Effect of Feeding Status on Mortality Response of Adult Bed Bugs (Hemiptera: Cimicidae) to Some Insecticide Products

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ABSTRACT Fresh and aged residual deposits of several insecticide products were tested against bed bug adults to determine if a recent bloodmeal affected their mortality response to the residues. The bed bugs with a recent bloodmeal survived significantly longer compared with the unfed ones on their exposure to fresh or aged residual deposits of chlorfenapyr and aged residual deposits of deltamethrin on a wooden substrate. Even though the survival time of fed bed bugs was significantly longer than that of unfed ones on their exposure to fresh residue of deltamethrin and aged residue of desiccant pyrethrin dust, these treatments resulted in similarly high final mortalities regardless of feeding status of the insects. Mortality responses of fed and unfed bed bugs were similar to fresh or aged residual deposits of imidacloprid + cyfluthrin combination and fresh residual deposits of desiccant pyrethrin dust. Topical application assays indicated that a recent bloodmeal significantly increased the bed bug’s survival time for chlorfenapyr, but not for deltamethrin. Pyrethroid-resistant bed bugs also showed a similar increase in their survival time for chlorfenapyr after a bloodmeal. The comparison of mortality responses between fed and unfed bed bugs treated with similar amount of chlorfenapyr per fresh body weight indicated that increased body mass was not the primary cause for this bloodmeal-induced tolerance increase for chlorfenapyr. Because the surviving bed bugs can continue ovipositing, the effectiveness of chlorfenapyr residual deposits in bed bug harborage could be significantly affected by the feeding status of the adult bed bug populations.

KEY WORDS deltamethrin, chlorfenapyr, imidacloprid, β-cyfluthrin, Cimex lectularius

Several insecticides currently available for bed bug control include pyrethroids, a pyrrole, neonicotinoids, and natural oils that are derived from various botanical sources. Even though pyrethroids typically provide fast knockdown and kill of bed bugs, the use of alternative insecticides with different modes of action is also important because of the widespread pyrethroid resistance in bed bug populations (Romero et al. 2007a,b; Yoon et al. 2008). Mixtures of pyrethroids and neonicotinoids have been recently introduced to combat resistance. Several different types of desiccant agents such as silica gel dust and diatomaceous earth are also available for bed bug management as a residual treatment option.

Because of the practical difficulty of finding every bed bug’s location in infested items or structures, the use of effective residual insecticides for potential bed bug harborage sites (i.e., cracks and crevices in the furniture or infested room) has been suggested (Meyers 2012). This type of treatment might potentially improve bed bug control by targeting bed bugs moving into the harborage after feeding (Romero et al. 2009a). In particular, deltamethrin and chlorfenapyr have been recently evaluated for their repellency and efficacy to treat bed bug harborage. Romero et al. (2009a) reported that bed bugs avoided deltamethrin residues on clean filter paper substrate, but natural harborage containing fecal materials and eggs, remained attractive to bed bugs even after being treated with a deltamethrin-based product. While being relatively slow-acting, residual deposits of chlorfenapyr were also found to be effective against four different bed bug strains, including two strains that were highly resistant to pyrethroids without apparent avoidance behavior (Romero et al. 2009a, 2010).

Previous evaluations of insecticides applied to harborage sites have been conducted with unfed adult bed bugs (i.e., no bloodmeal for 7–12 d after adult emergence) because their responses to insecticides were more consistent than those that had recently molted or fed (Romero et al. 2009a, 2010). However, both adult and immature stages of bed bugs typically take regular and frequent bloodmeals for their normal growth, longevity, and reproduction (Usinger 1966). For example, female bed bugs in a highly infested room kept at a constant temperature (26°C) were found to take a bloodmeal every 2.5 d (Reinhardt et al. 2010). Because bed bugs typically return to their harborage immediately after obtaining a bloodmeal (i.e., <30 min; Reis and Miller 2011), it is likely that at least

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some individuals that would contact the residual deposits in the harborage would be the ones that have taken a recent bloodmeal.

In several species of mosquitoes, a recent bloodmeal appears to be one of the factors influencing insecticide tolerance levels (Brown and Pal 1971). Hadaway and Barlow (1956) reported that tolerance of female Anopheles stephensi Liston and Aedes aegypti (L.) to dichlorodiphenyltrichloroethane and dieldrin increased about twofold at 24 h after a bloodmeal. Haliday and Feyereisen (1987) and Spillings et al. (2008) also reported similar phenomena in female Culex pipiens L. and a pyrethroid-resistant strain of Anopheles funestus Giles. Thus, it was of interest to determine if bed bugs that are recently fed would show different mortality response to insecticide residual deposits compared with starved ones.

In this study, we examined the effect of a recent bloodmeal on the survival time of adult bed bugs on their exposure to fresh and aged residual deposits of several insecticide products that are currently registered for bed bug control. Topical application studies were also conducted on the insecticide products to which the fed and unfed bed bugs showed different mortality responses. The potential implications for bed bug management are discussed.

Materials and Methods

Insects. Two strains of bed bugs, “Earl” and “Jersey,” were purchased from Sierra Research Laboratories (Modesto, CA). The Earl strain was originally collected in Modesto, CA, in 2007. Bioassays with technical-grade insecticide residues on a glass surface indicated that this strain was susceptible to technical-grade permethrin and deltamethrin (i.e., 90 and 100% knockdown in 2 h on a glass surface treated with technical-grade permethrin and deltamethrin, respectively; B. Donahue, unpublished data). Jersey strain is a relatively pyrethroid-resistant strain collected in Jersey City, NJ, in 2010 (i.e., 30 and 50% knockdown in 2 h on a glass surface treated with technical-grade permethrin and deltamethrin, respectively; B. Donahue, unpublished data). The bed bugs were kept in screened containers, and fed with a grafting tape membrane (Aglis & Co., Ltd., Yame City, Fukuoka, Japan) feeder containing rabbit blood plus sodium citrate (Hema Resource and Supply, Inc., Aurora, OR) once a week. All colonies were kept at 26°C, 35–38% RH, and a photoperiod of 12:12 (L:D) h.

To examine the effect of recent bloodmeals on the survival time of adult bed bugs on their exposure to various insecticide residual deposits, we prepared two groups of bed bug that differed in the periods since their most recent bloodmeal: 1 and 9 d postfeeding. Bed bugs fed the night before the experiment were considered as the 1 d postfeeding group. Bed bugs were anesthetized with carbon dioxide.

Bed bugs were obtained by feeding a Japan feeder containing rabbit blood plus sodium citrate (Hema Resource and Supply, Inc., Aurora, OR) once a week. All colonies were kept at 26°C, 35–38% RH, and a photoperiod of 12:12 (L:D) h. To examine the effect of recent bloodmeals on the survival time of adult bed bugs on their exposure to various insecticide residual deposits, we prepared two groups of bed bug that differed in the periods since their most recent bloodmeal: 1 and 9 d postfeeding. Bed bugs fed the night before the experiment were considered as the 1 d postfeeding group. Bed bugs were obtained by feeding a Japan feeder containing rabbit blood plus sodium citrate (Hema Resource and Supply, Inc., Aurora, OR) once a week. All colonies were kept at 26°C, 35–38% RH, and a photoperiod of 12:12 (L:D) h.

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Residual Activity Assay. The residual activity assay was conducted with the Earl strain to determine if fed and unfed bed bugs differed in their survival time when they contacted insecticides applied in their harborage sites. The top inner surface of the wooden shelter was treated with one of four different insecticides: deltamethrin 0.06% (D-Force HPX pressurized aerosol, Waterbury Companies, Inc., Waterbury, CT), chlorfenapyr 0.5% (Phantom pressurized aerosol, BASF Corporation, Research Triangle Park, NC), imidacloprid 21% and β-cyfluthrin 10.5% combination (Temprid SC, Bayer Environmental Science, Research Triangle Park, NC), and silica gel dust with pyrethrins 1% and piperonyl butoxide (PBO) 10% (Drione, Bayer Environmental Science). For the pressurized aerosol formulations (deltamethrin and chlorfenapyr), aliquots of the liquids were dispensed from pressurized containers into glass vials, and all the vol-

Fig. 1. Wooden shelter (75 by 40 by 6 mm) built with balsa wood panels and sticks for the residual activity assay.
atle propellant gases were removed from the liquids by shaking the vial vigorously and releasing the pressure by opening the cap. For the suspension concentrate formulation (imidacloprid + cyfluthrin combination), a 0.075% (label rate for indoor bed bug infestation) based on the combined active ingredient (AI) aqueous preparation was used. An aliquot of 700 μl liquid was evenly applied on the top inner surface of the wooden shelter (24.4 cm²), providing approximately 0.01, 0.6, 0.015, and 0.007 mg AI/cm² treatment rates for deltamethrin, chlorfenapyr, imidacloprid, and cyfluthrin, respectively. For the desiccant dust with pyrethrins and PBO (Drione), 8 mg of dust was evenly spread on the top inner surface of the wooden shelter with a cotton-tip applicator, providing 0.003 and 0.03 mg/cm² treatment rates for pyrethrins and PBO, respectively. Control shelters were treated with 700 μl of water.

Treated wooden shelters were allowed to dry in ambient conditions (26°C, 35–38% RH) for 6 h (referred to as fresh residual deposit hereafter), and attached on the bottom of the petri dishes using hot glue (thermoplastic adhesive). The insecticides were also tested as a 34-d-old residue. For the aging process, the treated wooden shelters were kept in ambient conditions (referred to as aged residual deposit hereafter). Approximately 10 adult bed bugs (10–12 individuals, 1:1 sex ratio) were introduced onto the top of the wooden shelter, and the number of dead or knocked down bed bugs was recorded every 24 h for 10 d. The 10-d observation time was selected based on a study by Romero et al. (2010), in which LT₅₀ and LT₉₀ values of four different bed bug strains for dry residues of chlorfenapyr (fresh and aged) were within the range of 3.5 and 8.9 d. Other insecticides containing pyrethroids and pyrethrins were considered to be quicker in their action compared with the chlorfenapyr insecticide. If live bed bugs were found at the bottom of the petri dish away from the wooden shelter, the bed bugs were moved to the top of the shelter. The bed bugs were considered to be dead or knocked down if they were not able to cling to the wooden shelter with their legs. Each trial was replicated five times, resulting in ~50 bed bugs being tested per feeding status per insecticide treatment. Control was replicated 10 times per feeding status.

Topical Application Assay. The topical application assay was conducted to determine if fed and unfed bed bugs differed in their survival time when known amounts of AIs were topically applied to their cuticle. First the Earl strain was tested with a subset of insecticides that were selected based on the residual activity assay results. Deltamethrin and chlorfenapyr were chosen because the residual activity assay result indicated that the fed and unfed bed bugs differed in their survival time when they contacted the fresh residues of the insecticides. The Jersey strain was subsequently tested with chlorfenapyr that was chosen based on the results of the first topical application assay with the Earl strain.

The topical application assay was conducted with the methods developed by Romero et al. (2009b). Aliquots of deltamethrin and chlorfenapyr aerosols were dispensed from pressurized containers into glass vials, and subsequently dissolved in acetone so that 0.5 μl of the acetone preparation applied to the insect provided the required quantity of AIs. The concentrations tested were 1, 2, and 5 ng (AI) per insect for deltamethrin, and 100, 200, and 500 ng (AI) per insect for chlorfenapyr. These concentrations were selected based on LD₅₀ values reported for a highly pyrethroid-susceptible strain of bed bug (0.03 ng deltamethrin/insect for the Harlan strain of bed bug; Adelman et al. 2011) and another insect species of similar size (29.98 ng chlorfenapyr/insect for subterranean termite; Rust and Saran 2006). A microapplicator equipped with a glass syringe (Micro Metric Instrument Co., Cleveland, OH) was used to apply the acetone preparation containing insecticide to the dorsal abdominal surface of the insects. A control group was treated with an identical amount of acetone only. Ten or twenty adult bed bugs (1:1 sex ratio) were treated and placed in individual cells (16 by 19 mm) of 24-well cell culture plates (Corning Inc., Corning, NY) with the bottoms lined with small disks of filter paper. The treated bed bugs were kept at 26°C. The number of dead or knocked down bed bugs was recorded every 24 h for 10 d. The bed bugs were considered to be dead or knocked down if the bed bugs could not cling onto the filter paper lining and respond to the mechanical stimuli provided by gentle touch with a wooden probe.

Statistical Analysis. The survival time data in the residual and topical studies were analyzed with survival analysis. The distribution of survival times was described using the survivorship function S(t), the probability that an individual survives longer than time t. PROC LIFETEST statement was used to compute nonparametric estimates of the survivor functions, to compare survival curves, and to compute log-rank test for association of the failure time variable (i.e., time until kill) with covariates (i.e., insecticide treatment; SAS Institute 2009). Survival curves were generated using the Statistix 9 program (Analytical Software 2008). Day 10 cumulative mortalities were compared between fed and unfed bed bugs using the one-tailed Fisher exact test to determine if the ability to tolerate the insecticide treatment is dependent on the feeding status of bed bugs (Analytical Software 2008).

Results

Residual Activity Assay. Upon their release on the top of the treated wooden shelter, most (>50%) of the bed bugs (Earl strain) readily moved into the structure in <30 min, contacting the insecticide deposits on its inner surface. Because overall control mortalities were low and identical between fed and unfed bed bugs (e.g., 3.5% for both fed and unfed bed bugs at day 10), all statistical comparisons were made between fed and unfed bed bugs from the insecticide treatments without correction based on corresponding control mortalities.
When Earl strain bed bugs were tested on the fresh residual deposits, the survival times of the fed and unfed bed bugs were significantly different for deltamethrin and chlorfenapyr ($\chi^2 = 5.784$, df = 1, $P = 0.016$ for deltamethrin; $\chi^2 = 10.94$, df = 1, $P = 0.001$ for chlorfenapyr; Fig. 2). For deltamethrin, 10-d cumulative mortalities for fed and unfed bed bugs were 92% (46 of 50) and 100% (50 of 50), respectively (Fisher exact test, $P = 0.059$). For chlorfenapyr, 10-d cumulative mortalities for fed and unfed bed bugs were 64% (32 of 50) and 88% (43 of 49), respectively (Fisher exact test, $P = 0.005$). The survival times of the fed and unfed bed bugs were not significantly different for the fresh residual deposits of imidacloprid + cyfluthrin combination and desiccant pyrethrin dust with PBO ($\chi^2 = 3.085$, df = 1, $P = 0.079$ for imidacloprid + cyfluthrin combination; $\chi^2 = 0.075$, df = 1, $P = 0.785$ for desiccant pyrethrin dust; Fig. 2). The 10-d cumulative mortalities of fed and unfed bed bugs were not significantly different for these treatments (60% [31 of 52] vs. 76% [38 of 50] for imidacloprid + cyfluthrin combination; 100% [50 of 50] vs. 98% [49 of 50] for desiccant pyrethrin dust) (Fisher exact test, $P > 0.05$).

When Earl strain bed bugs were tested on the 34-d-old residual deposits, the survival times of the fed and unfed bed bugs were significantly different for deltamethrin and chlorfenapyr aerosols and desiccant pyrethrin dust with PBO ($\chi^2 = 42.226$, df = 1, $P < 0.0001$ for deltamethrin; $\chi^2 = 66.655$, df = 1, $P < 0.0001$ for chlorfenapyr; $\chi^2 = 19.757$, df = 1, $P < 0.0001$ for desiccant pyrethrin dust; Fig. 3). By day 10, total cumulative mortalities for fed and unfed bed bugs were 53% (26 of 49) and 98% (50 of 51) for deltamethrin (Fisher exact test, $P < 0.0001$), 48% (24 of 50) and 98% (52 of 53) for chlorfenapyr (Fisher exact test, $P < 0.0001$), and 98% (49 of 50) and 100% (50 of 50) for desiccant pyrethrin dust with PBO (Fisher exact test, $P = 0.5$), respectively. For aged residual deposits of imidacloprid + cyfluthrin combination, the survival times and 10-d cumulative mortalities were not significantly different between fed and unfed bed bugs (survival analysis: $\chi^2 = 1.203$, df = 1, $P = 0.273$; final mortality: Fisher exact test, $P = 0.393$; Fig. 3).

Topical Application Assay. Because overall control mortalities were low and either similar or identical between fed and unfed bed bugs (e.g., 10 and 5% for fed and unfed Earl strain bed bugs, and 0% for both fed and unfed Jersey strain bed bugs at day 10, respectively; Fisher exact test, $P > 0.05$), all statistical comparisons were made between fed and unfed bed bugs from the insecticide treatments without correction based on corresponding control mortalities.

When three different doses of deltamethrin (i.e., 1, 2, and 5 ng per insect) were topically tested on the Earl strain bed bugs, survival times of the fed and unfed bed bugs were not significantly different ($\chi^2 = 0.944$, df =
1, \( P = 0.331 \) for 1 ng per insect; \( \chi^2 = 0.677, df = 1, P = 0.411 \) for 2 ng per insect; \( \chi^2 = 0.057, df = 1, P = 0.811 \) for 5 ng per insect; Fig. 4, left). Total cumulative mortalities of the treated bed bugs at day 10 ranged from 50 to 90% depending on the dose, without significant difference between the fed and unfed bed bugs (Fisher exact test, \( P > 0.05 \)).

When three different doses of chlorfenapyr (i.e., 100, 200, and 500 ng per insect) were topically tested on the Earl strain bed bugs, survival times of the fed and unfed bed bugs were significantly different at all rates tested (\( \chi^2 = 11.497, df = 1, P < 0.001 \) for 100 ng per insect; \( \chi^2 = 3.988, df = 1, P = 0.046 \) for 200 ng per insect; \( \chi^2 = 7.959, df = 1, P = 0.005 \) for 500 ng per insect; Fig. 4, right). Total cumulative mortalities of the fed and unfed bed bugs were 25% (5 of 20) versus 75% (15 of 20) for 100 ng/insect dose (Fisher exact test, \( P = 0.002 \)), 35% (7 of 20) versus 65% (13 of 20) for 200 ng/insect dose (Fisher exact test, \( P = 0.056 \)), and 70% (14 of 20) versus 100% (20 of 20) for 500 ng/insect dose (Fisher exact test, \( P = 0.01 \)), respectively.

Because the fed bed bugs were almost twice as heavy as the unfed bed bugs (the weights of fed and unfed bed bugs were 7.3 ± 0.6 and 4.0 ± 0.2 mg, respectively [mean ± SEM, \( n = 10 \) for each]), it was of interest to compare the survival time and final mortality data between the unfed bed bugs treated with \( X \) amount of AI and the fed bed bugs treated with \( 2X \) the amount. The survival analysis and final mortality comparison indicated that the fed bed bugs that received 27.4 ng chlorfenapyr per mg of fresh body weight (data from 200 ng per insect dose) survived significantly longer than did the fed bed bugs treated with 25 ng chlorfenapyr per mg of fresh body weight (data from 100 ng per insect dose; survival analysis: \( \chi^2 = 8.511, df = 1, P = 0.004 \); final mortality: Fisher exact test, \( P = 0.012 \)).

Chlorfenapyr was also topically tested on the Jersey strain at three different rates (i.e., 100, 200, and 500 ng per insect). The survival times of the fed bed bugs were significantly different from those of the unfed bed bugs at all rates tested (Fig. 5). Total cumulative mortalities of the fed and unfed bed bugs were 30% (3 of 10) versus 100% (10 of 10) for 100 ng/insect dose (Fisher exact test, \( P = 0.002 \)), 40% (4 of 10) versus 90% (9 of 10) for 200 ng/insect dose (Fisher exact test, \( P = 0.029 \)), and 70% (7 of 10) versus 100% (10 of 10) for 500 ng/insect dose (Fisher exact test, \( P = 0.105 \)), respectively. The weights measured from fed and unfed bed bugs immediately before the assay were 7.5 ± 0.5 and 3.9 ± 0.1 mg, respectively (mean ± SEM, \( n = 10 \) for each). The survival analysis and final mortality comparison indicated that the fed bed bugs that received 26.7 ng chlorfenapyr per mg of fresh body weight (data from 200 ng per insect dose) survived signifi-
significantly longer than did the unfed bed bugs treated with 25.6 ng chlorfenapyr per mg of fresh body weight (data from 100 ng per insect dose; survival analysis: \( \chi^2 = 14.791, df = 1, P = 0.0001 \); final mortality: Fisher exact test, \( P = 0.005 \)).

**Discussion**

The current study demonstrates that mortality responses of fed and unfed bed bugs could be substantially different when they are exposed to some residual insecticide deposits. Based on the survival time and final mortality data at day 10 posttreatment, adult bed bugs with a recent bloodmeal survived significantly longer than did unfed ones when exposed to fresh or aged residual deposits of chlorfenapyr and aged residual deposits of deltamethrin. Even though our analysis indicated that survival times of fed and unfed bed bugs were different on their exposure to the fresh residue of deltamethrin and aged residue of desiccant pyrethrin dust with PBO, both feeding groups resulted in similarly high final mortalities (92–100%) at day 10. For all the other treatments (i.e., fresh or aged residual deposits of imidacloprid + cyfluthrin, and fresh residual deposits of desiccant pyrethrin dust with PBO), the fed and unfed bed bugs were similar in their survival time and 10-d posttreatment mortality. Studies with technical-grade insecticides at varying con-

![Fig. 4. Kaplan-Meier survival analyses for Earl strain bed bugs (fed and unfed) when deltamethrin and chlorfenapyr insecticides were topically applied to the insects. Survivorship function \( S(t) \) was defined as the probability that an individual survives longer than time “\( t \).” (Online figure in color.)](image-url)
centrations would be helpful to understand if the phenomenon is specific for certain types of AIs.

For the treatments with deltamethrin and chlorfenapyr aerosols and desiccant pyrethrin dust with PBO, the degree of difference between fed and unfed groups in their mortality responses varied depending on the age of the insecticide residual deposits. For deltamethrin and chlorfenapyr aerosols, we found that differences in day 10 survivorships between fed and unfed bed bugs were more pronounced when they were tested on the 34-d-old residual deposits compared with the fresh ones. For example, the final mortality of fed bugs was lower than that of unfed bed bugs by 8 and 45% for the fresh versus aged residues of deltamethrin, respectively. For chlorfenapyr, the final mortality of fed bugs was lower than that of unfed bed bugs by 20 and 50% for the fresh versus aged residues, respectively.

Fig. 5. Kaplan–Meier survival analyses for Jersey strain bed bugs (fed and unfed) when a chlorfenapyr insecticide was topically applied to the insects. Survivorship function $S(t)$ was defined as the probability that an individual survives longer than time $t$. (Online figure in color.)
bugs by 24 and 50% for the fresh versus aged residues, respectively. For desiccant pyrethrin dust with PBO, survival times of the fed bed bugs were significantly longer than that of the unfed ones only when they were tested on the aged residual deposits.

The aforementioned results could be explained by possible differences between the fresh and aged residual deposits of these insecticide products. For deltamethrin and desiccant pyrethrin dust with PBO, it is likely that the aged residues had relatively lower toxicity compared with the fresh ones as a result of potential degradation or other unknown processes. Anderson and Cowles (2012) reported that residual deposits of D-Force HPX aerosol (deltamethrin) displayed reduced residual toxicity against bed bugs as the deposits aged (2–24 wk). Ebeling (1971) reported that the lethal action of pyrethrin in Drione was slightly slowed down when the dust deposits aged for 146 d inside of a wall void (i.e., aged dust required longer time to achieve 100% kill of German cockroaches compared with the fresh material). For chlorfenapyr, however, it is less clear what could be the possible difference between the fresh and aged residual deposits. Romero et al. (2010) reported that aged (4 mo) deposits of chlorfenapyr aqueous spray on filter paper were as toxic as fresh deposits for bed bugs based on a continuous exposure study. However, the persistency of residual toxicity and rate of degradation of AlS of the insecticide residue can vary depending on the substrates (Chadwick 1985). Arthur (2008) also reported that residual efficacy of chlorfenapyr is significantly influenced by the kinds of substrates treated. In addition to these potential toxicity differences, other chemical differences between the fresh and aged residual deposits such as the amount of solvent left in the residues might have direct or indirect effects on the mortality responses by influencing insecticide transfer or bed bugs’ behavior in the treated shelter. According to their material safety data sheet, all the three products (D-Force HPX and Phantom pressurized aerosols and Drione) contain petroleum-based solvents as their ingredients. Chemical differences between fresh and aged residues of these pesticide products and their impacts on the mortality responses of fed and unfed bed bugs warrant further study.

Even though the residual activity assay indicated that the survival times of the fed and unfed bed bugs were different when they were tested on the fresh and aged residual deposits of deltamethrin, the two feeding groups did not differ in their survival time and final mortality based on the topical application assays. This apparent discrepancy could be explained by a difference in study design between the residual activity and topical application assays. Unlike the topical application assays, the residual activity assay settings allowed the bed bugs to move away from the treated surface if the residual deposits of deltamethrin were repellent to them. If prolonged or repetitive exposure of bed bugs to a lower rate of deltamethrin residue is necessary to show the survival time difference between fed and unfed bed bugs, the difference could be detected only in our residual activity assays. Other behavioral differences between the fed and unfed bed bugs might be in part responsible for the different mortality responses observed for the deltamethrin residual activity assay. For example, the unfed bed bugs might have moved more frequently while searching for a bloodmeal compared with the fed bed bugs (How and Lee 2010, Reis and Miller 2011), potentially contacting the insecticide residues more often compared with the fed bed bugs.

In contrast to the deltamethrin data, results from topical application assays with chlorfenapyr were consistent with the residual activity assays, showing the survival times and 10-d cumulative mortalities of fed and unfed bed bugs were significantly different for most of the doses tested (except final mortality data for 200 and 500 mg/insect dose for Earl and Jersey stains, respectively). Because the fed bed bugs were more tolerant to chlorfenapyr than unfed ones even when they were treated with a similar dose per unit of fresh body weight, it is unlikely that the increased body mass and subsequent dilution of internalized insecticides ("vigor tolerance," Hoskins and Gordon 1956) are playing a central role in this bloodmeal-induced tolerance increase for chlorfenapyr. Then, what could be possible explanations for this phenomenon? First, it is possible that some kinds of detoxification mechanisms stimulated by the intake and digestion of the bloodmeal inadvertently reduced the toxicity of chlorfenapyr. In mosquitoes, it was suggested that a series of enzymatic detoxification pathways activated by the ingestion of a bloodmeal and its digestion were responsible for their elevated tolerance level to insecticides (Spillings et al. 2008). For example, upregulation of cytochrome P450s in response to a bloodmeal has been shown in Cx. pipiens and Ae. aegypti mosquitoes (Baldridge and Feyereisen 1986, Sanders et al. 2003). Alternatively, it is possible that the digestion and processing of the recent bloodmeal might inhibit the timely conversion of chlorfenapyr to its toxic form. Chlorfenapyr is a pro-pesticide that needs to be converted to a toxic form by a target organ via an oxidative process probably catalyzed by P450s (Black et al. 1994). If most multifunction oxidases such as P450s are preoccupied with the digestion of the recent bloodmeal, the conversion of chlorfenapyr to the toxic form by the fed bed bugs could be substantially delayed. Investigation of chlorfenapyr insecticidal action by elementary processes such as penetration, distribution, metabolism, and interaction with target sites, along with careful examination of resistance- or detoxification-associated gene products, would help to understand how the feeding status influences the bed bug's response to this particular insecticide.

Continued survival of blood-fed bed bugs after contacting the insecticide residues would be problematic not only because the surviving bed bugs might continue feeding on humans, but also because the fed adult female bed bugs may continue producing eggs. Moore and Miller (2006) reported that continuous exposure to a chlorfenapyr residue on a hardboard
panel did not prevent bed bugs from mating and laying eggs. Similarly, most of the fed female bed bugs in the current study produced eggs even after being exposed to the chlorfenapyr insecticide. For example, in the chlorfenapyr residual activity assays with the Earl strain, 9 of 10 replicates observed from the fresh and aged residue trials had eggs by day 10. Also, in the chlorfenapyr topical application assays with the Earl strain, 76.7% of the fed females observed (n = 30) laid eggs by day 10. In contrast, only 1 of 10 replicates from the fresh and aged deltamethrin residue trials had eggs by day 10. The topical application of deltamethrin also substantially inhibited the oviposition of the fed females, resulting in only 46.7% of the fed females (n = 15) having laid eggs by day 10. Deltamethrin typically knocks down the pyrethroid-susceptible bed bugs within 1–2 h, so this fast neurotoxic action might be, at least in part, responsible for the oviposition inhibition.

Based on the current study, we suspect that an eradication effort using chlorfenapyr insecticide as a sole residual treatment option could be negatively impacted if target bed bug populations can get frequent bloodmeals. For bed bug populations with recent bloodmeals, longer exposure time on the insecticide residues or higher application rate might be necessary to achieve a similar level of control as for a relatively starved bed bug population. Frequent feeding of a bed bug population could be partially prevented by setting up barriers or pitfall traps (e.g., interceptor devices) at the base of legs of couches or bed frames, where taking of the bloodmeal is most likely to occur. Where it is economically viable, an evacuation of the infested structure for several days before the insecticide treatment would also decrease the proportion of bed bugs with a recent bloodmeal within the population. Further study is still required to determine if the observed difference between fed and unfed bed bugs’ mortality responses to chlorfenapyr is a temporary phenomenon and, if so, how long it would last after a bloodmeal.

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