



Egg Hatch Rate and Nymphal Survival of the Bed Bug (Hemiptera: Cimicidae) After Exposure to Insecticide Sprays

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Abstract

Few studies have addressed the efficacy of insecticides used against eggs and first-instar nymphs of the bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae). Insect eggs are often resistant to insecticides; therefore, information on which products are effective is important. We evaluated the efficacy of four commonly used insecticide sprays applied directly to bed bug eggs. We also evaluated the efficacy of these insecticides to first-instar nymphs exposed to residuals resulting from directly spraying eggs. Temprid SC (beta-cyfluthrin, imidacloprid) was the most effective insecticide at preventing egg hatch (13% hatch rate) for pyrethroid-resistant, field-strain (Jersey City) bed bugs compared with a control (water [99% hatch rate]), Bedlam (MGK-264, sumithrin [84% hatch rate]), Demand CS (lambda-cyhalothrin [91% hatch rate]), and Phantom SC (chlorfenapyr [95% hatch rate]). Demand CS and Temprid SC were most effective at preventing egg hatch (0%) for an insecticide-susceptible (Harold Harlan) strain, followed by Bedlam (28%). Phantom SC produced a hatch rate similar to the control (97% and 96%, respectively). Harold Harlan-strain nymphs showed 100% survival for the control but 0% survival for Bedlam and Phantom SC. Jersey City-strain nymphs showed 100% survival for the control, 99% survival for Bedlam, 0% survival for Demand CS, 4% survival for Phantom SC, and 38% survival for Temprid SC. Demand CS was less effective at preventing hatch (91% hatch rate) of Jersey City-strain nymphs but was the only product to kill all nymphs (0% survival). One of the least effective products for preventing Jersey City-strain egg hatch (Phantom SC, 95% hatch rate) was the second most effective at killing nymphs, leaving only six of 141 alive. These findings indicate that survival of directly sprayed eggs and residually exposed, first-instar nymphs varies by strain, life stage, and product used.

Key words: Cimicidae, *Cimex*, urban pest

The majority of studies examining the efficacy of insecticides against the bed bug, *Cimex lectularius* L., have focused on adults or middle-late instars (Moore and Miller 2006; Romero et al. 2007, 2009, 2010; Seong et al. 2010; Adelman et al. 2011; Kilpinen et al. 2011; Tawatsin et al. 2011; Zhu et al. 2013). Although such studies are important for understanding control, only four studies (Callaway and Musgrave 1940, Goddard 2013, Singh et al. 2014, Campbell and Miller 2015) have examined the impact of directly applied liquid insecticides on the hatch rate of bed bug eggs. Callaway and Musgrave (1940) evaluated several compounds applied to bed bug eggs as direct sprays, but their findings have limited relevance to modern bed bug control; as of 1940, there were no documented cases of bed bug resistance to insecticides, which suggests that bed bugs used by Callaway and Musgrave (1940) were more susceptible to insecticides than are most modern populations. Furthermore, their dilution of all tested compounds in “highly refined kerosene-type oil” before application limits the study’s field applicability, as

these methods are no longer permitted due to safety concerns. Goddard (2013) used bed bugs from a local poultry house to evaluate the efficacy of several modern products applied as direct sprays; the results suggested that the population was an insecticide-susceptible strain. The same insecticide-susceptible population was used to assess egg hatch rates (Goddard 2013). As Steelman et al. (2008) also found that bed bugs collected in poultry houses were susceptible to modern insecticides (pyrethroids), it is possible that insecticide resistance was low or absent in Steelman et al. (2008) and Goddard (2013) due to the limited number of pyrethroids registered for use in poultry houses. Although Goddard’s (2013) work is valuable for understanding egg hatch rates for susceptible strains, most wild bed bug populations show some level of insecticide resistance (Zhu et al. 2010). Campbell and Miller (2015) also examined egg hatch and nymph survival based on product and concentration, but evaluated hatch rate by dipping eggs in different concentrations of insecticides. This approach improves our understanding of

resistance based on strain and concentration, but the methodology differs from the type of insecticide exposure eggs would receive when treated in the field. The method of Campbell and Miller (2015) for evaluating nymph survival on a treated surface had more field applicability, but was limited to only two products (Temprid and Transport). Singh et al. (2014) assessed bed bug egg hatch and nymph survival using Temprid SC and Demand CS, but focused primarily on essential oil and detergent-based insecticides.

We have chosen to expand upon these earlier works by evaluating bed bug egg hatch rate and nymph survival using insecticides selected based on their recent popularity (Potter and Haynes 2014) and unique chemical profiles, which included pyrethroids (sumithrin, beta-cyfluthrin, lambda-cyhalothrin), a pyrethroid synergist (MGK-264), a neonicotinoid (imidacloprid), and a halogenated pyrrole (chlorgfenapyr) as active ingredients.

Materials and Methods

We evaluated four commonly available insecticides applied as direct sprays to the eggs of the insecticide-susceptible Harold Harlan strain, and the pyrethroid-resistant Jersey City field strain. We also evaluated the mortality of emerged nymphs. The Harold Harlan strain has been maintained in colony for >40 yr. The Jersey City strain was originally collected on 16 May 2008 from Jersey City, NJ. It was screened for pyrethroid (deltamethrin) resistance in 2010 and 2016. Both screenings showed that the Jersey City strain was >300× more resistant than the Harold Harlan strain (Rick Santangelo [Coby Schal Urban Entomology lab manager, North Carolina State University], personal communication). Both strains were provided by North Carolina State University. Evaluated products included one aerosol formulation (Bedlam [MGK Corporation, Minneapolis, MN]) and aqueous dilutions of Demand CS (Syngenta, Basel, Switzerland), Phantom SC (BASF, Ludwigshafen, Germany), and Temprid SC (Bayer Corporation, Kansas City, MO). Bedlam contained sumithrin (0.40%), MGK-264 (1.53%), and 98.07% other ingredients. Demand CS (at 0.03%) consisted of 9.7% lambda-cyhalothrin and 90.3% other ingredients. Phantom SC (at 0.5%) consisted of 21.45% chlorgfenapyr and 78.55% other ingredients. Temprid SC (at 0.075%) included imidacloprid (21.0%), beta-cyfluthrin (10.5%), and other ingredients (68.5%). We hypothesized that these products would produce different hatch rates, and that the rate would be lower in the insecticide-susceptible Harold Harlan strain. We also hypothesized that all products would be effective against first-instar nymphs.

All bed bugs were maintained at 26°C, ~40% RH, and a reversed photoperiod of 12:12 (L:D) h before being removed from colony. All bed bugs were fed defibrinated rabbit blood (Hemostat Laboratories, Dixon, CA) through an artificial feeding system 5 d before being placed on filter paper. Approximately 1 wk before treatment, 300 adult male and 300 adult female pyrethroid-resistant, field-strain bed bugs (hereafter, Jersey City-strain bugs), and 230 adult male and 230 adult female Harold Harlan-strain bed bugs (hereafter, Harlan-strain bugs) were removed from colony and housed by sex in 125-ml glass jars (five jars per sex for each strain) with five pieces of folded envelope paper in each jar for harborage. A hole (~5 cm diameter) was created in the plastic lid of each jar. The hole was covered with white organza fabric (Mary Jo's Cloth Store, Gastonia, NC) and was attached to the plastic lid using super glue. The hole provided fresh air and enabled bed bugs to feed through the mesh on the artificial feeding system.

Nine days before insecticide applications, all male bed bugs of both strains were allowed to feed to repletion (~30 min) on defibrinated rabbit blood. Removal of males that did not feed ensured that all males had at least one bloodmeal in their adult lifetime and that their most recent meal occurred 3 d before being mated with females. All females were fed to repletion (~30 min) 3 d after feeding all males. Both sexes were combined in 125-ml glass jars 0–2 h after females had fed and were housed together for 4 d before males were removed. This method promoted mating and egg fertility; recently fed females are highly attractive to males (Cragg 1920, Mellanby 1939a, Stutt and Siva-Jothy 2001, Siva-Jothy 2006).

Through preliminary tests using the methodology described above, we determined that bed bug oviposition peaked for both strains at ~5–7 d post feeding. Based on these findings, 250 female Jersey City-strain bugs and 230 female Harlan-strain bugs were transferred to a total of 50 altered 59-ml Sysco plastic condiment cups (2.5 cups per strain, 9 or 10 bugs per cup) 5 d after feeding. Fewer Harlan-strain females were used due to limited colony size. Each container that housed bed bugs was created by removing the bottom of a (~60 mm diameter by ~30 mm height) condiment cup with scissors before using melted wax to fasten one piece of 90-mm Whatman (Vernon Hills, IL) filter paper to the bottom of the cup, forming a new base. The base was created to absorb excess product and prevent pooling and envelopment of bed bug eggs. A snap-on lid was modified for each cup by removing a circle ~2.5 cm diameter from the lid's center. A small circle of mesh was waxed over the hole. This design prevented escape and provided bed bugs with a fresh air supply. Condiment cups containing female bed bugs were placed on top of a plastic container situated within a large, soapy water-filled Tupperware container to prevent escapees from infesting the laboratory.

Female bed bugs were removed from condiment cups two days after introduction. Eggs were counted, then sprayed directly using Bedlam aerosol spray at 30 cm/s. A Pistol Pro (B&G Equipment Company, Jackson, GA) was used for the other four treatments at 25 psi at 30 cm/s, with a volume equivalent to 3.78 liter/92.9 m² (1 gal/1000 ft²). The total number of eggs hatched, plus live and dead nymphs, present 2 wk after insecticide application was determined for each strain and product combination. Eggs ranged 0–2 d old at the time of treatment, and the average egg hatched ~7–9 d after treatment. Nymphs, therefore, were subjected to ~5–7 d of insecticide exposure. The number of nymphs hatching for each product and strain combination was divided by the number of eggs determined before spraying to calculate average egg hatch rates. Nymph survival rate per product and strain combination was determined by dividing the number of living nymphs by the total number of hatched nymphs. Average proportions of egg hatch rate and nymph survival rate per product and strain combination were compared using ANOVA, followed by Fisher's LSD. Models used for analyses of egg hatch rate and live nymph proportions contained terms for product ($df=4$), block ($df=4$), and error ($df=16$). Assumptions necessary for the ANOVA and LSD results to be valid were carefully assessed. All calculations were performed using SAS procedure GLIMMIX (SAS 9.3). Voucher specimens for both strains are deposited in the Clemson University Arthropod Collection, and bear the label "Hinson dissertation, Chapter 4."

Results

Harlan-strain bugs produced 1,031 eggs, whereas Jersey City-strain bugs produced 753 eggs (Table 1). Egg hatch rate and proportion of nymphs living 2 wk after direct-spray applications varied by product

Table 1. Egg total and proportion of hatch rates of Harlan- and Jersey City-strain bed bug eggs when exposed to direct spray applications

Product	Harlan strain		Jersey City strain	
	Egg total	Proportion hatched	Egg total	Proportion hatched
Control	203	0.96 ± 0.019 ^A	152	0.99 ± 0.009 ^A
Bedlam	213	0.28 ± 0.079 ^B	145	0.84 ± 0.050 ^B
Demand CS	206	0.00 ± 0.000 ^C	152	0.91 ± 0.043 ^{AB}
Phantom SC	216	0.97 ± 0.016 ^A	148	0.95 ± 0.022 ^{AB}
Temprid SC	193	0.00 ± 0.000 ^C	156	0.13 ± 0.072 ^C

Female bed bugs were placed on filter paper by strain and were removed after 48 h. Filter paper containing eggs was treated by directly spraying eggs with distilled water (Control), Bedlam, Demand CS, Phantom SC, or Temprid SC. The number of eggs hatched was evaluated 2 wk after insecticide applications.

Standard errors are given for each proportion hatched. Values sharing the same superscript are not significantly different using Fisher's LSD test. Comparisons for statistical significance are applicable within strain only.

and strain (Table 1, Table 2). Both controls produced high hatch rates (96% for Harlan strain, 99% for field strain). Hatch rates among insecticide-treated Harlan-strain bugs were generally lower than those for Jersey City-strain bugs. Bedlam was moderately effective at preventing hatch of Harlan-strain bugs (28% hatch rate) and ineffective at preventing hatch of Jersey City-strain bugs (84% hatch rate). Demand CS was highly effective at preventing Harlan-strain egg hatch (0% hatch rate), but ineffective at preventing hatch of the Jersey City strain (91% hatch rate). Phantom SC hatch rates did not differ from the controls for either Harlan (97% hatch rate [$F=0.00$, $df=1,16$, $P>0.05$]) or Jersey City strains (95% hatch rates [$F=0.35$, $df=1,16$, $P>0.05$]). Temprid SC produced a 0% hatch rate for Harlan-strain eggs and was the only product to demonstrate a high level of control (13% hatch rate) against Jersey City-strain eggs (all $F > 144.00$, $df=1,16$, $P<0.05$).

Nymph survival rate after hatching differed by strain and product tested (Table 2). Insecticides affected Harlan-strain more than Jersey City-strain bugs. All Harlan- and Jersey City-strain nymphs in control treatments survived the duration of the study. No Harlan-strain nymphs survived any treatments other than the control. Bedlam failed to differ significantly from the control ($F=0.01$, $df=1,16$, $P>0.05$) for Jersey City-strain nymphs, with a 99%

survival rate. Demand CS resulted in a 0% survival rate for Jersey City-strain nymphs, but did not differ significantly from Phantom SC ($F=0.10$, $df=1,16$, $P>0.05$), with a 4% survival rate for Jersey City-strain nymphs. Temprid SC differed from all other products (all $F > 12$, $df=1,16$, $P<0.05$) but was only moderately effective at killing hatched nymphs, resulting in a survival rate of 38%. All products were ultimately effective against Harlan-strain bugs; Demand CS and Temprid SC produced an egg hatch rate of 0%, whereas Bedlam and Phantom SC produced a nymph survival rate of 0%.

Discussion

Temprid SC was the most successful product at preventing egg hatch and is appropriately labeled for bed bug eggs. The poor performance of other products containing pyrethroids suggests that Temprid SC's efficacy might be due in part to its other active ingredient, imidacloprid. Bedlam also was labeled for eggs, but performed poorly at preventing egg hatch, and was ineffective at killing nymphs. The true efficacy of this insecticide is difficult to evaluate as an aerosol product. Bedlam's label for bed bug eggs states that the product should be applied "for 13 seconds or until damp." Applying the product as we applied all other products (~30 cm/s) resulted in a damp surface, but this application method differed greatly from applying the product for 13 s, which produced a large, visible puddle of insecticide. This latter approach might be feasible for a few small spot treatments but would be difficult and costly to apply to a larger infestation.

In terms of proportion killed, Phantom SC and Demand CS were least effective at inhibiting hatch rates, but we note that neither is specifically labeled for bed bug eggs. These products were successful at killing first-instar nymphs, but have not been evaluated in the field; our nymphs were confined to small containers with residually acting insecticides for ~5–7 d. In a field setting, nymphs might wander from the treated oviposition sites or seek a host and receive little exposure to insecticides. Future researchers might directly spray eggs on filter paper and transfer this paper to a larger arena to provide emerging bed bugs with access to a larger nontreated surface.

A notable trend was that some products either killed the majority of Jersey City-strain eggs while leaving some nymphs alive, or killed few eggs and almost all nymphs. Despite high hatch rates among Jersey City-strain nymphs, Demand CS and Phantom SC were effective at killing emerged nymphs. Although Demand CS and Phantom SC did not differ significantly, Demand CS killed all nymphs,

Table 2. Number of eggs hatched and number of nymphs living 2 wk after insecticide treatments using Harlan- and Jersey City-strain bed bugs

Product	Harlan strain			Jersey City strain		
	No. of nymphs		Proportion survived	No. of nymphs		Proportion survived
	Hatched	Survived		Hatched	Survived	
Control	195	195	1.00 ± 0.000 ^A	150	150	1.00 ± 0.000 ^A
Bedlam	59	0	0.00 ± 0.000 ^B	122	121	0.99 ± 0.010 ^A
Demand CS	0	N/A	N/A	138	0	0.00 ± 0.000 ^B
Phantom SC	209	0	0.00 ± 0.000 ^B	141	6	0.04 ± 0.018 ^B
Temprid SC	0	N/A	N/A	21	8	0.38 ± 0.200 ^C

Female bed bugs were placed on filter paper by strain and were removed after 48 h. Filter paper containing eggs was treated by directly spraying eggs with distilled water (Control), Bedlam, Demand CS, Phantom SC, or Temprid SC. The number of nymphs surviving was evaluated 2 wk after insecticide applications.

Standard errors are given for each proportion of nymphs survived. Values sharing the same superscript are not significantly different using Fisher's LSD test. Comparisons for statistical significance are applicable within strain only.

whereas Phantom SC left six alive in this study. Why Demand CS and Phantom SC performed so poorly at preventing egg hatch cannot be fully explained. Demand CS contains a pyrethroid as the only active ingredient, which may explain why it was ineffective against the eggs of the pyrethroid-resistant, Jersey City strain. However, the active ingredient in Phantom SC (chlorfenapyr) belongs to a class known as halogenated pyrroles. Romero et al. (2010) found that chlorfenapyr was effective against adults of an insecticide-susceptible strain and two strains that were highly resistant to pyrethroids. Multiple mechanisms of resistance have been documented among bed bugs (Adelman et al. 2011, Zhu et al. 2013), which suggests that the pyrethroid-resistant, Jersey City strain may possess different resistance ratios compared with the strains examined by Romero et al. (2010). Resistance of eggs to insecticides has been documented in other groups of insects (Horowitz and Ishaaya 1994, Toloza et al. 2008) but is poorly understood for bed bugs. What is known about resistance in adult and immature bed bugs is difficult to compare to resistance mechanisms in eggs, as the stages are fundamentally different.

An important factor to consider is Phantom SC's inability to kill Harlan-strain eggs. Although Harlan embryos might be more resistant to insecticides than nymphs or adults, hatch rate more likely depends on the interaction of various factors. If products are highly toxic and weakly permeable, the products might fail to kill eggs but kill most nymphs after emergence. High toxicity but weak permeability might be the case for products such as Phantom SC. Demand CS was effective against Harlan-strain eggs, yet ineffective against field-strain eggs, perhaps due to resistance by strain, though these strains also might exhibit differences in egg shell composition that contributes to resistance. Hatch rate might need to be viewed as the result of an interaction between the type of resistance exhibited by strain, the active ingredient of the product, whether this active ingredient is capable of penetrating eggs, and the general permeability of a particular strain's eggs. Some ingredients, whether active or inert, might be capable of penetrating multiple layers of the egg chorion or gaining access to the embryo via aeropyles or micropyles (Campbell and Miller 2015).

Because of the unknown variability of bed bug chorionic permeability, nymph survival cannot be viewed simply as exposure to insecticides upon emergence. Depending on the chorionic permeability of a given product, it is likely that some first instars were not exposed to insecticides in the egg stage, whereas others were exposed as eggs and nymphs. As our assay was designed to evaluate survivorship of the early life stages of bed bugs in the context of a thoroughly treated infestation, it was necessary to directly spray eggs and expose surviving nymphs to residual insecticides. Although it was beyond the scope of our study, future researchers may wish to evaluate the survivorship of directly sprayed eggs and nymphs exposed to residual insecticides, and compare nymph survivorship to nymphs which are not sprayed as eggs, but transferred to an insecticide-treated surface immediately upon hatching. This approach would allow researchers to determine whether products that are highly effective at killing eggs are similarly effective at killing nymphs.

Goddard (2013) was the first of recent studies to investigate the effects of liquid insecticides sprayed directly on bed bug eggs. In addition to several other products, Goddard tested Phantom SC and Bedlam. Goddard's (2013) hatch rate for Phantom SC was lower (80%) than our rates for either strain, but still showed that the product was ineffective at preventing hatch. Goddard's (2013) hatch rate for Bedlam (24%) was similar to our hatch rate for the Harlan strain (28%), perhaps because both were insecticide-

susceptible strains. Hatch rates using Bedlam, however, were much higher for our Jersey City strain (84% hatch rate), probably due in part to the strain's pyrethroid resistance. Despite the similarity of our findings for insecticide-susceptible strains, Goddard (2013) might have used a different application rate; he applied products "according to rate," so Bedlam perhaps was applied to each group of eggs for 13 s.

Although the methodology of Campbell and Miller (2015) of dipping eggs in insecticides differed from our direct-spray applications, their use of late embryonic bed bugs (4–5 d development) complements our use of early embryonic (0–2 d) bed bugs. Campbell and Miller (2015) also found that Harlan-strain eggs and nymphs were more susceptible to insecticides (Temprid SC and Transport) than resistant-strain eggs and nymphs. They similarly found that eggs of two insecticide-resistant strains (Epic Center and Richmond) were not highly resistant to Temprid SC.

Singh et al. (2014) was methodologically similar to our study; they examined the efficacy of Demand CS and Temprid SC against eggs and first-instar nymphs of a moderately pyrethroid-resistant (Indy) bed bug strain over a 14-d period. Although the Jersey City strain used in our study is also moderately pyrethroid resistant, some of our results differed greatly from Singh et al. (2014). Using the Abbott formula (1925) to correct for mortality, and after applying arcsine square root transformation to mortality percentages, Singh et al. (2014) found that Demand CS produced a hatch rate of 32.6% and a nymph survival rate of ~25%, whereas our hatch rate for Demand CS was 91%, with a nymph survival rate of 0%. Our results for Temprid SC also differed from those of Singh et al. (2014); Singh et al. (2014) found that Temprid SC produced an egg hatch rate of 42% and nymph survival rate of ~28% compared with our hatch rate of 13%, and a nymph survival rate of 38%. The dramatic difference in hatch rate when applying the same product to different strains may be due to some of the previously mentioned characteristics regarding product and chorionic permeability by bed bug strain.

Compared with the number of studies focusing on adult bed bugs, few studies have focused on the application of liquid insecticides to bed bug eggs, even though such studies could be conducted with limited resources. Several popular commercial products have not been examined by independent researchers. Simple applications could be extended to surface-type assays in which eggs could be directly sprayed on wood, metal, or carpet to evaluate the effects of product and surface. Surfaces that absorb product more rapidly might decrease the exposure of eggs to insecticides, thereby increasing hatch rates.

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