten bed bugs were placed at the far end of each plexiglas container, the lid was replaced, and the bugs were left for 24-48 hours.

RESULTS

Trials are still ongoing but preliminary results (Type 1, 20 Type 2, 20 Type 3, 10 Type 4, and 10 Type 5 trials) indicate that there were no discernible differences in the test and control for Type 1 and 3 trials; but for Type 2, 4, and 5 trials, the bed bugs tended to avoid the test solution by staying outside of the box that contained the paper towel in Type 2 trials, remained in the paper towel shelter in Type 4 trials, and stayed outside of the box in Type 5 trials.

DISCUSSION

Throughout this series of trials, modifications were continually made to improve efficiency and efficacy. Type 1 trials first made use of large cotton balls instead of swabs. The cotton balls were unwieldy and left a residue which could trap and kill the bed bugs. The switch to swabs greatly reduced the residue and made it easier to record results. Multiple materials were tried for the test strip in Type 2 trials, but a simple rolled up paper towel yielded the best results. Type 3 trials began with a circle of paper towel rather than chromatography paper, but the paper towel tended to leave spaces allowing the bugs to crawl underneath. The rigidity of the chromatography paper prevented this. Type 1-3 trials were conducted in the winter, with the lab in which we were conducting the trials cooler. Therefore, the bed bugs were less active, making it harder to obtain meaningful results. Type 4 trials remained mostly unchanged. Type 5 trials allowed for a lot of variation in trial duration, and, in the most recent attempts, the separator between each half of the two plexiglas containers was removed and bed bugs were placed a greater distance from the test and control solution. General trends seem to indicate that the longest trial duration of 24 or more hours yielded the most discernible results for bug behavior. These longer trials introduced an added impediment by allowing for the measurements of distance and number of bugs in certain locations rather than perceived behavior. Statistics were not utilized for results as the experiment is still in its early stages and few notable trends were evident. Future plans are to continue Type 5 trials with a new box holding the test and control bulbs so that the bugs are not able to take refuge in small cracks or crevices.

ACKNOWLEDGEMENTS

Special thanks to Dan Short of Ashrethold and Hicki Hicks of Layer Industries One for providing bed bugs, essential oils, pheromones, and other materials and the Department of Biology and Chemistry for providing the lab space.

REFERENCES


