Behavioral Activity of Methyl Benzoate Against the Common Bed Bug (Hemiptera: Cimicidae)[1]

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J. Entomol. Sci. 55(3): 344–349 (July 2020)

Abstract We evaluated a botanical compound, methyl benzoate, which was previously shown to have insecticidal activity on several agricultural pests, for its behavioral action on the common bed bug, *Cimex lectularius* L. Methyl benzoate, along with acetophenone and Cirkil™, a commercially available bed bug control product, exhibited repellent action against bed bugs in an EthoVision video system designed to track the movement of individuals.

Key Words common bed bug, methyl benzoate, behavior, repellency

The common bed bug, *Cimex lectularius* L., is a blood-sucking insect pest of humans whose incidence is apparently on the rise worldwide (Doggett et al. 2012, 2018, Potter et al. 2015, Doggett et al. 2018). Bed bug infestations are a public health concern even though they are not known to transmit human disease. Bites from these hematophagous insects can cause no reaction or severe cutaneous and systemic allergic responses (Goddard and deShazo 2009, deShazo et al 2012, Doggett et al. 2012). In addition to these physical issues, infestations with bed bugs can negatively affect an individual's mental health. Not only can anxiety and depression develop from a bed bug infestation but also the social stigma that is associated with bed bug remediation can result in additional undue stress (Goddard and deShazo 2009, Doggett et al. 2012, Susser et al. 2012, Romero et al. 2017).

Certain compounds isolated from the common bed bug were originally thought to function only as alarm pheromones (see Levinson and Bar Ilan 1971), although recent studies have demonstrated that these compounds can act as attractants, primarily at lower concentrations (Gries et al. 2015, Ulrich et al. 2016). Here, we evaluated methyl benzoate, Cirkil™ (a commercially available botanical pesticide), and acetophenone (structurally similar to methyl benzoate) for behavioral effects on the common bed bug. Methyl benzoate has been previously shown to kill several taxa of insects in laboratory bioassays (Feng and Zhang 2017, Zhang and Feng 2017, Chen et al. 2019, Feng et al. 2018, Larson et al. 2019, Morrison et al. 2019). Additionally, acetophenone and Cirkil have been shown to be toxic to bed bugs (see Feldlaufer and Ulrich 2015, Larson et al. 2019). Bed bug movement was characterized through use of EthoVision® XT software to record positional data of individuals within a defined arena when exposed to individual compounds.

[1]Received 19 September 2019; accepted for publication 21 October 2019.

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Materials and Methods

Insects. Harlan strain (Harold Harlan, Crownsville, MD) bed bugs, which are susceptible to pyrethroid insecticides, were reared and maintained in glass Mason jars (480 ml) containing filter paper harborages at the USDA-ARS facility in Beltsville, MD, as previously described (Feldlaufer et al. 2014). Using an *in vitro* artificial feeding system (Feldlaufer et al. 2010), we fed the colony expired human red blood cells and plasma weekly to engorgement. The colony was maintained at ambient conditions (25 ± 2°C and 40 ± 15% relative humidity). All assays used bed bugs that had not fed in 8 days.

Chemicals. The commercially available compounds methyl benzoate and acetophenone were purchased from Sigma-Aldrich (St. Louis, MO). Cirkil ready-to-use was purchased from an online source (http://ePestSolutions.com).

EthoVision behavioral assays. To determine whether the tested compounds altered bed bug behavior (i.e., were attractive, repellent, or neutral), a modified video tracking protocol reported by Ulrich et al. (2016) using an EthoVision XT recording system and software (Noldus Information Technology Inc. Leesburg, VA) was used to record positional data of individual bed bugs. Briefly, recordings were performed using a Basler acA 1300-60 gm high-resolution monochrome camera (Basler AG, Ahrensburg, Germany) suspended 30 cm above a glass petri dish arena (150 × 20 mm). The arena was illuminated with an Axton AT-8 infrared LED illuminator (AxtonTech, North Salt Lake, UT). The petri dish arenas were lined with a single sheet of filter paper (Whatman® no. 4; diameter, 15 cm; GE Healthcare Life Sciences, United Kingdom). Four filter paper disks (Whatman, grade AA; diameter, 6 mm) were placed into the arena at the 3, 6, 9, and 12 o'clock positions. One disk was treated with 10 μl of a control (acetone) or test compound. The treated disk was dried for 15 min prior to placement in the arena. A circular area around the treated disc (diameter, 15.6 mm) was delineated as a treatment zone within the EthoVision software, and time spent within this zone was calculated for determination of attractancy or repellency (or neutrality). An individual bed bug was placed into the center of the arena and covered with an inverted pipette tip for a 5-min acclimation period. After the bed bug was released, video recording was performed for 1 h under ambient temperature and humidity. Acclimation periods and video recordings were conducted in darkness. Sixteen replicates (eight per sex) were run for each tested compound. A new individual was used for each replicate, and following each replicate, the filter paper lining was replaced after the glass petri dish was cleaned with acetone. To reduce positional effects, the treated disks occupied different positions for each replicate. GraphPad Prism version 8 (GraphPad Software, San Diego CA) was used for all statistical analyses and for figure creation.

Results

EthoVision behavioral assays. Methyl benzoate, acetophenone, and Cirkil were repellent to adult *C. lectularius* over a 1-h period (Fig. 1A–D). The average amount of time that an adult individual would spend within the target zone of an untreated control disc was approximately 70 (± 13 SEM) s. All treatments resulted in the reduction of the time spent within the target zone to under 5 s. Methyl

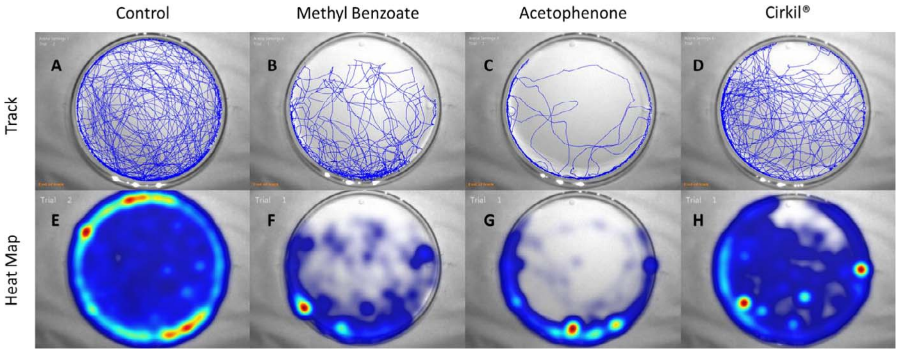

Fig. 1. Representative tracks (A–D) and corresponding "heat maps" (E–H) of individual *C. lectularius* movement within a petri dish arena for 1 h. A and E represent control movement, and B, C, and D represent methyl benzoate, acetophenone, and Cirkil, respectively. Heat maps were generated within EthoVision XT to visualize the location frequency of individuals based on a color gradient, where red indicates the most time spent by an individual. All treated discs are located at the 12 o'clock position.

benzoate and Cirkil reduced the average time spent within the target zone by 16- to 17-fold to 4.2 ($\pm$ 3.3 SEM) and 4.3 ($\pm$ 1.6 SEM) s, respectively. While acetophenone reduced the average time spent within the target zone by 44-fold to 1.6 ($\pm$ 0.78 SEM) s, this was not significantly different from that of methyl benzoate or Cirkil. Visualizations of individual bed bug tracks and "heat maps" (representing the time spent within certain areas of the petri dish arenas) are given in Fig. 1. Typical control tracks show the individual walking around the entire arena (Fig. 1A), and the corresponding heat map shows that the individual spent more time in several areas within the arena, indicated by the color gradient (Fig. 1E). Arenas containing either a methyl benzoate disc or a disc treated with either acetophenone or Cirkil (Fig. 1B–D) show the individual tracks avoiding the area at the top of the petri dish, and the corresponding heat maps (Fig. 1F, H) show that the individuals avoided the areas near the treated discs. This was most apparent for methyl benzoate and acetophenone (Fig. 1F, G) where the individual spent most of the time as far away from the treated disc as physically possible. For Cirkil, it appeared that the individual spent most of the time in two areas away from the treated disc (Fig. 1H)

Discussion

Botanical compounds have been increasingly studied for their insecticidal activity against a variety of insect pests, including those of stored products, urban/medical pests, and household pests, such as termites and bed bugs (Tong and Bloomquist 2013, Feldlaufer and Ulrich 2015, Kedia et al. 2015, Lee et al. 2018). The increase in popularity is likely due to their low mammalian and environmental toxicity and lower cost for control formulation development, as well as the potential for effective control against species that have developed resistance to current synthetic chemical controls (Kannathasan et al. 2011, Tong and Bloomquist 2013, Kedia et al. 2015, Santos et al. 2017). There are several successful methods, both chemical and nonchemical, that are used to control bed bugs (Kells 2018, Lee et al. 2018).

Botanical pesticides, created from essential oil products, are typically complex mixtures of a variety of volatile constituents and not all of the components will have a toxicological effect on insects. However, these botanical "pesticides" can have additional desirable attributes, such as acting as a behavior modifier through attraction or repellency (Isman 2006, Isman et al. 2011, Miresmailli and Isman 2014). Because repellency or attraction cannot be measured directly, as they are variables that are not directly observable, we can infer their levels based upon behaviors we can measure. For instance, "avoidance" is a measurable behavior that is often used to infer repellency. In this study, we use avoidance by a bed bug to demonstrate that methyl benzoate, acetophenone, and Cirkil have repellent activity by significantly reducing the amount of time an individual adult bed bug spent near a treated target. Although insect repellent development has been primarily focused on creating personal-use products that repel biting arthropods associated with disease transmission (see Katz et al. 2008), repellents could additionally be used to repel bed bugs (Wang et al. 2013). Personal repellent use may not be feasible for bed bug control due to the need to treat either a very large area or the time of day required to treat (bedtime). However, although the use of a repellent would ultimately not solve a bed bug infestation, there are several other potential ways for repellents to be used against this blood-sucking pest. For example, travelers' belongings could be treated with a repellent to prevent bed bugs from taking refuge within them or clothes of workers who have an increased chance of visiting infested locations could be treated to lessen the chance of a bed bug being unwittingly transported to another destination (Hentley et al. 2017, Anderson et al. 2018). Additionally, a repellent could be used to increase the efficiency of bed bug pesticide treatments (see Benoit et al. 2009) or cause individuals to move into a more centrally located area within a dwelling, allowing for a concentrated application of the pesticide.

Research on plant-derived chemicals will continue to be conducted to further the goal of developing affordable, effective, and safe pest management tools. This current study provides evidence that methyl benzoate, a botanical compound found to be a part of the scents of several different flowers (see Negre et al. 2003), has repellent activity and is toxic to the common bed bug when in a confined space (Larson et al. 2019).

Acknowledgments

We thank members of the Armed Forces Pest Management Board (Silver Spring, MD) for their help in obtaining blood products to maintain all strains of bed bugs used in this study. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an Equal Opportunity Employer.

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