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Research article

Evaluation of the Potential for Secondary Kill for Ingested Insecticides in the Common Bed Bug (Hemiptera: Cimicidae)

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Abstract

Baits are a preferred method of urban pest management. Baits enable more targeted insecticide applications with a fraction of the active ingredient used in residual sprays. Bait translocation by foragers, and consequent secondary kill of nonforagers, enhances bait effectiveness in social insects, and in other group-living species like German cockroaches (*Blattella germanica* L.). We investigated the potential for secondary kill in bed bugs (*Cimex lectularius* L.), another gregarious species, using a liquid bait. We first investigated whether blood-fed adults enhance nymph survivorship within aggregations by increasing the local relative humidity (RH) and providing fecal nutrients. Higher RH (50% and 95%) resulted in greater survivorship of first instars compared with 0% RH. Therefore, in subsequent experiments, we controlled RH to decouple its effect on nymph survivorship from effects of fecal nutrients. The presence of fed or unfed adults did not increase unfed first instar survivorship, suggesting that if nymphs ingested feces, its nutritional benefits were minimal. Nymph survivorship was unaffected by the presence of adult males fed fipronil or clothianidin, suggesting that unlike in cockroaches, highly effective insecticides might not be effective as secondary kill toxicants in bed bugs. To directly compare secondary kill in first-instar bed bugs and *B. germanica*, we exposed both to insecticide-laden adult *B. germanica* feces. Whereas first-instar *B. germanica* died in the presence of insecticide-laden feces, bed bugs did not. We, therefore, conclude that secondary kill with neuroactive insecticides will likely not be a significant factor in bed bug population suppression.

Key words: bed bug, *Cimex lectularius*, secondary kill, German cockroach, *Blattella germanica*

Baits have increasingly become a preferred and effective control method for urban pest management, largely because they have several advantages over broad-spectrum residual sprays: namely, smaller amounts of active ingredient (AI) are used in baits, they can last longer than most residual sprays, generally have lower mammalian toxicity, can be made as low-odor formulations, and can be used across a range of environmental conditions (Reierson 1995, Schal 2011). Additionally, effective AIs that cannot be labeled in spray formulations or are photolabile have been formulated in baits (Harpaz 1987). Baits can deliver much larger amounts of AI by ingestion than residual sprays do through cuticular penetration (Reierson 1995, Sierras and Schal 2017). Therefore, large amounts of AI can also be translocated by foraging individuals and cause mortality (i.e., secondary kill) in relatively more sedentary members of the population (Silverman et al. 1991, Buczkowski et al. 2001). Baits are currently used successfully against a variety of urban pests, including cockroaches, termites, wasps, ants, and rodents (Reierson 1995, Rust and Su 2012, Buckle and Eason 2015).

Baits have been highly effective against the German cockroach (*Blattella germanica* L.), a widespread urban pest, that lives in difficult-to-treat cracks and crevices (Reierson 1995, Schal 2011). A factor contributing to the efficacy of baits is the horizontal transfer of AI to other cockroaches who have not visited the bait, via ingesting insecticide-laden feces (coprophagy; Silverman et al. 1991, Buczkowski et al. 2001), other excretions, or feeding on dead insects (Gahlhoff et al. 1999, Buczkowski et al. 2001). Coprophagy is particularly important for survivorship of first-instar *B. germanica* (Kopanic et al. 2001), and it is an important mechanism for the horizontal transfer of hydramethylnon to the nymphs, leading to secondary kill (Silverman et al. 1991, Kopanic and Schal 1999). Secondary kill has been demonstrated in *B. germanica* with other AIs including boric acid, chlorpyrifos, fipronil, and indoxacarb (Buczkowski et al. 2001, 2008; Ko et al. 2016).

Bed bugs (*Cimex lectularius* L.), another household pest, have been making a recent global resurgence (Doggett et al. 2004, Hwang et al. 2005, Romero et al. 2007, Bencheton et al. 2011, Wang and

Wen 2011). These blood-sucking ectoparasites can have several adverse direct and indirect effects on humans, including bites that can lead to secondary infections, sleeplessness, anxiety, and social isolation (Hwang et al. 2005, Goddard and de Shazo 2012). Current control methods for bed bugs are limited to just a few options by efficacy, cost, and safety concerns. Residual insecticide treatments can be problematic because bed bug harborage sites are often on mattresses and other areas that are in close contact with humans (Pereira et al. 2009). Similar to German cockroaches, bed bugs also live in cracks and crevices which may be difficult to reach with residual sprays (Usinger 1966). Moreover, there is extensive resistance to pyrethroid insecticides, which are commonly used for bed bug control, and even to the more recently introduced neonicotinoids (Romero et al. 2007, Zhu et al. 2010, Kilpinen et al. 2011, Romero and Anderson 2016). Heat treatments of a whole room, apartment, or building (Pinto et al. 2007) and fumigation of infested objects (Wang et al. 2012) often provide greater efficacy if properly implemented, but these approaches can be cost prohibitive, as heat treatment of an entire home can cost US$3,000 or more, about two to five times the cost of treatment with residual sprays. Current control methods require multiple visits from pest control technicians, especially to eradicate broadly distributed infestations (Pinto et al. 2007, Pereira et al. 2009).

Bed bugs are an excellent candidate for control with a liquid bait, which could provide an effective and more affordable intervention. They take very large bloodmeals, which would deliver a large amount of AI even when the AI is formulated in relatively low concentration. For example, bed bugs experienced high mortality when they fed on humans who had taken a prescription-based dose of ivermectin, a ubiquitous antiparasitic drug (Sheele et al. 2013), or moxidectin, a related macrocyclic lactone (Sheele and Ridge 2016). Additionally, bed bugs can be stimulated to ingest large amounts of water augmented with phagostimulants (Romero and Schal 2014), and various insecticides, including fipronil, clothianidin, and abamectin, cause high mortality at relatively low concentrations when incorporated into an artificial feeding system (Sierras and Schal 2017). Horizontal transfer of synthetic and botanical insecticides, and biopesticides via contact has been demonstrated in bed bugs (Barbarin et al. 2012, Akhtar and Isman 2013), but not by ingestion.

We assessed the potential for secondary kill via an ingested insecticide in bed bugs by evaluating potential benefits of first instars cohabiting with adults and their direct exposure to the excreta of adults fed with insecticides. First, we sought to determine if adult bed bugs affect the survivorship of first instars by assessing 1) the potential of adults to prolong survivorship by increasing relative humidity (i.e., blood-fed adult conditioning of the microhabitat), and 2) the effects of blood-fed or unfed adults on unfed first-instar bed bugs (i.e., do nymphs gain nutritional benefits from adult excreta?). We then evaluated the direct effects of exposure of first instars to the excreta of adult male bed bugs fed with insecticide. If secondary kill occurs in bed bugs, it could enhance the effectiveness of a liquid bait, once one is developed.

Materials and Methods

Insects

A pyrethroid-susceptible strain of *C. lectularius* (Harold Harlan = Ft. Dix strain, maintained since 1973 on human blood, and on defibrinated rabbit blood in our lab since 2008) and two pyrethroid-resistant strains were used: Jersey City and Winston-Salem (collected in Jersey City, NJ, and Winston-Salem, NC, in 2008, and maintained in culture on defibrinated rabbit blood in our lab). Further selection with insecticides was not imposed in the laboratory. Bed bugs were maintained in an incubator at 27°C and a photoperiod of 12:12 (L:D) h. Virgin females and males were obtained by separating fifth instars from the colony and maintaining them in isolation through the adult molt.

Blattella germanica were from an insecticide-susceptible laboratory colony (Orlando Normal = American Cyanamid strain, collected >70 yr ago in Orlando, FL, and maintained in our lab since 1989) and a field-collected fipronil-resistant strain (PR-712, collected in 2012 from an apartment in Carolina, Puerto Rico) that was selected in the lab for higher fipronil resistance (Ko et al. 2016). We used a fipronil-resistant strain to ensure that individuals that consumed the bait would survive long enough to defecate. Both strains of *B. germanica* were provided ad libitum with water and rodent chow (LabDiet 5001, PMI Nutrition International, Brentwood, MO).

Artificial Feeding System

Bed bugs were fed defibrinated rabbit blood (Quad Five, Ryegate, MT) in an artificial feeding system. Custom-built water-jacketed glass feeders were connected to a thermal circulator water bath (B. Braun Biotech, Inc., Allentown, PA) heated to 37 °C. Feeders could hold up to 4 ml of blood, which was held in place by a membrane stretched across the bottom of the feeder (NESCOFILM, Karlan, Cottonwood, AZ).

Experimental Chambers and Vials

Experimental chambers for relative humidity (RH) studies were 32 oz. (946 ml) glass straight-sided jars with metal caps (Uline, Pleasant Prairie, WI; Fig. 1). Saturated salt solutions were prepared to maintain uniform RH in each chamber. Reagent grade potassium nitrate (Product number S25494A, Thermo Fisher Scientific, Waltham, MA), which was used to maintain 95% RH at 27 °C, was prepared by dissolving 38.4 g of potassium nitrate in 86 ml of deionized water on a 300 °C hot plate stirrer (Thermo Fisher Scientific). Likewise, reagent grade magnesium nitrate hexahydrate (Product number 423885000, Acros Organics, Thermo Fisher Scientific), which was used to maintain 50% RH at 27 °C, was prepared by dissolving 165 g of magnesium nitrate in 50 ml of deionized water. After the salt solutions cooled, a 6-cm section of PVC pipe (6 cm OD, United States Plastic Corp., Lima, OH) was placed in the bottom of each chamber. The pipe held an 8.3-cm-diameter circular metal screen that separated the salt solution from the vials containing bed bugs.

A glass desiccator jar (Model 3081150, Corning, Inc., Corning, NY) with 150 g of Drierite anhydrous calcium sulfate (Product number 23001, W.A. Hammond Drierite Co. Ltd., Xenia, OH) served as the experimental chamber to maintain 0% RH. A ceramic plate on top of the Drierite separated it from the bed bug vials. Vaseline petroleum jelly (Unilever, London, United Kingdom) was used to coat the edges of the desiccator lid to create a tight seal. HOBO Temperature and Relative Humidity Data Loggers (Model UX100-003, Onset Computer Corporation, Bourne, MA) were used to monitor the temperature and RH within all experimental chambers.

Bed bugs were contained in 7-ml borosilicate glass scintillation vials (Thermo Fisher Scientific). An ~5-mm hole in the center of each vial cap allowed for ventilation through plankton netting fabric (0.3 mm mesh opening, 0.2 mm fabric thickness; BioQuip Products,

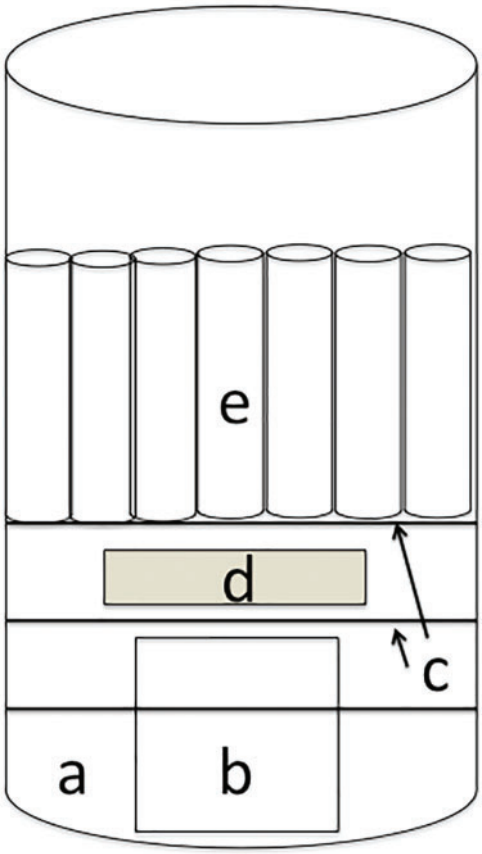

Fig. 1. Experimental chambers for controlled RH. Saturated salt solutions (**a**) were in the bottom of a sealed glass jar, with a section of PVC pipe (**b**) as a spacer. Metal screening (**c**) separated the salt solutions, HOBO temperature and RH loggers (**d**), and experimental vials containing bed bugs (**e**).

Inc., Compton, CA). Another piece of plankton netting (28 by 4 mm) inside the vial served as a substrate for bed bugs to grasp.

Experimental Design

Five newly hatched first-instar bed bugs were transferred into each experimental 7-ml vial using a sliver of filter paper (Whatman number 1, GE Healthcare Bio-Sciences, Pittsburgh, PA) held in a glass 100-µl capillary (Thermo Fisher Scientific). Each daily cohort of hatched nymphs was distributed evenly among all five treatments for a total of 50 nymphs (10 vials) in each treatment. Treatments consisted of nymphs alone at 0%, 50%, and 95% RH, nymphs with two blood-fed virgin adults, and nymphs with two unfed virgin adults at 95% RH. Mortality of nymphs was recorded twice daily. The experiment ended when all nymphs were dead.

Co-Habitation With Fed or Unfed Adults

Virgin females or males (~7-d old) were blood-fed as a group and allowed to fully engorge for 30 min. Two fully fed females or males were then placed in each 7-ml experimental vial with five unfed nymphs. Every 5 d, adults were removed from the experimental vials and fed in pairs according to their experimental vials for 30 min. Care was taken not to disturb or remove the nymphs. If an adult died or did not feed, it was replaced with another virgin adult of the same sex.

Similar experiments were conducted with ~7-d-old unfed virgin females or males. Adults were not removed from vials for the duration of the experiment, but dead insects were replaced.

First-Instar Bed Bug Survivorship in the Presence of Insecticide-Fed Adults

Insecticide solutions were prepared according to the procedure described in Sierras and Schal (2017). Briefly, technical grade fipronil (88.7%, Bayer CropScience, Research Triangle Park, NC) and clothianidin (99.5%, Chem Service, Inc., West Chester, PA) were dissolved and serially diluted in dimethyl sulfoxide (DMSO). The AI in DMSO solutions were then mixed with defibrinated rabbit blood, resulting in a 0.75% DMSO in 2 ml blood.

Five first-instar Harold Harlan bed bugs that remained unfed for 5–10 d since they hatched were placed into each 2-ml autoinjection vial (Thermo Fisher Scientific). A 17- by 4-mm manila folder insert was placed in the vial as substrate and the Teflon septum in the vial cap was replaced with plankton netting to allow for ventilation. In a separate treatment, five German cockroach first instars were starved for 24 h (but provided water) and transferred into 2-ml vials. Starved adult male bed bugs of the Winston Salem and Jersey City populations were allowed to feed for 20 min on blood containing fipronil or clothianidin, respectively, and an additional control group was fed on blood only. Immediately after feeding, groups of five fully engorged adult bugs were added to each 2-ml experimental vial containing either bed bug or cockroach nymphs. Mortality of nymphs of both species was monitored daily for 7 d. Dead adults and nymphs were not removed from experimental vials.

Nymph Survivorship in the Presence of Insecticide-Laden German Cockroach Feces

Approximately 60 *B. germanica* adult males from the moderately fipronil-resistant PR-712 colony were starved for 24 h and then allowed to feed for 1 h on Maxforce FC Magnum (0.05% fipronil; Bayer Environmental Science, Research Triangle Park, NC, EPA Reg number 432-1460). The males were then transferred to a 10- by 10-cm plastic box (Althor Products, Windsor Locks, CT), the bottom of which was covered with four accordion-folded 65-mm-diameter filter paper discs (Whatman number 1, GE Healthcare Bio-Sciences), provided with water, and allowed to defecate for 24 h. All bait-fed PR-712 males died during this 24-h period. The filter paper discs and a 0.5-ml microcentrifuge tube of water (Thermo Fisher Scientific) were then placed inside a 65-mm petri dish (Thermo Fisher Scientific). Ten first instar insecticide-susceptible Orlando Normal *B. germanica* nymphs and 10 insecticide-susceptible Harold Harlan *C. lectularius* first instars were placed in each of the four petri dishes. Nymph mortality of both species was recorded every 24 h for 7 d. The same procedure was followed with another group of PR-712 males fed rodent chow instead of bait as control.

To reduce coprophagy and, therefore, indirectly assess the importance of contact with feces, the same experiment was conducted as described above with another group of bait-fed PR-712 males and 10 *B. germanica* first instars in each of the four petri dishes, but no bed bugs. However, this time along with water, a 0.5 g dollop of creamy peanut butter (Jif, The J.M. Smucker Company, Orrville, OH) was placed in each petri dish as an alternative, and presumably preferable, food source to feces. A rodent chow-fed, instead of bait-fed, group was again used as control. Sample size in all feces assays was 40 first instars of each species.

Data Analysis

Data were analyzed using a Kaplan–Meier survivorship curve (Parmar and Machin 1995) generated in SPSS 22 (IBM 2013). This option for analysis was chosen over regression owing to the presence of censored data. The log-rank option was selected to identify differences among treatment groups, and pairwise comparisons were conducted across treatments.

Results

Effects of RH and Fecal Nutrients on Nymph Survivorship

Relative humidity affected the survivorship of first-instar nymphs. Overall, the length of nymph survivorship varied with the percentage of RH, with significant differences among treatments ($\chi^2 = 64.40$; df = 2; $P < 0.0001$; Fig. 2A). Pairwise comparisons between the 0% and 50% RH groups and 0% and 95% RH groups were highly significant ($\chi^2 = 54.55$; df = 1; $P < 0.0001$ and $\chi^2 = 30.32$; df = 1; $P < 0.0001$, respectively), but a pairwise comparison between the 50% and 95% RH groups was not significant ($\chi^2 = 1.02$; df = 1; $P = 0.312$). These results suggest that under low environmental RH conditions, if fresh liquid excreta from recently fed adults increased RH within the microhabitat, the survival of nymphs could also increase.

To control for the effects of RH and investigate the potential nutrient contribution of fed adults to starved nymphs, we placed unfed nymphs with blood-fed or unfed adult males or females at 95% RH. Cohabitation of blood-fed adult males or females with unfed nymphs did not increase nymph survivorship. Indeed, pairwise comparisons indicated that first instars survived longer alone ($\chi^2 = 121.90$; df = 2; $P < 0.0001$; Fig. 2B) than with blood-fed males ($\chi^2 = 86.74$; df = 1; $P < 0.0001$), but not with unfed males ($\chi^2 = 2.39$; df = 1; $P = 0.122$). Likewise, first instars did not live longer in the presence of adult females ($\chi^2 = 148.79$; df = 2; $P < 0.0001$; Fig. 2C). In pairwise comparisons, first instars lived significantly shorter in the presence of either fed ($\chi^2 = 94.52$; df = 1; $P < 0.0001$) and unfed ($\chi^2 = 5.43$; df = 1; $P = 0.02$) females than when alone. These results suggest that unfed first-instar bed bugs would not benefit significantly from either the water or nutrients in the excretions of recently blood-fed adults. These results also predict that secondary kill might be minimal in bed bugs.

Secondary Kill Effects in Bed Bugs

To maximize the excretion of insecticide in feces, we used adult bed bugs of the Winston Salem and Jersey City populations which are moderately resistant to fipronil and clothianidin, respectively (A.S and C.S., unpublished data). Therefore, with the concentrations of AIs we used, these bugs were expected to fully engorge and defecate without succumbing to the AIs. Indeed, only ~5% adults died in the fipronil treatment and ~65% in the clothianidin treatment during the 7-d observation period, while 100% of Harold Harlan strain bed bugs died during this period (Sierras and Schal 2017). Survivorship of insecticide-susceptible (Harold Harlan) first-instar bed bugs, however, was unaffected by the presence of adult bed bugs that were fed fipronil or clothianidin ($\chi^2 = 2.11$; df = 2; $P = 0.348$; Fig. 3A).

To bioassay the relative amount of AI in bed bug excreta, we exposed Orlando Normal German cockroach first instars, which are known to be highly susceptible to these AIs, to similarly treated bed bugs and their excreta. Notably, German cockroaches are much more susceptible to starvation, and there was a general decline in survival in all treatment groups over the 7-d experiment (Fig. 3B). However, the presence of insecticide-fed adult bed bugs and their excrement did not affect the survivorship of first-instar German cockroaches. In bed bugs fed three concentrations of fipronil and two concentrations of clothianidin, an overall comparison showed a marginally significant difference among treatments ($\chi^2 = 11.46$; df = 5; $P = 0.043$; Fig. 3B). However, pairwise comparisons between insecticide-fed and the respective blood-only control did not reveal any significant differences in any of the fipronil treatments ($\chi^2 = 0.27$; df = 1; $P = 0.601$ for 5.5 ng/ml; $\chi^2 = 0.33$; df = 1; $P = 0.568$ for 10 ng/ml; $\chi^2 = 1.08$; df = 1; $P = 0.300$ for 25 ng/ml) or clothianidin treatments ($\chi^2 = 0.08$; df = 1; $P = 0.785$ for 43.4 ng/ml; $\chi^2 = 3.35$; df = 1; $P = 0.067$ for 86.8 ng/ml).

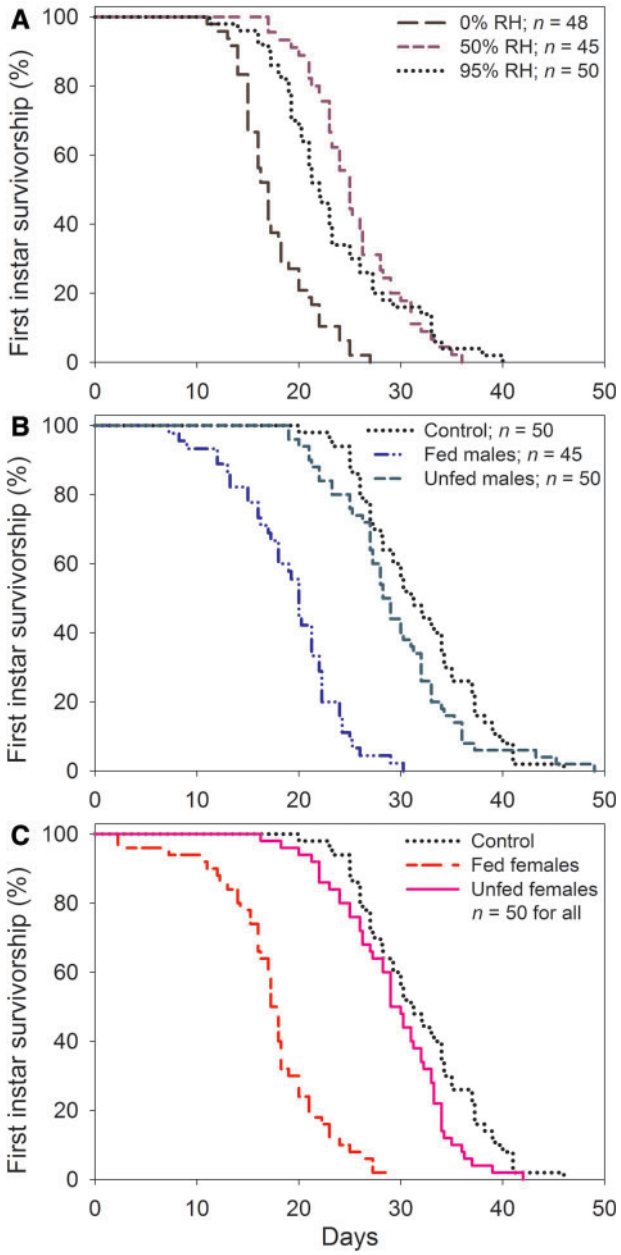

Fig. 2. Kaplan–Meier survivorship curves of unfed first instars of *C. lectularius* alone under different RH conditions (**A**; $\chi^2 = 64.40$; df = 2; $P < 0.0001$), and in the presence of males (**B**; $\chi^2 = 121.90$; df = 2; $P < 0.0001$) and females (**C**; $\chi^2 = 148.79$; df = 2; $P < 0.0001$). Relative humidity was 95% in all treatments in (**B**) and (**C**).

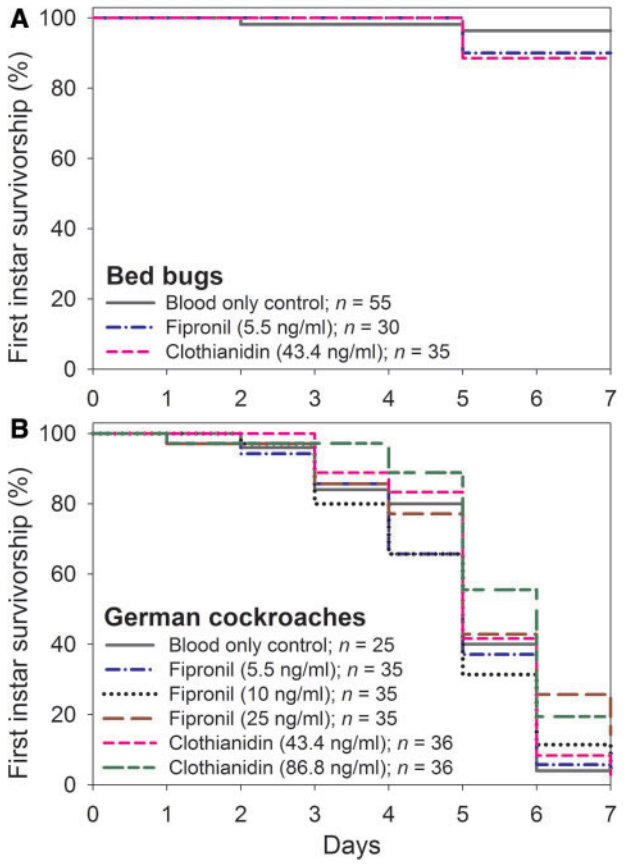

Fig. 3. Kaplan–Meier survivorship curves of unfed first instars of *C. lectularius* (**A**; $\chi^2 = 2.11$; df = 2; $P = 0.348$) and *B. germanica* (**B**; $\chi^2 = 11.46$; df = 5; $P = 0.043$) in the presence of fipronil- or clothianidin-fed adult male bed bugs. Each curve represents a different species and treatment combination. Concentrations denote the concentration of AI fed to adult male bed bugs.

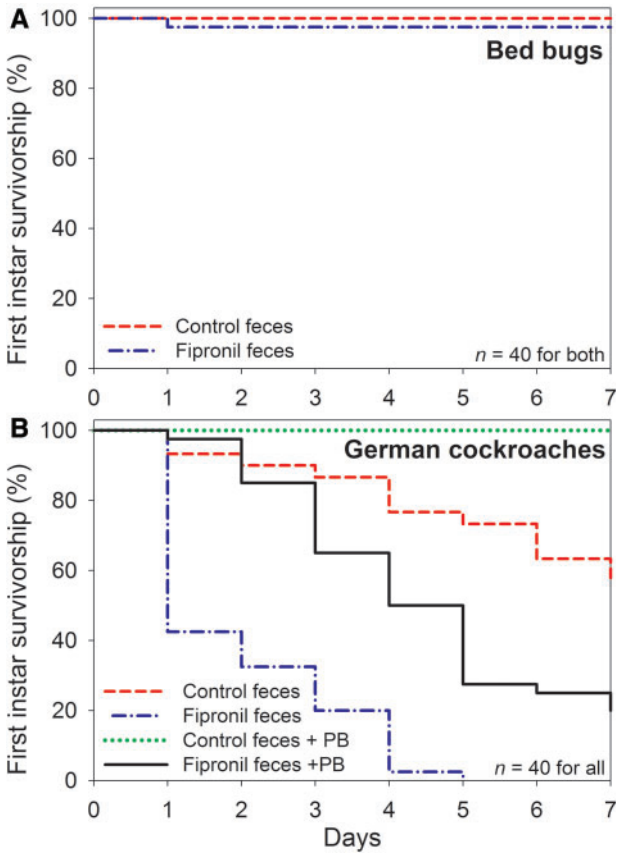

Fig. 4. Kaplan–Meier survivorship curves of unfed first instars of *C. lectularius* and *B. germanica* exposed to insecticide-laden feces of German cockroach adult males ($\chi^2 = 136.23$; df = 3; $P < 0.0001$). Each curve represents a different species and treatment combination. PB is peanut butter offered as an alternative food to feces, and control feces do not contain insecticide.

To further increase the excretion of insecticide-laden feces, we used moderately fipronil-resistant German cockroaches. All treated adults died within 24 h. An overall comparison of mortality in insecticide-susceptible first-instar German cockroaches and insecticide-susceptible bed bugs revealed a highly significant difference among treatments ($\chi^2 = 136.23$; df = 3; $P < 0.0001$; Fig. 4). The first-instar *B. germanica* nymphs exposed to fipronil-laden feces died significantly more quickly than those exposed to insecticide-free feces ($\chi^2 = 49.76$; df = 1; $P < 0.0001$). Yet, the insecticide-susceptible first-instar bed bugs in both groups were unaffected, with only one dead.

To determine whether mortality in first-instar *B. germanica* was owing to contact with or ingestion of feces, we offered the starved experimental nymphs peanut butter. Although none of the control peanut butter-fed nymphs died, only 20% of those exposed to both peanut butter and fipronil-laden feces survived ($\chi^2 = 10.40$; df = 1; $P = 0.001$). There was also a highly significant difference in survivorship in first-instar *B. germanica* between groups offered fipronil-laden feces and fipronil-laden feces plus peanut butter ($\chi^2 = 34.48$; df = 1; $P < 0.0001$). It thus appears that secondary kill owing to contact with fipronil-laden feces is negligible in bed bugs and slight in cockroaches, whereas coprophagy facilitates most of the secondary kill observed in cockroaches and none in bed bugs.

Discussion

The direct effect of baits on population suppression is substantial, as evidenced by the effectiveness of bait formulations in a wide array of social and solitary insects (Reierson 1995, Vargas et al. 2002, Klotz et al. 2003, Rust and Su 2012). Nevertheless, in pest management of social insects (ants, termites, and wasps) and cockroaches, delivery of bait to the colony and its distribution among colony members contribute significantly to effective pest management (Buczkowski et al. 2001, Rust and Su 2012). Three common features to all these species are as follows: 1) aggregation of foragers with nonforagers; 2) chewing mouthparts that facilitate coprophagy, trophallaxis, and other means of sharing nutrients and microbes; and 3) fitness benefits accrued from the behaviors in 1) and 2). Bed bugs also live in aggregations that apparently facilitate their development (Saenz et al. 2014). But their piercing–sucking mouthparts may not be conducive to coprophagy and it is not known whether nonforagers benefit from forager-mediated delivery of nutrients and microbes. First, we investigated the effects of recently blood-fed adults on survivorship of unfed nymphs, and then the effects of adult ingestion of large amounts of insecticides on survivorship in cohabiting nymphs.

Adults might benefit cohabiting nymphs by altering the microhabitat and by delivering to nymphs' fitness-promoting materials, such as nutrients and beneficial microbes. Given the first instar's high surface-to-volume ratio and thin cuticle, RH is expected to be an important factor. Indeed, unfed first-instar bed bugs lived longer

at medium and high RH than at low RH. Nevertheless, they did show a remarkable tolerance to desiccation. These findings are not surprising, as other studies found that first instars were most susceptible to desiccation (Usinger 1966, Benoit et al. 2007, Polanco et al. 2011). Aggregation increases the water-retention abilities in first instars, and it follows therefore that the presence of blood-fed defecating adults could locally increase the RH and prolong first-instar survivorship (Benoit et al. 2007). Moreover, nymphs might directly ingest and, thus, benefit from copious excretions of adults. However, we did not specifically investigate the potential of adult excreta to locally increase RH in experimental vials, as we were more interested in addressing coprophagy. Instead, we controlled RH using saturated salt solutions in subsequent experiments to investigate the effects of cohabitation with blood-fed and unfed adults.

Cohabitation with either blood-fed or unfed adults did not affect the survivorship of first-instar bed bugs. On the contrary, survivorship of nymphs was lower in the presence of fed adults. It is possible that our frequent manipulation of the experimental vials had a detrimental effect on nymphs despite our efforts not to disturb them. We tried several approaches to allow adults to blood-feed while preventing nymphs in the same vial from feeding, including using a thicker feeding membrane, multiple membrane layers, and using multiple layers of plankton mesh; but in all cases, adults did not feed as readily. Had we been able to feed adults while excluding nymphs cohoused in the same vial, these manipulations could have been prevented. However, because it was imperative that we maximize the number of adults that fully engorged, we opted to remove adults from vials every 5 d to refeed them, and then returned the freshly fed adults into the respective vials containing nymphs.

It is also possible that other features of the experimental design might have contributed to the decline in first-instar survivorship in the presence of blood-fed adults. For example, first instars could have gotten stuck in feces deposited by adults as the fecal spots dried, rendering them immobile. Indeed, this was observed in several cases, but the phenomenon did not appear to be widespread (Y.K.M., personal observation). The presence of either unfed males or females (who were not removed from experimental chambers) also slightly decreased survivorship of first instars, suggesting that perhaps adults somehow damaged first instars. First instars have a thin cuticle (Polanco et al. 2011) that could easily be pierced by adult tarsal claws as they move about, leaving first instars susceptible to desiccation.

The lack of survivorship benefits to nymphs from cohabitation with freshly blood-fed adults suggests that first instars likely do not accrue macronutrient benefits from adults. Behavioral observations also indicate that starved adults and nymphs do not feed on isolated droplets of blood in a petri dish, even when placed on or directly next to the drop (Y.K.M., personal observation), but they accept and feed readily on the artificial feeding system. Starved bed bugs on occasion, however, have been observed to drink from free-standing droplets of water (Benoit et al. 2007), although this does not appear to be a common behavior. Lacking a direct benefit of coprophagy, secondary kill from ingestion of adult excretions is therefore unlikely in bed bugs. This is in contrast to the German cockroach, where starved first instars live longer when allowed to feed on adult feces (Kopanic et al. 2001). It is also in contrast to the situation in the closely related triatomines that engage in coprophagy, hematophagy, and cannibalism. In fact, these behaviors are mechanisms that facilitate the transmission of pathogens such as *Blastocrithidia triatomae* (Schaub et al. 1989) and *Triatoma virus* (Muscio et al. 2000). Additionally, *Triatoma virus* can be transmitted to uninfected bugs that feed on chickens whose skin had been previously contaminated with dried and liquid feces of infected bugs (Muscio et al. 2000).

The prediction that secondary kill would be minimal in first-instar bed bugs was confirmed by directly exposing them to adult males fed various concentrations of fipronil in blood: survivorship was unaffected compared with nymphs exposed to unfed males or first instars not exposed to any adult bugs. These results indicate not only that first-instar bed bugs are not ingesting adult fecal products, but also that they appear to be unaffected by close contact with insecticide-treated adults and insecticide-laden feces. Fipronil and clothianidin are potent AIs on bed bugs both by ingestion and contact (Sierras and Schal 2017). The fipronil ingested LC_{50} values for adults and first instars of the Harold Harlan strain are 13.4 and 6.84 ng/ml blood, respectively; the LD_{50} for adult males by topical application is 2.21 ng. For clothianidin the corresponding ingestion values are 14.2 and 20.7 ng/ml of blood for adult males and first instars, respectively (Sierras and Schal 2017). It is possible that the maximum amounts of these two AIs that were ingested by the adult bed bugs were insufficient to kill first instars via contact. Using an empirically measured ingested volume of 3.92 µl blood per male and 0.46 µl blood per nymph, Sierras and Schal (2017) calculated LD_{50} values for adult males and first instars of 52 pg/adult male and 3 pg/first-instar nymph. Thus, at the highest concentration of fipronil that we used (25 pg AI per µl blood), a male would ingest 98 pg of fipronil. It is unknown how much AI adult bed bugs would defecate, but even 10% would be sufficient to kill nymphs, if they ingested it. By contact alone, however, this is not nearly enough fipronil to kill bed bugs. Based on the same calculations using clothianidin (the highest concentration of AI we used was 86.8 ng/ml blood, which was twofold the d 3 LC_{90} generated from probit analysis; calculated ingested 339 pg AI/adult male; calculated twofold the LC_{90} or 40 pg/first-instar nymph) we reach a similar conclusion—nymphs would likely die if they ingested the AI but there was likely not enough AI in feces to kill nymphs by contact.

We attempted to increase the amount of AI in feces by feeding adult German cockroaches' fipronil bait and exposing first-instar bed bugs to insecticide-laden feces of German cockroaches. Here too, however, first-instar bed bugs were unaffected. As calculated by Ko et al. (2016), the LD_{50} of fipronil was 2.12 ng AI per adult male, so a male that ingested a typical daily intake of 2.5 mg (Hamilton and Schal 1988) of the bait (0.05% AI) would ingest 1,250 ng of fipronil, ~600-fold the LD_{50}. Again, if 10% of the ingested AI were defecated, the 125 ng of fipronil would be ~40-fold the amount needed to kill first-instar bed bugs by ingestion. Yet, although first-instar *B. germanica* were killed by these fipronil-laden feces, first-instar bed bugs, which are smaller and more susceptible to fipronil, did not die. The German cockroach nymphs died because they ingested the feces, whereas the bed bug nymphs did not. This inference is supported by the observation that fewer first-instar cockroaches died when offered peanut butter in addition to fipronil-laden feces. Finally, their survival on relatively high concentrations of fipronil in feces may suggest that bed bugs might employ some other behavioral mechanisms to avoid contact with AI-laden feces.

The concept of secondary kill has gained prominence in urban pest management. In social insects, where trophallaxis and other food-sharing tactics are central features of the social organization, horizontal transfer of AIs and secondary kill are pivotal for colony elimination of pest insects. In sub- and nonsocial insects, however, the magnitude and efficacy of secondary kill varies with the biology of the pest, and especially with the degree of dependence of larvae on coprophagy. For example, cat flea (*Ctenocephalides felis* Bouché)

larvae feed on the feces of adults, and the relatively undigested blood excreted in adult feces contributes to larval nutrition and development (Silverman and Appel 1994, Hsu et al. 2002). In fact, larvae that do not feed on adult feces do not achieve pupation (Bruce 1948, Moser 1989). Consequently, when flea larvae consume the feces of adults that fed on host blood containing insecticide, they in turn die if enough AI is present in the feces. Systemic insecticides, such as avermectins, which are ingested by vertebrate hosts and then by adult fleas of both sexes (Rust 2005), serve both as flea adulticides and larvicides because of the dependence of larvae on adult feces, dried blood, and infertile eggs for nutrients. German cockroach nymphs are much less dependent on coprophagy than fleas. However, the combination of frequent (daily) large meals by adults, rapid defecation of ingested baits, mandibulate mouthparts that can ingest solid feces, and the propensity of first instars to engage in some coprophagy, all combine to facilitate high secondary kill. Nevertheless, the significance of coprophagy and secondary kill has never been quantified in the field (Schal 2011). Bed bugs take even larger meals than cockroaches (relative to body mass), but meals are much less frequent, and it could be possible to ingest feces via the piercing–sucking mouthparts only transiently while it is in liquid form. Moreover, the concentration of AI in solid and gel baits is substantially greater than in systemic use of the same AI to protect vertebrate hosts.

As we progress toward the design of liquid baits for bed bugs and other hematophagous arthropods such as mosquitoes, sand flies, fleas, and some flies, these biological factors must be taken into account to optimize secondary kill. A major advantage to an artificial liquid bait over the use of a live host is the concentration of AI can be elevated markedly to facilitate greater secondary kill effects in systems that exhibit secondary kill.

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