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Behavioral Responses of the Bed Bug to Insecticide Residues

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ABSTRACT The recent resurgence of bed bugs, Cimex lectularius L. (Heteroptera: Cimicidae), has increased the demand for information about effective control tactics. Several studies have focused on determining the susceptibility of bed bug populations to insecticides. However, behavioral responses of bed bugs to insecticide residues could influence their efficacy. The behavioral responses of bed bugs to deltamethrin and chlorfenapyr, two commonly used insecticides for bed bug control in the United States, were evaluated. In two-choice tests, grouped insects and individual insects avoided resting on filter paper treated with deltamethrin. Insects did not avoid surfaces treated with chlorfenapyr. Harborages, containing feces and eggs and treated with a deltamethrin-based product, remained attractive to individuals from a strain resistant to pyrethroids. Video recordings of bed bugs indicated that insects increased activity when they contacted sublethal doses of deltamethrin. Insecticide barriers of chlorfenapyr or deltamethrin did not prevent bed bugs from reaching a warmed blood source and acquiring blood meals. We discuss the impact of these responses on bed bug control practices.

KEY WORDS Cimex lectularius, behavioral responses, insecticide avoidance, locomotor activity

The recent resurgence of bed bugs, Cimex lectularius L. (Heteroptera: Cimicidae), has renewed interest in effective control tactics, particularly on the efficacy of insecticides on different bed bug populations. Several studies have focused on the determination of susceptibility of bed bug populations to commonly used insecticides (Boase et al. 2006, Moore and Miller 2006, Karunaratne et al. 2007, Romero et al. 2007b). However, behavioral responses of bed bugs to insecticide residues could also influence their efficacy.

Bed bugs have cryptic habits and are usually found assembled along mattress and box spring seams, or cracks, crevices, or edges of furniture and other locations (Potter et al. 2006). A typical harborage contains adults, nymphs, eggs, egg shells, shed skins, and feces. Aggregation seems to be promoted by thigmotaxis (Usinger 1966) and/or the presence of chemical cues (Marx 1955, Levinson and Bar Lan 1971, Siljander et al. 2007). Even with thorough inspections, some bed bugs go undetected and therefore are not directly treated. Thus, insecticide efficacy relies in part on insects contacting the insecticide residues while searching for a blood meal or returning to harborages. Little is known about the interaction between bed bugs and insecticide residues.

Insecticides can influence insect behavior through their detection by a functional sensory system or by disrupting the normal function of the sensory or central nervous system or hormonal system (Georghiou 1972, Haynes 1988). Insects avoid prolonged exposures to insecticides by moving away from the treated area either because of repellency (after perceiving insecticides at some distance) or because of irritancy (after contacting the treated area).

Sensory-mediated "repellency" or "irritancy" of sublethal neurotoxic effects can complicate the interpretation of laboratory evaluations of insecticide residues. These behavioral responses can increase or decrease exposure to potentially lethal residues. The role of irritancy and/or repellency of pyrethroid insecticides has been studied in other medically important and household insects, e.g., cockroaches, ants, termites, kissing bugs, mosquitoes (Ebeling et al. 1966, Knight and Rust 1990, Su and Scheffran 1990, Diotaiuti et al. 2000, Chareonviriyaphap et al. 2004).

The objective of this study was to evaluate the behavioral responses of bed bugs to residues of a pyrethroid (deltamethrin) and a pyrrole (chlorfenapyr), two of the most commonly used insecticides in bed bug control in the United States.

Materials and Methods

Insects. Colonies of bed bugs were maintained at 26° C, $65 \pm 5\%$ RH, and a photoperiod of 14:10 (L:D) h. The bed bug strains were collected from human dwellings in Los Angeles, CA (LA-1), Cincinnati, OH (CIN-1, CIN-3), and Lexington, KY (LEX-1). The strain LA-1 is susceptible to deltamethrin, whereas the other strains are highly resistant to the same pyrethroid (Romero et al. 2007a, b). Insects were fed with a parafilm-membrane feeder containing citrated rab-

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bit blood that was heated to 39°C with a circulating water bath (Montes et al. 2002). Bed bugs were evaluated in behavioral bioassays 7–12d after adult emergence. Bed bugs were not fed as adults. This procedure standardized both age and hunger level. Because bugs are adapted to survive long periods (months) without taking a blood meal (Usinger 1966), our test insects were hungry but not severely stressed.

Choice Tests. Insects were offered two tents made of filter paper (15 by 12 mm, Whatman no. 2) folded in the middle to offer a tent-like shelter of 15 mm length by 5 mm height with two open ends. Group responses were carried out in flat-bottomed Pyrex bowls (12.4 cm diameter by 6.0 cm height; Corning, Corning, NY) whose surfaces were covered with a white filter paper (110 mm diameter; Whatman no. 2), fixed to the glass with double-sided tape. After each assay, papers were removed and bowls were rinsed with acetone. During the photophase, arenas were illuminated with 40-W fluorescent tubes placed 60 cm above the arena surfaces, which provide a light intensity of ≈660 lux. Under these conditions, bed bugs would seek harborages during the day.

Individual responses were carried out in 500-ml glass beakers whose bottom-inside surfaces were covered with white filter paper (70 mm diameter; Fisherbrand, quality P4). Paper was fixed to the glass with double-sided tape to prevent bed bugs from crawling under the paper. After each assay, beakers were cleaned as mentioned earlier for bowls. During the photophase, each one of three blocks of 16 arenas was illuminated with a 19-W fluorescent light that was placed 60 cm above the arena surfaces (light intensity of ~300 lux at arena level).

In the first experiment, 10 bed bugs (1:1 sex ratio) were offered tents that had been sprayed until the paper was saturated with a suspension of 0.06% Suspend SC (Bayer Environmental Science, Montvale, NJ), 0.5% Phantom (BASF, Research Triangle Park, NC), or distilled water (\approx 50 μ l per tent). Tents were allowed to dry for 2 h before being placed in arenas. Six replicates were performed for each insecticide.

In a second experiment, assays were conducted to measure individual responses to deltamethrin or chlorfenapyr (technical grade) that corresponded to the maximum labeled rate (Suspend) or the regular recommended labeled rate (Phantom) of the commercially available formulations. Individual insects were offered a tent impregnated with 50 μ l of an insecticide-acetone solution of 0.06% deltamethrin (99% purity; Chem Service, West Chester, PA) or 0.5% chlorfenapyr (99.3% purity; Chem Service) and a control tent treated with 50 μ l of acetone. The acetone was allowed to evaporate. Unless otherwise stated, 60 replicates (30 males and 30 females) were performed.

In a third experiment, individuals from the CIN-1 pyrethroid-resistant strain (1:1 sex ratio, 48 replicates) or groups of 10 insects (1:1 sex ratio, 12 replicates) were exposed to arenas (beakers for individuals or bowls for groups) that contained an established harborage and a clean tent. The established harborage was obtained by placing 10 fed insects (1:1 sex ratio)

in a tent for 48 h to allow them to produce body secretions, fecal matter, and eggs. In follow-up experiments, established harborages were sprayed with Suspend and offered to 10 insects (1:1 sex ratio, 12 replicates) along with a water-treated tent. Finally, 20 insects (1:1 sex ratio, six replicates) were offered two established harborages: one treated with Suspend and the other treated with distilled water.

All assays lasted ≈ 16 h (from ≈ 1630 to 0830 hours the next day) with the following light-dark regimen: lights off at 2100 hours and lights on at 0700 hours (the same light cycle used during rearing). Room temperature remained at $24 \pm 2^{\circ}$ C. Insects were acclimated to the environment for 15 min by restricting them in a shell vial (21 mm diameter by 70 mm height) which was placed inverted in the center of the arena. Insects were released by lifting up the shell vial. At the end of the test, the location of insects resting on a tent or wandering in the arena was recorded. Number of responses was analyzed by a binomial test with exact two-tailed P values, with the null hypothesis that the

tent were chosen with equal probability.

Video Recording of Bed Bug Activity. Activity of individuals from the pyrethroid-resistant CIN-1 and -susceptible LA-1 strains was recorded while exposed to a single tent treated with a dry deposit of 0.06% deltamethrin, 0.5% chlorfenapyr, or an acetonetreated (50 μ l) control tent. The susceptible strain LA-1 was also exposed to a lower concentration of deltamethrin (0.006%) on a tent. Evaluations were conducted in glass bowl arenas as described in the group choice response experiments. The recording was carried out with a black-and-white video camera (model MC3651H-2; Pelco, Clovis, CA) suspended 1 m above the arena surfaces and a time lapse recorder (model AG-RT850; Panasonic, Osaka, Japan). Six arenas were simultaneously recorded. Individual insects in shell vials were acclimated for 15 min in the arena. During the photophase, three 19-W fluorescent lights were place above arenas pointing toward the walls and ceiling of the room where assays were conducted. The light intensity at arena level was ≈40 lux. During the scotophase, a dim red light (20 W) pointing toward the ceiling provided a homogeneous illumination required for recording. Time that insects spent outside tents was divided by total time of the test period, and the square roots of these proportions were arcsine transformed before analysis of variance (ANOVA) and Tukey's pairwise comparison (at 5% level of significance) with MINITAB (2005). Twelve replicates were performed for each strain.

Responses of Host-seeking Insects to Insecticide Barriers. This experiment was conducted in plastic containers (5 cm diameter by 5 cm height); the open end of each container was covered with a fine mesh fabric (organza; Fashion Fabrics Club, St. Louis, MO). This fabric top made contact with a parafilm-membrane feeder described earlier. Insects reached the heated surface by crawling up a strip of cardboard (20 mm wide by 70 mm height). A paper strip (20 mm wide by 50 mm height, Whatman no. 2) was impregnated with an insecticide-acetone solution (200 µl of 0.06%

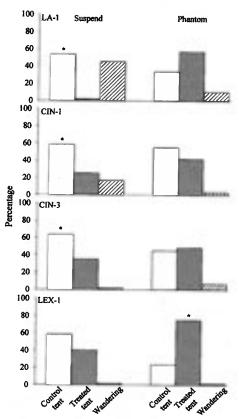


Fig. 1. Preference of a group of bed bugs (five females, five males) from four strains for a paper tent sprayed with an insecticide [Suspend, 0.06% (AI) or Phantom, 0.5% (AI)] or a tent sprayed with distilled water. After a 16-h test period, the number of insects resting on any of the treatment harborages or wandering on the arena was recorded. *Significant differences between control and insecticide-treated tents (P < 0.05; N = 6).

deltamethrin or 0.5% chlorfenapyr) or acetone and was wrapped around the cardboard strip and attached with staples. To reach the blood source, bed bugs would need to cross the insecticide-treated band. Ten insects (1:1 sex ratio, four replicates) were released and allowed to respond for 15 min. Then, the number of fed and unfed insects was recorded for each treatment and analyzed with a χ^2 goodness-of-fit test (MINITAB 2005).

Results

Choice Tests. In assays with groups of insects, three of four strains (LA-1, CIN-1, and CIN-3) significantly preferred to settle in water-treated tents rather than in tents treated with Suspend (P < 0.05; Fig. 1, left). The LEX-1 strain showed no preference. Insects from LA-1 were least likely to occupy Suspend-treated tents because they were often found either residing in insecticide-free tents (53%) or wandering in the arena (45%). No individuals from any strain avoided resting in tents treated with Phantom (Fig. 1, right).

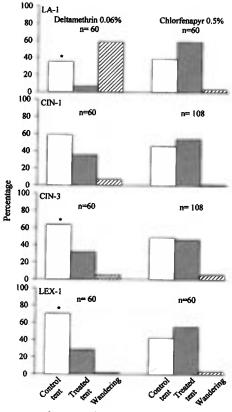


Fig. 2. Preference of individual bed bugs from four strains for a paper tent impregnated with insecticide-acetone solution (0.06% deltamethrin or 0.5% chlorfenapyr in 50 μ l acetone) or a tent treated with acetone (50 μ l, control). After a 16-h test period, the place where the insect was found was recorded (resting on insecticide or acetone treated tent or wandering in the arena). *Significant differences between control and insecticide-treated harborages (P < 0.05). Wandering insects were not included in this analysis.

Assays with individuals showed that three of four strains (LA-1, CIN-3, and LEX-1, but not CIN-1) significantly preferred to settle in acetone-treated rather than in deltamethrin-treated tents, at a ratio of at least 2:1 (acetone:deltamethrin; P < 0.05; Fig. 2, left). In the deltamethrin assays, >93% of insects settled in one or the other of the offered tents with the exception of individuals from the susceptible strain LA-1. At the end of the test period, 58% of the individuals from this susceptible strain were wandering in the arena with symptoms of pyrethroid poisoning, including ataxia or increased locomotor activity. Insects from all strains did not significantly avoid resting in tents with dry deposits of 0.5% chlorfenapyr (P > 0.05; Fig. 2, right).

Individuals and groups of bed bugs from CIN-1 assembled significantly more in established harborages (P < 0.05) than in control tents (Fig. 3A, B). Similarly, groups assembled significantly more in Suspend-treated established harborages than in control tents (P < 0.05; Fig. 3C). No preference was observed for the established harborages treated with Suspend or the one treated with water (P < 0.05; Fig. 3D).

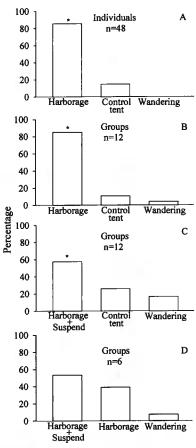


Fig. 3. Bed bug preference (CIN-1 strain) to different treatments in two-choice tests. (A) Individuals offered a tent with feces, eggs, and body secretions (harborage products) and a tent sprayed with distilled water (control). (B) Groups (five females, five males) offered harborage tents and control tents. (C) Groups offered Suspend (0.06%)-treated harborage and a tent sprayed with distilled water. (D) Group offered a Suspend (0.06%)-treated harborage or harborage tent sprayed with distilled water. In all experiments, harborage products were obtained by placing 10 recently fed bed bugs (1:1 sex ratio) on tents during the 48 h before assays. After a 15-h test period, insects resting on any of the treatment harborages or wandering in the arena was recorded. *Significant differences between treatment and control (P < 0.05).

Video Recording of Bed Bug Activity. Overall, the amount of time that individuals from CIN-1 spent away from tents differed significantly among treatments (F=6.33; df = 2, 33; P<0.005). CIN-1 individuals spent significantly more time away from deltamethrin-treated tents (36.7%) than acetone-treated control tents (6.2%; P<0.05), but no differences were found in the amount of time outside of tents in arenas with deltamethrin- and chlorfenapyr-treated tents (36.7 versus 16.4%; Fig. 4A).

The mean percentage of the time spent outside tents varied with treatment for LA-1 (F = 7.52; df = 2,15; P < 0.05). Interaction with deltamethrin-treated tents caused individuals from this strain to spend most

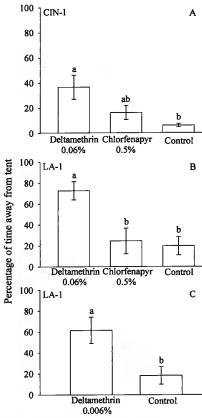


Fig. 4. Mean \pm SE percentage time that insects from CIN-1 (A) and LA-1 (B and C) strains spent away (wandering in the arena) from insecticide-treated tents (delta-methrin or chlorfenapyr) during the 15-h testing period in no-choice tests. Bars with the same letter are not significantly different (ANOVA followed by Tukey's test, P > 0.05).

of the time outside the treated tents (72.7%) in comparison to time spent outside the treatments with control (20.0%) or chlorfenapyr tents (24.6%; Tukey's test, P < 0.05; Fig. 4B). Individuals from the LA-1 strain also spent more time outside in the arena when exposed to tents treated with lower doses of deltamethrin (0.006%; 1/10th maximum label rate; F = 5.03, df = 1,16, P < 0.05; Fig. 4C).

Response of Host-seeking Bed Bugs to Insecticide Barriers. In both CIN-1 and LA-1 strains, there were no significant differences in the percentage of fed insects between control and deltamethrin (for CIN-1, 55 versus 52.5% respectively, $\chi^2 = 0.05$, P = 0.823; for LA-1, 52.5 versus 57.5%, $\chi^2 = 0.02$, P = 0.653) and between control and chlorfenapyr (for CIN-1, 55 versus 50%, $\chi^2 = 0.201$; P = 0.654; for LA-1, 52.5 versus 35%, $\chi^2 = 2.489$, P = 0.115). No mortality was observed among the fed individuals from the resistant and susceptible strain in the chlorfenapyr assays (7 d after exposure).

Exposure to deltamethrin barriers was also insufficient to cause significant mortality in fed individuals from both resistant and susceptible strain (0 and 5% mortality 7 d after exposure, respectively).

Discussion

Bed bug locomotor activity occurs mostly during the night and they tend to remain hidden in refuges during the day. Given their cryptic behavior, it is not always possible to find and treat all their harborages. Thus, when bed bug control methods include the application of insecticides, it is advantageous that insecticide formulations leave toxic residues for insects to encounter when seeking blood meals or returning to their refuges. Insecticide residues can cause changes in behavior of bed bugs. The study of behavioral responses is important because these responses can affect insecticide efficacy and ultimately provide a better understanding of the overall impact of insecticide treatment in bed bug management programs.

We evaluated the behavioral responses of bed bugs to deltamethrin and chlorfenapyr, two of the most commonly used insecticides for their control. Deltamethrin is a pyrethroid that acts at sodium channels, thereby disrupting nerve transmission. It is widely used against urban and agricultural pests (Casida and Quistad 1998). Chlorfenapyr is a halogenated pyrrole that uncouples oxidative phosphorylation processes in mitochondria (Hollingworth and Gadelhak 1998).

Insects tended to avoid dry residues of Suspend and preferred to rest in insecticide-free tents. Although LA-1-susceptible individuals avoided the insecticide (Figs. 1 and 2), ≈50% of individuals were found wandering in the arena, some with irreversible symptoms of pyrethroid poisoning. However, no evidence of acute intoxication was observed in individuals from the resistant strains (CIN-1, CIN-3, and LEX-1), even in those insects that were resting on the deltamethrintreated tent (Figs. 1 and 2). All these individuals survived. Avoidance responses displayed by insects indicated that bed bugs have behavioral mechanisms that reduce exposure to insecticides. These behavioral responses to insecticides have been reported in cockroaches, which, along with physiological resistance. may be partly responsible for insecticide treatment failures (Lockwood et al. 1984, Rust et al. 1993, Hostetler and Brenner 1994).

The responses of bed bugs to the active ingredient of Suspend, deltamethrin, were consistent with those found with the formulated material, indicating that deltamethrin is at least one constituent of the formulation responsible for the avoidance responses of bed bugs. If bed bugs avoid insecticides and retreat to insecticide-free areas, it would reduce the efficacy of insecticide treatments. Avoidance behavior to pyrethroid insecticides has been documented in other crawling household pests such as ants, termites, cockroaches, and kissing bugs (Knight and Rust 1990, Su and Scheffran 1990, Hostetler and Brenner 1994, Diotaiuti et al. 2000).

Bed bugs spend most of their time aggregated in harborages and only abandon them when the time comes to search for a blood meal. Marx (1955) suggested that bed bugs are driven by scent glands odors and feces to return to harborages. Levinson and Bar Lan (1971) showed that bed bugs aggregated in filter

papers previously exposed to adult conspecifics and suggested the existence of an aggregation pheromone. Aggregation may be mediated by contact pheromones produced by males and immature stages (Siljander et al. 2007). In our study, filter papers that had been previously occupied by adults attracted both individual and groups of bed bugs (Fig. 3A, B). Harborages remained attractive to bed bugs after being treated with Suspend (Fig. 3C). This indicates that either attracting or arresting factors were unaltered after insecticide treatment. These factors could include pheromones or physical characteristics of frass or eggs (thigmotactic cues). Our laboratory findings of nonavoidance behavior by bugs to treated harborages correspond with field observations after Suspend applications in which insects are found resting in treated harborages (Romero et al. 2007a). The continued occupancy of insects in such treated areas might increase exposure of bugs to the insecticide. This effect, however, might have limited benefits when populations are resistant to pyrethroids. Insecticide assays showed mortality of <30% in individuals from the CIN-1 pyrethroid-resistant strain when they were exposed continuously for more than a week to dry deposits of Suspend (A.R., unpublished data).

Insects from strains that avoided deltamethrin deposits in the absence of any aggregating stimuli (see choice tests, Figs. 1 and 2) would crawl over deltamethrin or chlorfenapyr barriers to reach a heat source and take a blood meal. Fed insects from the resistant and susceptible strain survived the exposure to chlorfenapyr residues. Insect mortality in deltamethrin barrier assays was minimal for both strains. Moore and Miller (2006) reported no avoidance behavior of bed bugs to insecticides when a heat source was nearby. Our studies confirm that responses of bed bugs to a close-range heat source may take precedence over avoidance responses to deltamethrin.

Video recordings over the course of a night and day allowed us to observe the activity of individual insects while they interacted with a tent impregnated with dry deposits of insecticides. A highly pyrethroid-resistant and a -susceptible strain showed an increase in locomotor activity as a result of contacting the deltamethrin-treated tent. An indication of this increase in activity was shown by the longer time insects spent outside of treated tents (Fig. 4). Hyperactivity has been reported as the first symptom of poisoning by some types of pyrethroids in blood-feeding insects, including Aedes aegypti L., Anopheles maculipennis atroparous van Thiel (Kennedy 1947), and Triatoma infestans Klug (Alzogaray et al. 1997). Hyperactivity for type I pyrethroids could be caused by repetitive discharges of nerves and associated muscular contraction (Scott and Matsumura 1983). However, because deltamethrin is a type II pyrethroid, the avoidance of residues may involve other mechanisms. An increased rate of movement caused by a sublethal dose of insecticides can have beneficial or adverse effects depending on the circumstances. For example, in control programs of T. infestans in South America, a spray with certain pyrethroids is part of pre- and post-insecticide

inspections because these insecticides are routinely used to flush out bugs from harborages. Among different pyrethroids tested, deltamethrin proved to be the most active agent in stimulating this response for T. infestans and Rhodnius prolixus Stål (Wood et al. 1993). In addition, locomotor hyperactivity caused by insecticides can increase the chance of insects to move across insecticide-treated surfaces, which would accelerate the acquisition of lethal doses of insecticide (Kennedy 1947). However, little benefit of this effect can be expected when individuals are resistant to the insecticide used, when individuals encounter sublethal doses, or when there is incomplete insecticide coverage that allows bugs to move into insecticidefree areas. This might explain, in part, patterns of bed bug spread observed under field conditions where adjoining locations become infested overtime.

Individual insects did not avoid dry deposits of chlorfenapyr. Similar results were achieved when groups were exposed to dry deposits of Phantom (chlorfenapyr). These results indicated that neither the technical nor the formulated material prevented individual or groups of insects from establishing residence in chlorfenapyr-treated tents. These findings are not surprising because other studies report non-repellent effects of chlorfenapyr in other insects, e.g., ants and termites (Buczkowski et al. 2005, Rust and Saran 2006). No avoidance by bed bugs of chlorfenapyr leads them to pick up a greater dose of the insecticide, and eventually this exposure causes mortality (A.R., unpublished data).

Behavioral responses of bed bugs to insecticides and their implications for control may vary depending on several factors including, insecticide susceptibility of populations, insecticide coverage, and the presence of other stimuli in the environment. The presence of aggregating factors in harborages and attraction to a heat source might reduce the avoidance behavior of bed bugs to deltamethrin. Survival of bed bugs after their contact with deltamethrin residues, with the subsequent increase in locomotor activity, represents a potential problem for the spread of bed bugs to adjoining areas. This is a behavioral effect that should be considered with all insecticides used for bed bug management.

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