JULY 2020 - VOL 57 - NO 4

PRINT ISSN 0022-2585 ONLINE ISSN 1938-2928

JOURNAL OF MEDICAL ENTOMOLOGY

ENTOMOLOGICAL SOCIETY OF AMERICA
SHARING INSECT SCIENCE GLOBALLY

ACADEMIC.OUP.COM/JME
INSECTSCIENCE.ORG

OXFORD UNIVERSITY PRESS

Journal of Medical Entomology, 57(4), 2020, 1199–1206
doi: 10.1093/jme/tjaa038
Advance Access Publication Date: 26 February 2020
Research

Lethal and Sublethal Effects of Ingested Hydroprene and Methoprene on Development and Fecundity of the Common Bed Bug (Hemiptera: Cimicidae)

Angela Sierras[1] **and Coby Schal**[1,2]

[1]Department of Entomology and Plant Pathology, North Carolina State University, Campus Box 7613, Raleigh, NC 27695-7613 and [2]Corresponding author, e-mail: coby@ncsu.edu

Subject Editor: Michael Rust

Received 8 January 2020; Editorial decision 4 February 2020

Abstract

In the last two decades, bed bugs (*Cimex lectularius* L. and *Cimex hemipterus* F.) have become perennial and difficult to control indoor pests. Current pest control options are severely constrained by high prevalence of insecticide resistance and availability and relatively high costs of alternative interventions. Among various measures to counter the drawbacks of insecticide resistance include efforts to diversify the modes of action of insecticides with residual applications of combinations of insecticides, which include a juvenile hormone analog (JHA). JHAs, such as hydroprene and methoprene, have a desirable safety profile and are effective against a variety of indoor pests. We evaluated the potential of hydroprene and methoprene to be incorporated into an ingestible bait, with dose–response studies on fifth-instar male and female bed bugs. Females were more susceptible than males to both JHAs, and methoprene was more effective by ingestion than hydroprene at inducing both lethal and sublethal effects. Ingestion of ≥10 µg/ml blood of either JHA by last instar nymphs reduced oviposition; untreated females that mated with males exposed to high concentrations of either JHA also exhibited lower oviposition. We suggest that methoprene could be incorporated into integrated pest management programs in liquid baits and residual sprays in combination with other active ingredients.

Key words: Urban IPM, bed bugs, *Cimex*, juvenile hormone analog, insect growth regulator

Bed bugs are ubiquitous, obligatory blood feeding insects. Their preferred host are humans, but they readily feed on other vertebrates (Usinger 1966). Like lice, but unlike other blood feeding urban pests, such as fleas, mosquitoes, sand flies, and other hematophagous flies, every mobile life stage is dependent on a bloodmeal for growth, development and reproduction. When a host is available, bed bugs feed every 3–7 d (Reinhardt and Siva-Jothy 2007, Pereira et al. 2013). Indoors, their obligate ectoparasitic association with humans makes bed bugs a particularly loathed pest. During feeding, they introduce salivary secretions that can elicit local cutaneous or systemic immune responses in the host; these responses can be serious in some individuals (Goddard and deShazo 2009, Hwang et al. 2018). Subsequent scratching of bite sites can lead to secondary infections (Thomas et al. 2004).

As bed bugs have become more prevalent, so has their impact on people and their public health importance. Bed bugs have been shown to adversely affect quality of life and psychological health, causing depression, anxiety, and paranoia (Rossi and Jennings 2010, Perron et al. 2018). Bed bug feces contains histamine, and significant amounts levels of histamine accumulate and persist in household dust even after successful heat interventions (DeVries et al. 2018). Although the health impacts of environmental histamine remain unknown, adverse respiratory and dermatological reactions are possible because of the close proximity of bed bug feces to our breathing space. As with histamine, the importance of bed bugs as pathogen vectors is largely unknown. Although >40 pathogens have been found in different body parts or excretions of the bed bug (Lai et al. 2016), transmission of these harmful pathogens has not been documented under natural conditions (Doggett 2018).

Indirect health and environmental concerns include the large amounts of insecticides used to control bed bug infestations. Although desiccant dusts can be efficacious when properly used, residual insecticide sprays, often consisting of combinations of pyrethroids and neonicotinoides, are the primary approach to bed bug control because this approach is less expensive than fumigation or heat treatment, and residual sprays are readily available for do-it-yourself pest control (Doggett et al. 2012, Lee et al. 2018). High levels of resistance to these insecticides (Zhu et al. 2010, Adelman et al. 2011, Romero and Anderson 2016, Agnew and Romero 2017, Romero 2018), however, necessitate multiple applications, leading to

greater insecticide use and strong selection for higher resistance and multiple resistance mechanisms.

The practice of combining insecticides with different modes of action to combat resistance, includes insect growth disruptors (IGDs), formerly termed insect growth regulators (IGRs) (Pener and Dhadialla 2012). Although IGDs are slower acting than neurotoxic insecticides, they can be highly effective as they disrupt the insect life cycle, particularly at juvenile stages (Pener and Dhadialla 2012). The most common IGDs used within the urban environment are chitin synthesis inhibitors, such as lufenuron and novaluron (Su et al. 2014, Seccacini et al. 2018), which interfere with the formation of chitin, and juvenile hormone mimics (e.g., pyriproxyfen, hydroprene, and methoprene), which disrupt metamorphosis (Invest and Lucas 2008, El-Sheikh et al. 2016). The juvenile hormone analogs (JHAs), namely hydroprene and methoprene, are structurally similar to naturally occurring juvenile hormones (JHs), and have a desirable safety profile, including low mammalian toxicity and relatively rapid degradation in the environment (Mohandass et al. 2006).

Both hydroprene and methoprene have been used in the successful control of various urban pests. Both have been shown to cause larval mortality and affect egg production and egg hatch in fleas (Moser et al. 1992, Graf 1993), and genitalia and ootheca deformities and sterility in German cockroaches (King and Bennett 1989, 1991; Atkinson et al. 1992) and oriental cockroaches (Short and Edwards 1992). Hydroprene has also been shown to effectively control several stored product pests (Mohandass et al. 2006). Likewise, methoprene, the first commercially registered JHA, has been used to control mosquito larvae, various flies, fleas, as a feed-through insecticide mainly against flies, and in baits to control Pharaoh ant (Lim and Lee 2005) and Fire ant (Williams et al. 2001) colonies.

Juvenile hormone analogs have been evaluated for bed bug control. Goodman et al. (2013) found that hydroprene and methoprene were ineffective against bed bugs when they were applied directly to various life stages at the label rates. Naylor et al. (2008) and Bajomi et al. (2011) found similar results with residual methoprene applications. Earlier studies have yielded similar results (Takahashi and Ohtaki 1975; Shaarawi et al. 1981, 1982).

Previously, we showed that adenosine triphosphate (ATP) and NaCl synergistically served as bed bug phagostimulants and facilitated the ingestion of water rather than blood (Romero and Schal 2014). We also reported proof of concept studies that insecticides delivered through ingestion could be much more effective at causing bed bug mortality than by topical application (Sierras and Schal 2017, Sierras et al. 2018). These findings suggest that it may be possible to develop an artificial liquid bait with further investigations of host attractants. In this study, we evaluate two JHAs, hydroprene and methoprene, and demonstrate their lethal and sublethal effects against fifth-instar male and female nymphs and adults that emerged from the treated nymphs.

Materials and Methods

Insects

Bed bugs (*Cimex lectularius* L.) from the HH (Harold Harlan; also known as Fort Dix) population were used for all experiments. This strain was collected in Fort Dix, NJ, in 1973, and maintained in our laboratory on defibrinated rabbit blood since 2008 as a standard insecticide-susceptible strain. Colonies were maintained in an incubator at ~27°C and ~50% RH on a 12:12 (L:D)-h regime, and experimental insects were held in the same conditions. Bed bugs were fed defibrinated rabbit blood using an artificial feeding system, as described in Sierras and Schal (2017), and all experimental chemicals were fed to bed bugs using this system.

Rearing Procedure for Experimental Insects

To generate staged fifth instars, we first separated a large cohort of eggs. Groups of blood-fed adult females (~75) and males (~25) were placed into 118-ml (4 oz) plastic wide-mouth jars (57-mm diameter × 70-mm; with 58-mm screw cap) (Consolidated Plastics Company, Stow, OH) containing pleated paper inserts that served as shelter and substrate for oviposition. The adults were removed from the jars after 10 d, and 10 d later first instars were fed and left for another 10 d to molt to second instars. Nymphs were similarly fed every 10 d to generate synchronous same-stage cohorts, until fifth instars were present. Nymphs were then separated by sex into 20-ml vials capped with modified plastic caps with a center hole that was sealed with plankton netting (BioQuip Products, Rancho Dominguez, CA) to enable feeding. The sexes of unfed fifth instars were distinguished by the tapering of the abdomen in males, and characteristics outlined by Usinger (1966): In the female, a small transverse spot at the sinuate (wavy) middle of the hind margin of the seventh sternite, and two median pairs of small pale spots, one pair on either side of the suture separating the eighth and ninth sternites, whereas in the male there is only one pair of pale spots near the anus.

Feeding Assays

To determine the concentration of dimethyl sulfoxide (DMSO) suitable for use as the solvent for JHAs, different concentrations of DMSO (0, 0.1, 0.25, 0.5, 0.75, and 1% [vol/vol]) were dissolved in defibrinated rabbit blood. Groups of 24–31 adult females per plastic container (59 ml, 2 oz; 54-mm diameter × 47.5-mm; with 53-mm screw cap; Consolidated Plastics) were starved 7–10 d prior to the feeding assay. Concurrently, adult males were starved 7–10 d and then fed untreated defibrinated rabbit blood. A paper substrate was enclosed in the container and plankton netting allowed blood-feeding. Females and males in separate containers were given 30 min to feed, but after 15 min a Pasteur pipette was used to mix the blood-DMSO solution. Only fully engorged females were placed four to six females per 4-ml glass vial, along with three to four males, with a strip of paper for shelter and oviposition substrate and capped with plankton netting. Females oviposited for 5 d and then males and females were moved as a cohort to a new vial with a new paper insert for another 5 d. Eggs were thus counted in each vial every 5 d, after adults were removed. First instars were counted 10 d later and the percentage eggs that hatched was determined.

Feeding Assays with Hydroprene and Methoprene: Effects on Mortality and Molting

Technical grade (*S*)-hydroprene (98%, Zoëcon, Schaumburg, IL) and (*S*)-methoprene (99%, Chem Service, West Chester, PA) were dissolved in DMSO to make a stock solution of 100 mg/ml DMSO and then diluted further in DMSO to obtain the desired concentrations. Fifth-instar male and female nymphs were used in separate vials (35–77 each) for each treatment group (0.1, 1, 10, and 100 µg JHA/ml blood) and for two control groups (defibrinated rabbit blood and 0.1% DMSO in blood). The experimental feeding procedure was the same as described above. Only fully engorged nymphs were placed individually into 2-ml microcentrifuge tubes with a small hole in the cap and a paper insert as substrate. Mortality was recorded daily for 10 d for both JHAs, but molting was recorded daily over the 10 d only for methoprene.

Feeding Assays With Hydroprene and Methoprene: Sublethal Effects

All bed bugs of the same treatment group and sex that survived to adults 10 d after ingesting JHA as fifth instars were placed into 20-ml vials and fed untreated defibrinated rabbit blood. Fully fed bugs were then placed individually into new 2-ml microcentrifuge tubes with a new paper insert and paired with a single untreated adult of the opposite sex of the same stage and feeding status. Pairs could mate and oviposit freely for 5 d, after which they were transferred to a new 2-ml microcentrifuge tube with a new paper insert and given another 5 d to oviposit. Eggs were counted on day 5 in the first vial, and again on day 10 in the second vial. First instars were counted 10 d after counting eggs and percentage egg hatch was calculated.

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) and Tukey's HSD to compare egg production over a 10-d period among treatments using SAS Institute (2016). Kaplan–Meier analysis was performed using SAS Institute (2012) to compare time until molting among methoprene treatment groups and Sidak correction was performed for all group comparisons.

Results

Effect of Ingested DMSO on Oviposition and Egg Hatch

To determine the amount of DMSO that would be suitable to use as a solvent for JHAs, without adversely affecting females, we conducted a dose–response study of DMSO in defibrinated rabbit blood. Overall, DMSO concentration in blood significantly affected egg production (ANOVA; $F_{5,29}$ = 4.24, P = 0.0051; Fig. 1A). The control females (0% DMSO) oviposited significantly more eggs per day (1.8 ± 0.27) than females that ingested 0.75% (1.3 ± 0.14; P = 0.035) and 1% (1.2 ± 0.40; P = 0.009) DMSO in blood. Likewise, females that ingested 0.1% DMSO oviposited more eggs (1.7 ± 0.12) than females that ingested 1% DMSO (P = 0.043). All other group comparisons did not significantly differ from one another ($P \geq 0.130$).

Eggs were given 10 d to hatch, first instars were counted, and the percentage hatch was calculated for each treatment group (Fig. 1B). The control, 0.1%, and 0.25% DMSO groups resulted in 87, 85, and 80% successful egg hatch, respectively. Higher concentrations of DMSO – 0.5, 0.75, and 1% – caused a sharp decline in egg hatch, with only 60, 40, and 15% of the eggs successfully hatching, respectively. Based on these results, we considered 0.1% DMSO in blood suitable for dose–response assays with hydroprene and methoprene.

Mortality of Fifth Instars After Ingesting Hydroprene or Methoprene

Newly ecdysed fifth instar male and female bed bugs were evaluated in an ingestion assay in which nymphs were fed hydroprene or methoprene in 0.1% DMSO using the artificial feeding system, and mortality was recorded daily for 10-d postingestion only for fully engorged nymphs. After ingestion of JHAs, bed bugs that successfully molted exhibited a less melanized cuticle than nymphs fed untreated blood (Fig. 2A and B). Mortality generally resulted from disruption of ecdysis (Fig. 2C and D). The control females fed only blood, or blood supplemented with 0.1% DMSO, experienced <6% mortality and male nymphs experienced <18% mortality (Fig. 3A). Low concentrations of both JHAs, 0.1 and 1 µg/ml blood, had negligible effects on both males and females, with <16% mortality (Fig. 3B and C). However, the two JHAs differentiated at higher concentrations (10- and 100-µg JHA/ml blood), with females experiencing higher mortality on methoprene (38 and 84%, respectively) than on hydroprene (0 and 40%). The highest dose (100-µg/ml blood) was not used on males, but results with the 10-µg methoprene/ml blood dose suggest that females might be more susceptible to the JHA than males (Fig. 3C).

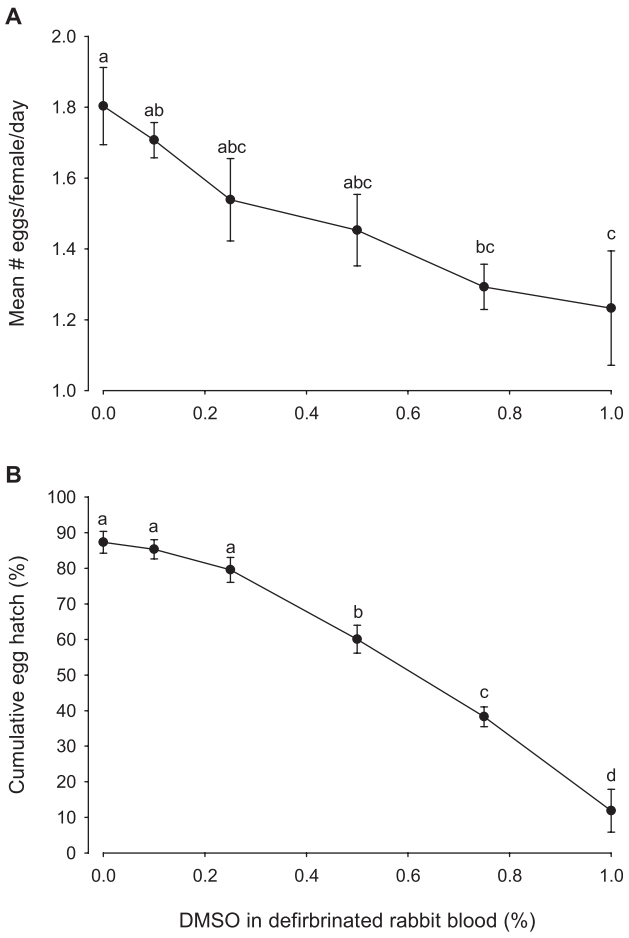

Fig. 1. Dose–response of DMSO in defibrinated rabbit blood on *Cimex lectularius*, showing (A) mean ± SEM number of eggs deposited by females per day during a 10-d period (n = 24–31 females per treatment group, 4–6 females per replicate), and (B) the percentage ± SEM of eggs that successfully hatched. Letters not shared by means indicate significant differences between the treatments (ANOVA, Tukey's HSD, $P < 0.05$).

Effect of Methoprene on the Imaginal Molt

We recorded daily for 7 d the number of insects that successfully molted to adults after ingesting methoprene as newly emerged fifth-instar males or females. Control females and males (no methoprene) synchronously ecdysed to adults between day 4 (0% adults) and d-5 (nearly 100% adults) (Fig. 4A and B). The lowest concentration of methoprene (0.1-µg/ml blood) did not affect the temporal pattern of ecdysis, with ≥96% of both sexes successfully molting to adults. Higher concentrations of methoprene (≥1 µg/ml blood) caused earlier molting, with 70–93% of the females and 58–96% of males molting to adults by day 4. Only females received the 100-µg methoprene/ml blood treatment and they experienced high mortality (Fig. 3C). Nevertheless, methoprene accelerated their imaginal molt, with 88% becoming adults by day 4. For both females (Fig. 4A) and males (Fig. 4B), there were significant differences among the treatment

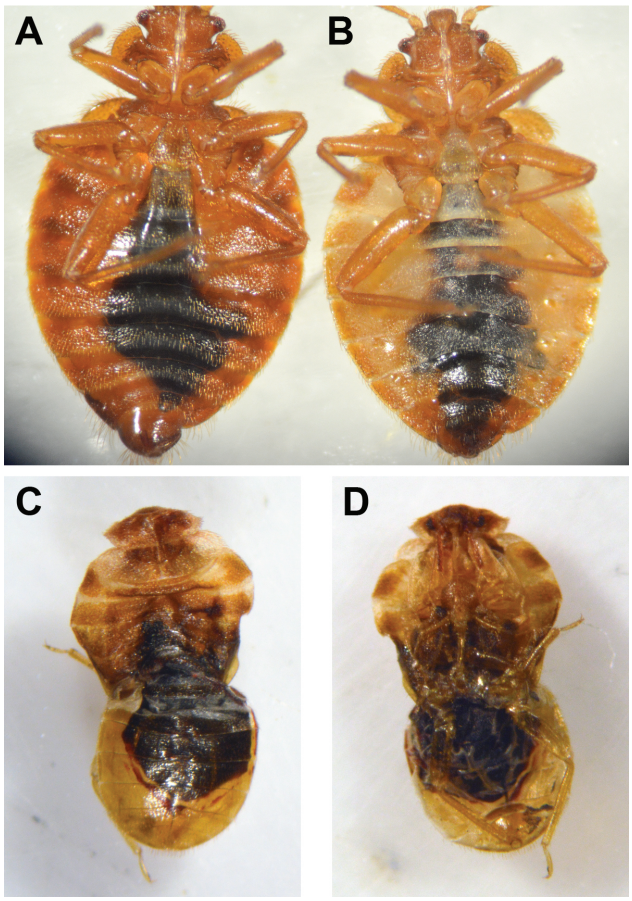

Fig. 2. Post-ingestion effects of hydroprene on last instar male *Cimex lectularius*. Compared to a control male fed untreated blood (A), hydroprene-bed bugs exhibited less melanization of the cuticle (B). Mortality generally occurred during the molt, as shown in dorsal (C) and ventral (D) views.

groups (log rank test; females: $\chi^2 = 101.28$, df = 4, $P < 0.0001$; males: $\chi^2 = 77.3$, df = 3, $P < 0.0001$). The time to molt did not differ for the females in the control (0) and 0.1 µg methoprene/ml blood, as well as for females in the 1 and 10 µg methoprene/ml blood groups ($P \geq 0.961$). However, all other female groups significantly differed from one another ($P \leq 0.021$). The same was true for males in the control and 0.1 µg/ml blood, as well as the males in the 1 and 10 µg methoprene/ml blood ($P \geq 0.13$). In all control and treatment groups for both males and females 100% of the nymphs molted by day 7.

Sublethal Effects of Hydroprene and Methoprene on Oviposition

After the imaginal molt, individuals that successfully molted were fed untreated rabbit blood and paired with an untreated adult of the opposite sex. The mean number of eggs per female per day was recorded over a 10-d period. Control bed bugs oviposited 1.3–1.7 eggs/d (Fig. 5A). Both males and females that survived JHA treatment as nymphs and allowed to mate with untreated bed bugs experienced sublethal effects (ANOVA; hydroprene: $F_{10,476} = 31.11$, $P < 0.0001$, Fig. 5B; methoprene: $F_{10,459} = 54.14$, $P < 0.0001$, Fig. 5C). Females that ingested the two lowest concentrations of hydroprene (0.1- and 1-µg/ml blood) were unaffected and produced as many eggs as control females. The two highest concentrations of hydroprene (10- and 100-µg/ml blood) significantly reduced egg production relative to control females, to 1.01 ± 0.73 and 0.00 ± 0.0 eggs/d, respectively ($P \leq 0.003$). Males were less affected by hydroprene than females at all concentrations (0.1-, 1-, and 10-µg hydroprene/ml blood). When these males mated with untreated females, the females' egg production was not significantly different from control females that mated with untreated males ($P \geq 0.148$).

Methoprene had more significant sublethal effects than hydroprene on both females and males. Only female nymphs that survived the lowest concentration of methoprene (0.1-µg methoprene/ml blood) and emerged as adults produced the same number of eggs as control females. All other females (fed 1-, 10-, or 100-µg

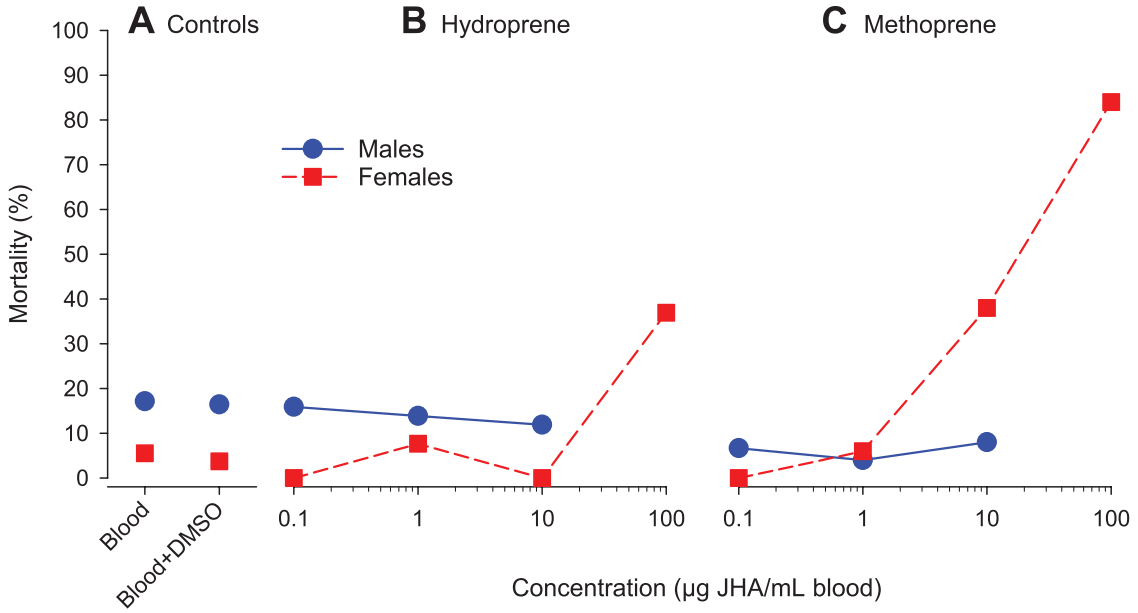

Fig. 3. Cumulative mortality (%) of fifth instar *Cimex lectularius* 10 d after ingesting a JHA. Only fully engorged fifth instars were assessed after feeding on untreated blood or blood supplemented with DMSO (A), or various concentrations of hydroprene (B) and methoprene (C). Each assay consisted of 29–66 males and 29–77 females. Males were not fed 100 µg/ml blood for either JHA.

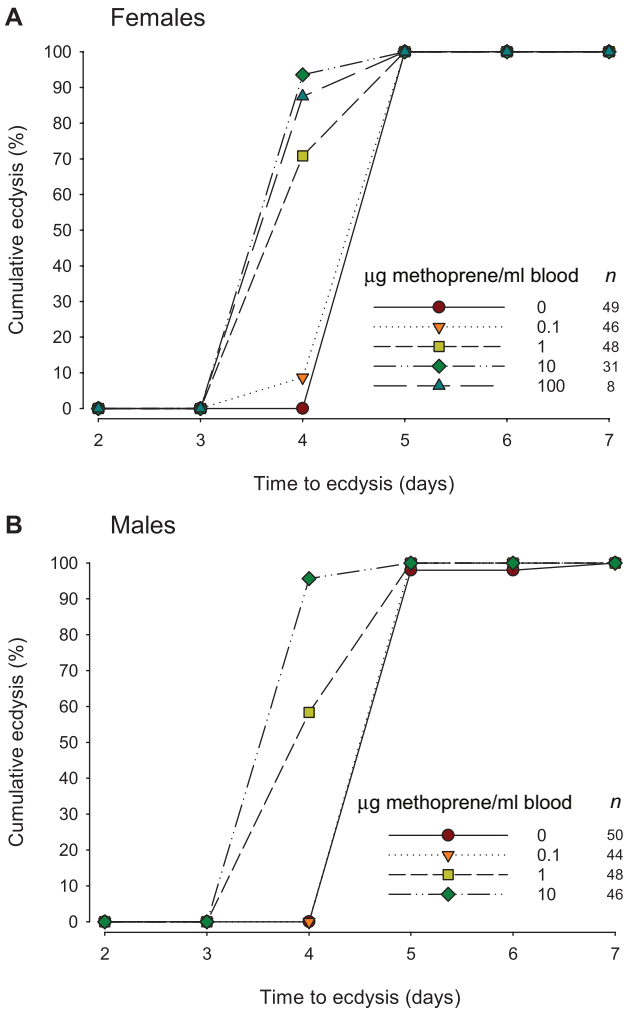

Fig. 4. Cumulative daily molting (%) of fifth-instar *Cimex lectularius* after ingesting methoprene. Only fully engorged fifth instar females (A) and males (B) were assessed after feeding on blood supplemented with 0.1% DMSO (0% methoprene), or various concentrations of methoprene in DMSO. Males were not fed 100 µg methoprene/ml blood.

methoprene/ml blood) oviposited significantly fewer eggs (0.11, 0.00, and 0.00 eggs/d, respectively; $P < 0.0001$) than females fed on 0.1-µg methoprene/ml blood (Fig. 5C). Females fed 1- and 10-µg methoprene/ml blood also oviposited significantly fewer eggs/d than females fed the respective concentrations of hydroprene ($P < 0.001$; Fig. 5B and C). Males also were more affected by methoprene than hydroprene. Untreated females that mated to males fed 0.1 µg methoprene/ml of blood as nymphs produced the most eggs (1.94 ± 0.51 eggs/d) of any treatment, including controls; egg production was significantly higher than in females mated to males that ingested 1 µg methoprene/ml of blood as nymphs (1.49 ± 0.72; $P = 0.022$). Ingestion of 10 µg methoprene/ml blood by males significantly reduced egg production in their untreated mates relative to blood-only controls (1.34 ± 0.49; $P = 0.002$) to only 0.60 ± 1.22 eggs/d. Overall, methoprene appeared to be more effective at reducing female oviposition at concentrations ≥1 µg/ml blood, whereas the sublethal effects of hydroprene on oviposition were evident at concentrations ≥10 µg/ml blood. The sublethal effects of methoprene were also more significant when female nymphs ingested the JHA and mated as adults with untreated males than on untreated females that mated with males that ingested the JHA as nymphs.

Discussion

Our results showed that higher concentrations of hydroprene and methoprene were needed to obtain significant lethal and sublethal effects than with neurotoxic active ingredients (Sierras and Schal 2017). Female bed bugs were more susceptible to both JHAs than males, and methoprene was more effective than hydroprene at causing mortality and reducing oviposition when either female nymphs were treated, or male nymphs were treated and (after molting to adults) mated with untreated females. Overall, we conclude that both JHAs are more effective by ingestion than by contact.

The lethal effects of the two JHAs were evident in fifth instar nymphs that ingested JHA-supplemented blood early in the instar and died during the imaginal molt. Mortality caused by hydroprene was low and not different from control mortality on untreated blood for both males and females. Even the highest dose of hydroprene (100-µg/ml blood; 0.01%) that we tested, caused only 40% mortality in females. In contrast, methoprene was more effective at causing mortality in females, with 10-µg/ml blood causing 40% mortality in females and 100-µg/ml blood causing nearly 90% mortality. We observed that most of the mortality occurred during ecdysis from the fifth instar to either adult, adultoid, or supernumerary nymph.

JHAs often delay development and increase melanization of the cuticle (Pener and Dhadialla 2012). In bed bugs, however, both hydroprene and methoprene accelerated development and reduced cuticular melanization. Some individuals that were treated with ≥1-µg JHA/ml blood molted to adults with lighter cuticle and patchiness of discoloration, as observed by Naylor et al. (2008). Almost all untreated fifth instar females and males molted to normal adults 5 d after ingesting blood. We observed a hitherto unreported dose-dependent acceleration of the imaginal molt. More than 80% of the females that survived 10- or 100-µg methoprene/ml blood molted on day 4, and >95% of males fed 10-µg methoprene/ml blood molted by day 4. We are not aware of similar findings with other insects.

Oviposition in females that survived exposure to JHAs in the last nymphal stadium was concentration dependent. The sublethal effects of hydroprene on reproduction were evident only in female bed bugs treated with ≥10-µg hydroprene/ml blood, with ingestion of 100-µg hydroprene/ml blood by nymphs completely suppressing reproduction in adult females over the 10-d observation period. Methoprene's sublethal effects were significantly greater than with hydroprene: treatment of female nymphs with 1-µg methoprene/ml blood greatly reduced reproduction of adults, and reproduction in females treated as nymphs with ≥10-µg methoprene/ml blood was completely suppressed. The indirect effects on female reproduction of treating male nymphs with JHAs were less severe. Male nymphs that survived ingesting up to 10-µg hydroprene/ml blood did not affect reproduction of untreated females, suggesting that relatively high doses of hydroprene did not affect sperm viability or related processes required for egg fertilization. However, males that ingested methoprene as nymphs and survived, significantly affected reproduction of their female mate in a dose-dependent manner. Thus, males fed 10-µg methoprene/ml blood as nymphs and mated with an untreated female, caused an 80% reduction in oviposition, indicating that either their genitalia morphology or sperm viability were adversely affected by methoprene. We did not dissect the females, but it is possible that lower oviposition was related to egg retention, as observed by Goodman et al. (2013). Certainly, delivery of inviable sperm could fail to induce females to switch from a virgin (low oviposition of unfertilized eggs) to mated state (elevated oviposition of fertilized eggs).

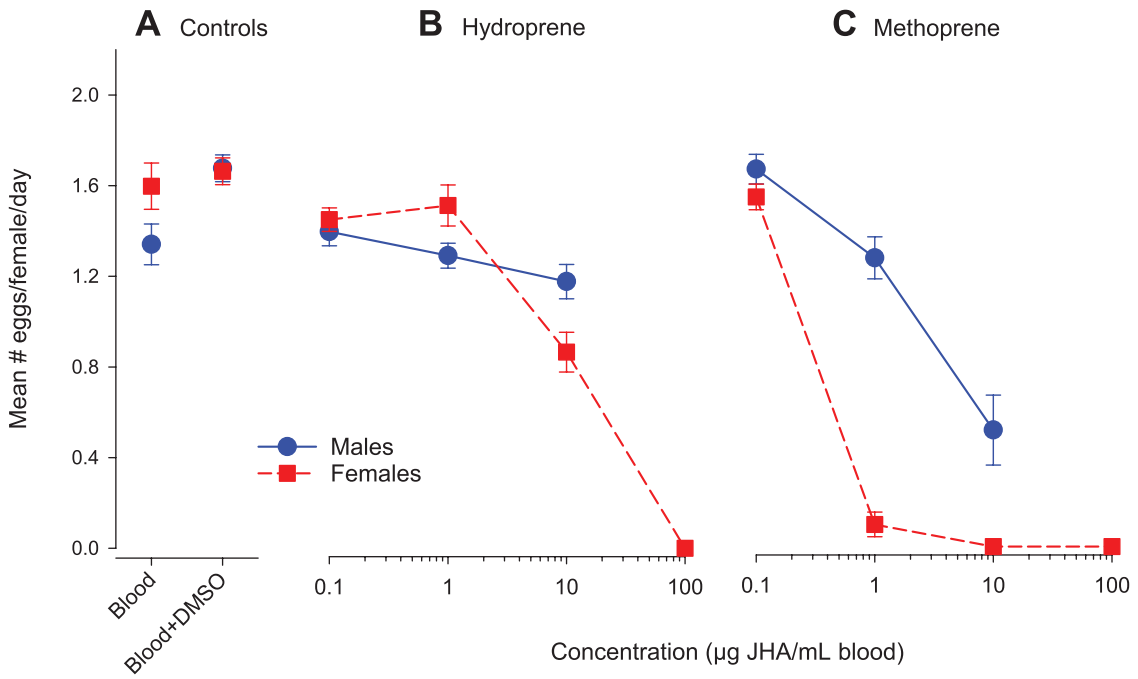

Fig. 5. Dose–response including two controls (A), hydroprene (B), and methoprene (C) in defibrinated rabbit blood fed to fifth instar *Cimex lectularius*, which were allowed to molt for 10 d and then fed untreated defibrinated rabbit blood. Mean ± SEM number of eggs deposited per d by females are shown. Males were not fed 100 µg/ml blood of either JHA.

Three important observations are worthy of further discussion: a) the relative activity of different JHAs, b) their relative effectiveness on males and females, and c) their relative efficacy by contact and ingestion. In the bed bug, methoprene was more effective than hydroprene, yet in other hemimetabolous insects, including the German cockroach (King and Bennett 1989, Kramer et al. 1989), oriental cockroach (Bao and Robinson 1990, Short and Edwards 1992, Edwards and Short 1993), and mole crickets (Parkman and Frank 1998), hydroprene has been shown to be more effective than methoprene. Both are isoprenoids, and structural analogs of natural JHs, whose mode of action is to provide excess JHA at the time in development when endogenous JH is absent, thus interfering with metamorphosis and embryogenesis. Methoprene has a broad-spectrum of activity against a variety of insects, including many dipterans (mosquitoes, flies, and sand flies) and stored products pests (moths and beetles), whereas hydroprene has a more limited activity spectrum on insects (Mohandass et al. 2006). The natural JH of hemipteran insects remains unknown, and it is possible that methoprene has greater affinity to the JH receptor in bed bugs than hydroprene. Regardless, two recommendations emerge from these observations: First, given its superior performance, it appears that hydroprene should be replaced by methoprene for bed bug control. Second, in this context, it would be important to also consider other JHAs, such as kinoprene, which is particularly effective against homopterans (Henrick 2007), and heterocyclic analogs of JH, such as pyriproxyfen, which is the most potent JHA available with perhaps the broadest spectrum of activity that likely would include bed bugs. Pyriproxyfen has been shown to be effective on various hemipterans (Pener and Dhadialla 2012), and it is already labeled for indoor use against cockroaches, fleas, ants, and beetles.

While both sexes of bed bugs are vulnerable to the effects of JHAs, *C. lectularius* females were more susceptible than males, whereas in other insects, males are especially affected. In the German cockroach, for example, hydroprene can cause melanization, morphological deformities of external appendages (e.g., genitalia, antennae, wings) and internal tissues (e.g., ovaries, testes), and ultimately sterilization (Bennett and Reid 1995). As little as 40-µg hydroprene/g body mass (ca. 2 µg per male), applied topically, can sterilize male nymphs, but double this dose is needed to sterilize female nymphs (King and Bennett 1989). In our investigation, females experienced greater lethal and sublethal effects than males. However, we caution that last instar females likely ingested more blood than males and therefore were exposed to higher doses at equivalent concentrations of the JHAs.

The bed bug is obviously much less sensitive to the effects of JHAs in topical application and residual assays; three- to eightfold label rate of hydroprene and methoprene were required for any effects on bed bugs (Naylor et al. 2008, Goodman et al. 2013). Ingestion assays, however, suggest otherwise. Based on the estimation that adult male bed bugs ingest ~3.9 µl of blood in a single bloodmeal (Sierras and Schal 2017), we estimate that ingestion of methoprene, the more effective of the two JHAs, at a concentration of 10-µg/ml blood, would expose the bed bug to only 39 ng of methoprene (390 ng at 100-µg methoprene/ml blood). Ingestion of a larger bloodmeal, for example, up to 6 µl (Pereira et al. 2013), would obviously increase exposure to ingested insecticides. Thus, the efficacy of methoprene by ingestion is much greater than by topical treatment. The highly apolar nature of most JHAs likely affects their cuticular penetration and presentation to receptors. Pener et al. (1997) reported that pyriproxyfen was over 1,000-fold more effective on locusts by injection in oil than by topical application. They attributed this enormous difference to both the delivery by injection and the use of oil as a slow-release solvent. Greater efficacy of neuro-active insecticides, inorganic insecticides and JHAs by ingestion (Sierras and Schal 2017, Sierras et al. 2018) should stimulate greater interest in bait development for bed bugs.

Notably, a wide range of insecticides can be used against bed bugs in either a liquid bait or in a systemically treated host. We used blood in our present assays, but bed bugs readily accept a saline solution supplemented with various phagostimulants, notably ATP (Romero and Schal 2014). Thus, an artificial bait would contain

water, phagostimulants, heat, attractants, and one or more insecticides. Our findings suggest that even DMSO has potential as an insecticide in an artificial aqueous bait. Our present assays showed that 1% DMSO fed to last instar females significantly reduced their fecundity and fertility as adults. The same concentration of DMSO fed to adult males resulted in >60% mortality (Sierras and Schal 2017, Sierras et al. 2018), and 5% caused 100% mortality (not shown).

In conclusion, formulating insecticides into ingestible aqueous baits would enable a dramatic reduction in the amount of insecticide needed to kill bed bugs, thus reducing potential exposure of humans and pets. Our results suggest that while methoprene may be effective at suppressing bed bug populations, a host of other insecticides kill bed bugs much faster and at much lower concentrations. Moreover, because JHAs target a narrow range of developmental stages, bed bugs may continue to bite if exposed only to JHAs. Nevertheless, JHAs, and more generally IGDs, should continue to be investigated against bed bugs because other IGDs may be more effective than methoprene, the JHAs might synergize the activity of other insecticides, and the unique mode of action of JHAs, such as activity on embryos (Takahashi and Ohtaki 1975), may serve to forestall resistance to other active ingredients. An important step would be to screen kinoprene, pyriproxyfen, and various chitin synthesis inhibitors not only on standard susceptible strains but also on recently field-collected bed bug populations.

Acknowledgments

We thank Rick Santangelo for maintaining the bed bug colonies, Zachary DeVries for assisting with statistical analysis, and Charles Apperson, Michael Reiskind, Michael Waldvogel, and Wes Watson for suggestions on an earlier draft of the manuscript. Funding for this study was provided by the Blanton J. Whitmire Endowment at North Carolina State University, and the US Department of Housing and Urban Development Healthy Homes program (NCHHU0017-13) to C. S., and a National Institute of Environmental Health Sciences grant to the Center for Human Health and the Environment (CHHE, P30ES025128). A. S. received an NIH Initiative for Maximizing Student Diversity (IMSD) Fellowship and scholarship awards from the North Carolina Pest Management Association, Pi Chi Omega, and North Carolina State University.

References Cited

Adelman, Z. N., K. A. Kilcullen, R. Koganemaru, M. A. Anderson, T. D. Anderson, and D. M. Miller. 2011. Deep sequencing of pyrethroid-resistant bed bugs reveals multiple mechanisms of resistance within a single population. PLoS One. 6: e26228.

Agnew, J. L., and A. Romero. 2017. Behavioral responses of the common bed bug, *Cimex lectularius*, to insecticide dusts. Insects. 8: 11.

Atkinson, T. H., P. G. Koehler, and R. S. Patterson. 1992. Volatile effects of insect growth regulators against the German cockroach (Dictyoptera: Blattellidae). J. Med. Entomol. 29: 364–367.

Bajomi, D., J. Szilágyi, R. Naylor, and L. Takács. 2011. S-methoprene formulations: Laboratory tests for efficacy against bed bugs, pp. 249–255. *In* W. H. Robinson and A. E. de Carvalho Campos (eds.), Seventh International Conference on Urban Pests. Instituto Biológico, São Paulo, SP. Brazil.

Bao, N. G., and W. H. Robinson. 1990. Morphogenetic effects of hydroprene on genitalia of the oriental cockroach (Dictyoptera: Blattidae). J. Econ. Entomol. 83: 1415–1421.

Bennett, G. W., and B. L. Reid. 1995. Insect growth regulators, pp. 267–286. *In* M. K. Rust, D. A. Reierson and J. M. Owens (eds.), Understanding and controlling the German cockroach. Oxford University Press, New York.

DeVries, Z. C., R. G. Santangelo, A. M. Barbarin, and C. Schal. 2018. Histamine as an emergent indoor contaminant: accumulation and persistence in bed bug infested homes. PLoS One. 13: e0192462.

Doggett, S. L. 2018. Bed bugs and infectious diseases, pp. 117–126. *In* S. L. Doggett, D. M. Miller and C-Y. Lee (eds.), Advances in the biology and management of modern bed bugs. John Wiley & Sons, Hoboken, NJ.

Doggett, S. L., D. E. Dwyer, P. F. Peñas, and R. C. Russell. 2012. Bed bugs: clinical relevance and control options. Clin. Microbiol. Rev. 25: 164–192.

Edwards, J. P., and J. E. Short. 1993. Elimination of a population of the oriental cockroach (Dictyoptera, Blattidae) in a simulated domestic environment with the insect juvenile-hormone analog (*S*)-hydroprene. J. Econ. Entomol. 86: 436–443.

El-Sheikh, e. l. -. S. A., S. G. Kamita, and B. D. Hammock. 2016. Effects of juvenile hormone (JH) analog insecticides on larval development and JH esterase activity in two spodopterans. Pestic. Biochem. Physiol. 128: 30–36.

Goddard, J., and R. deShazo. 2009. Bed bugs (*Cimex lectularius*) and clinical consequences of their bites. JAMA. 301: 1358–1366.

Goodman, M. H., M. F. Potter, and K. F. Haynes. 2013. Effects of juvenile hormone analog formulations on development and reproduction in the bed bug *Cimex lectularius* (Hemiptera: Cimicidae). Pest Manag. Sci. 69: 240–244.

Graf, J. F. 1993. The role of insect growth regulators in arthropod control. Parasitol. Today. 9: 471–474.

Henrick, C. A. 2007. Methoprene. J. Am. Mosq. Control Assoc. 23: 225–239.

Hwang, S. J. E., S. L. Doggett, and P. Fernandez-Penas. 2018. Dermatology and immunology, pp. 109–116. *In* S. L. Doggett, D. M. Miller and C-Y. Lee (eds.), Advances in the biology and management of modern bed bugs. John Wiley & Sons, Hoboken, NJ.

Invest, J., and J. Lucas. 2008. Pyriproxyfen as a mosquito larvicide, pp. 239–245. *In* W. H. Robinson and D. Bajomi (eds.), Proceedings of the Sixth International Conference on Urban Pests. OOK-Press Kf, Budapest, Hungary.

King, J. E., and G. W. Bennett. 1989. Comparative activity of fenoxycarb and hydroprene in sterilizing the German cockroach (Dictyoptera: Blattellidae). J. Econ. Entomol. 82: 833–838.

King, J. E., and G. W. Bennett. 1991. Sensitive developmental period of last-instar German cockroaches (Dictyoptera: Blattelidae) to fenoxycarb and hydroprene. J. Med. Entomol. 28: 514–517.

Kramer, R. D., P. G. Koehler, and R. S. Patterson. 1989. Morphogenetic effects of hydroprene on German cockroaches (Orthoptera, Blattellidae). J. Econ. Entomol. 82: 163–170.

Lai, O., D. Ho, S. Glick, and J. Jagdeo. 2016. Bed bugs and possible transmission of human pathogens: a systematic review. Arch. Dermatol. Res. 308: 531–538.

Lee, C. Y., D. M. Miller, and S. L. Doggett. 2018. Chemical control, pp. 285–310. *In* S. L. Doggett, D. M. Miller, and C-Y. Lee (eds.), Advances in the biology and management of modern bed bugs. John Wiley & Sons, Hoboken, NJ.

Lim, S. P., and C. Y. Lee. 2005. Effects of juvenile hormone analogs on new reproductives and colony growth of Pharaoh ant (Hymenoptera: Formicidae). J. Econ. Entomol. 98: 2169–2175.

Mohandass, S. M., F. H. Arthur, K. Y. Zhua, and J. E. Throne. 2006. Hydroprene: mode of action, current status in stored-product pest management, insect resistance, and future prospects. Crop Protection. 25: 902–909.

Moser, B. A., P. G. Koehler, and R. S. Patterson. 1992. Effect of methoprene and diflubenzuron on larval development of the cat flea (Siphonaptera: Pulicidae). J. Econ. Entomol. 85: 112–116.

Naylor, R., D. Bajomi, and C. Boase. 2008. Efficacy of (*S*)-methoprene against *Cimex lectularius* (Hemiptera: Cimicidae), pp. 115–121. *In* W. H. Robinson and D. Bajomi (eds.), Sixth International Conference on Urban Pests. OOK-Press Kf, Budapest, Hungary.

Parkman, J. P., and J. H. Frank. 1998. Development and reproduction of mole crickets (Orthoptera: Gryllotalpidae) after treatments with hydroprene and pyriproxyfen. J. Econ. Entomol. 91: 392–397.

Pener, M. P., and T. S. Dhadialla. 2012. An overview of insect growth disruptors; applied aspects, pp. 1–162. *In* T. S. Dhadialla (ed.), Advances in insect physiology, Vol 43: insect growth disruptors. Academic Press Ltd-Elsevier Science Ltd, London, United Kingdom.

Pener, M. P., A. Ayali, G. Kelmer, B. Bennettova, V. Nemec, M. Rejzek, and Z. Wimmer. 1997. Comparative testing of several juvenile hormone

analogues in two species of locusts, *Locusta migratoria migratorioides* and *Schistocerca gregaria*. Pestic. Sci. 51: 443–449.

Pereira, R. M., A. S. Taylor, M. P. Lehnert, and P. G. Koehler. 2013. Potential population growth and harmful effects on humans from bed bug populations exposed to different feeding regimes. Med. Vet. Entomol. 27: 148–155.

Perron, S., G. Hamelin, and D. Kaiser. 2018. Mental health impacts, pp. 127–132. *In* S. L. Doggett, D. M. Miller, and C-Y. Lee (eds.), Advances in the biology and management of modern bed bugs. John Wiley & Sons, Hoboken, NJ.

Reinhardt, K., and M. T. Siva-Jothy. 2007. Biology of the bed bugs (Cimicidae). Annu. Rev. Entomol. 52: 351–374.

Romero, A. 2018. Insecticide resistance, pp. 273–284. In S. L. Doggett, D. M. Miller, and C-Y. Lee (eds.), Advances in the biology and management of modern bed bugs. John Wiley & Sons, Hoboken, NJ.

Romero, A., and T. D. Anderson. 2016. High levels of resistance in the common bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), to neonicotinoid insecticides. J. Med. Entomol. 53: 727–731.

Romero, A., and C. Schal. 2014. Blood constituents as phagostimulants for the bed bug Cimex lectularius L. J. Exp. Biol. 217: 552–557.

Rossi, L., and S. Jennings. 2010. Bed bugs: a public health problem in need of a collaborative solution. J. Environ. Health. 72: 34–35.

SAS Institute. 2012. Statistical analysis system computer program, version 9.4. SAS Institute, Cary, NC.

SAS Institute. 2016. JMP Pro computer program, version 13.1.0. SAS Institute, Cary, NC.

Seccacini, E. A., L. W. Juan, C. V. Vassena, E. N. Zerba, and R. A. Alzogaray. 2018. Lufenuron kills deltamethrin-resistant *Blattella germanica* (Blattodea). Rev. Soc. Entomol. Argent. 77: 32–35.

Shaarawi, F., W. Radwan, and S. Soliman. 1981. Effects of juvenoids on some biological aspects of the bed bug, *Cimex lectularius* L. I. effects on female fecundity. Bull. Fac. Earth Sci. (King Abdulaziz Univ.). 5: 123–132.

Shaarawi, F., W. Radwan, and S. Soliman. 1982. Effects of juvenoids on some biological aspects of the bed bug, *Cimex lectularius* L. II. effect on egg viability and embryogenesis. Bull. Fac. Sci. (King Abdulaziz Univ.). 6: 35–47.

Short, J. E., and J. P. Edwards. 1992. Effects of hydroprene on development and reproduction in the oriental cockroach, *Blatta orientalis*. Med. Vet. Entomol. 6: 244–250.

Sierras, A., and C. Schal. 2017. Comparison of ingestion and topical application of insecticides against the common bed bug, *Cimex lectularius* (Hemiptera: Cimicidae). Pest. Manag. Sci. 73: 521–527.

Sierras, A., A. Wada-Katsumata, and C. Schal. 2018. Effectiveness of boric acid by ingestion, but not by contact, against the common bed bug (Hemiptera: Cimicidae). J. Econ. Entomol. 111: 2772–2781.

Su, T. Y., M. L. Cheng, A. Melgoza, and J. Thieme. 2014. Laboratory and field evaluations of Mosquiron 0.12crd, a new formulation of novaluron, against *Culex* mosquitoes. J. Am. Mosq. Control Assoc. 30: 284–290.

Takahashi, M., and T. Ohtaki. 1975. Ovicidal effects of two juvenile hormone analogs, methoprene and hydroprene, on the human body louse and the bed bug. Med. Entomol. Zool. 26: 237–239.

Thomas, I., G. G. Kihiczak, and R. A. Schwartz. 2004. Bedbug bites: a review. Int. J. Dermatol. 43: 430–433.

Usinger, R. L. 1966. Monograph of Cimicidae (Hemiptera, Heteroptera), Entomological Society of America, College Park, MD.

Williams, D. F., H. L. Collins, and D. H. Oi. 2001. The red imported fire ant (Hymenoptera: Formicidae): an historical perspective of treatment programs and the development of chemical baits for control. Am. Entomol. 47: 146–159.

Zhu, F., J. Wigginton, A. Romero, A. Moore, K. Ferguson, R. Palli, M. F. Potter, K. F. Haynes, and S. R. Palli. 2010. Widespread distribution of knockdown resistance mutations in the bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), populations in the United States. Arch. Insect Biochem. Physiol. 73: 245–257.