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Research article

Household and Structural Insects

Posttreatment Feeding Affects Mortality of Bed Bugs (Hemiptera: Cimicidae) Exposed to Insecticides

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Abstract

Insecticide sprays and dusts are used for controlling bed bugs, *Cimex lectularius* L. In natural environments, bed bugs have daily access to hosts after they are exposed to insecticides. The established laboratory insecticide bioassay protocols do not provide feeding after insecticide treatments, which can result in inflated mortality compared with what would be encountered in the field. We evaluated the effect of posttreatment feeding on mortality of bed bugs treated with different insecticides. None of the insecticides tested had a significant effect on the amount of blood consumed and percent feeding. The effect of posttreatment feeding on bed bug mortality varied among different insecticides. Feeding significantly reduced mortality in bed bugs exposed to deltamethrin spray, an essential oil mixture (Bed Bug Fix) spray, and diatomaceous earth dust. Feeding increased the mean survival time for bed bugs treated with chlorfenapyr spray and a spray containing an essential oil mixture (Ecoraider), but did not affect the final mortality. First instars hatched from eggs treated with chlorfenapyr liquid spray had reduced feeding compared with nymphs hatched from nontreated eggs. Those nymphs hatched from eggs treated with chlorfenapyr liquid spray and successfully fed had reduced mortality and a higher mean survival time than those without feeding. We conclude that the availability of a bloodmeal after insecticide exposure has a significant effect on bed bug mortality. Protocols for insecticide efficacy testing should consider offering a bloodmeal to the treated bed bugs within 1 to 3 d after treatment.

Key words: bed bug, *Cimex lectularius*, feeding, insecticide, efficacy

During the past 10 yr, bed bugs, *Cimex lectularius* L. (Hemiptera: Cimicidae), have reemerged as a serious pest throughout the United States as well as many other developed countries (Doggett et al. 2004, Hwang et al. 2005, Gangloff-Kaufmann et al. 2006). Like other hematophagous insects, blood feeding is an essential physiological process for growth and development in bed bugs (Usinger 1966). Both sexes of bed bugs feed exclusively on blood for development and reproduction. During nymphal development, a bloodmeal is required for molting into the subsequent instar. Moreover, egg production in females and sperm production in males require regular bloodmeals (Usinger 1966, Reinhardt and Siva-Jothy 2007). A recent bloodmeal by a bed bug may overcome the stress caused by insecticides, a phenomenon that has been demonstrated in different species of mosquitoes. Mortality in fed *Aedes aegypti* (L.) females exposed to a spray of pyrethrins or DDT was significantly lower than that in nonfed females (David and Bracey 1946). In another study (Hadaway and Barlow 1956), *Ae. aegypti* females were found to be less susceptible to DDT at 24 h after feeding. Similar trends were reported in northern house mosquito, *Culex pipiens* L., where a bloodmeal at 24 h prior to topical application of DDT, fenvalerate, and *trans*-permethrin resulted in decreased mortality (Halliday and Feyereisen 1987). The increased tolerance to permethrin following a bloodmeal has also been reported in *Anopheles funestus* Giles (Spillings et al. 2008). Recently, it was reported that fed bed bugs survived longer than starved bugs exposed to dry residues of chlorfenapyr and deltamethrin (Choe and Campbell 2014). These studies suggest that feeding can affect susceptibility when the insects have access to a bloodmeal just prior to insecticide treatments. However, studies examining the effect of posttreatment feeding on mortality of bed bugs are still lacking.

A recent bloodmeal has been associated with increased levels of detoxifying enzymes, including cytochrome *P450s* and glutathione S-transferases (*GSTs*). The upregulation of *P450s* in response to a bloodmeal has been shown in *Cu. pipiens* and *Ae. aegypti* (Baldridge and Feyereisen 1989, Sanders et al. 2003). Several *GSTs* genes were also upregulated after blood ingestion in *Ae. aegypti* (Marinotti et al. 2005). These detoxification enzymes play a key role in metabolizing insecticides in many insects (Scott et al. 1998, Hemingway and Ranson 2000, Che-Mendoza et al. 2009, Komagata et al. 2010, Bai et al. 2011). It is possible that bed bugs also produce increased transcripts of *P450s* and *GSTs* after feeding.

Insecticide sprays and dusts are used for controlling bed bugs in infested dwellings (Potter 2008). In natural environments, bed bugs have access to hosts upon which to feed, after they are exposed to

insecticides. As long as their foraging behavior is not impaired by the treatment, bed bugs feed approximately every 3 d (Reinhardt et al. 2010). However, the present recommended laboratory insecticide bioassay protocols do not provide feeding after treatment (U.S. Environmental Protection Agency 2012). The lack of feeding after treatment may result in inflated mortality compared with what would be encountered in the field. The present study was conducted to determine the effect of posttreatment feeding on mortality of bed bugs exposed to different classes of insecticides.

Materials and Methods

Insects

Two bed bug strains, Indy and Bayonne, were collected during 2008–2009 from infested apartments in Indiana and New Jersey, respectively. The Indy strain and the Bayonne strain were moderately resistant to deltamethrin (0.06%; Suspend SC, Bayer Environmental Science, Durham, NC) in a direct spray bioassay conducted 6 mo before this study (Singh et al. 2014). Deltamethrin caused <40% mortality in both strains at 7 d after treatment compared with 100% mortality in a laboratory susceptible strain (Ft Dix) at 3 h after treatment (Singh et al. 2014). Bugs were maintained in plastic containers (4.7 cm height and 5 cm diameter) with folded paper as harborages at 26 ± 1°C, 40 ± 10% relative humidity (RH), and a photoperiod of 12:12 (L:D) h, and fed weekly on defibrinated rabbit blood (Hemostat Laboratories, Dixon, CA) using a Hemotek membrane-feeding system (Discovery Workshops, Accrington, United Kingdom). The bed bugs were starved for 7 d before being used. Due to limited number of bed bugs, Bayonne strain large nymphs (fourth–fifth instars) or adult males were used for evaluating one insecticide (Bed Bug Fix, Nature's Innovation Inc., Buford, GA), whereas Indy strain large nymphs or adult males were used for evaluating all other insecticides. Females were not used for evaluating insecticide treatments due to limited number of females in the colonies. Indy strain females were only used for collecting eggs.

Insecticides

In total, four insecticide sprays from three classes were tested following the label rate: 1) pyrethroid: deltamethrin (0.06%; Suspend SC); 2) pyrrole: chlorfenapyr (0.5%; Phantom SC, BASF Corporation, Durham, NC); 3) essential oils: (geraniol [1%] + cedar oil [0.3%] + citronella oil [0.3%] + eugenol [0.2%] + 2-phenethyl propionate [2%] [Bed Bug Fix, NuSafe Floor Solutions Inc., Walton, KY]) and (geraniol [1%] + cedar extract [1%] + sodium lauryl sulfate [2%] [Ecoraider, Reneotech Inc., North Bergen, NJ]). Two dust insecticides were tested: 100% inorganic diatomaceous earth dust (DE; MotherEarth D, BASF Corporation, Durham, NC) and 1% cyfluthrin (pyrethroid; Tempo D, Bayer Environmental Science, Durham, NC). The products were obtained either directly from manufacturers or from commercial distributors. Suspend SC and Phantom SC were diluted to desired concentrations with water following the label directions. Essential oil sprays and dusts were ready-to-use products.

Bioassay Methods

Direct Spray Bioassay

To determine the effect of feeding on mortality in large nymphs (fourth–fifth instars) and adult males, 20 nymphs or 20 adult males were placed on filter paper contained in a small plastic Petri dish (5.5 cm diameter and 1.5 cm height; Fisher Scientific, Pittston, PA; Fig. 1A). Bed bugs in the dishes were then sprayed with an insecticide using a Potter spray tower (Burkard Scientific Ltd, Herts, United Kingdom) at the rate of 1 gallon/1,000 ft^2 of diluted or ready-to-use solution. We used this application rate for all insecticides as it is a standard "point of run off" and is the commonly used application rate by professionals. Bed bugs in the control group were sprayed with water. Fourteen dishes of large nymphs were used for deltamethrin. Fourteen dishes were used for both large nymphs and adult males for chlorfenapyr and Bed Bug Fix, whereas 15 dishes of large nymphs and 18 dishes of adult males were used for Ecoraider. Twenty males (3–4 wks old) in each dish were treated with chlorfenapyr, Bed Bug Fix, and Ecoraider in a similar manner as nymphs (Table 1). Treated bed bugs were immediately transferred to clean plastic Petri dishes (3.8 cm diameter and 1 cm height) with a 1.5-cm-diameter screened area on the lid. Folded red construction paper (3 cm long by 1 cm wide; Universal Stationers Supply Co., Deerfield, IL) was added as a harborage in each dish to provide resting and hiding places for bed bugs. The Petri dishes were held in a room at 26 ± 1°C, 40 ± 10% RH, and a photoperiod of 12:12 (L:D) h. Mortality data were recorded daily. A bed bug was considered dead if there was no movement when it was prodded with forceps.

Dust Exposure Bioassay

The two treatments were DE and cyfluthrin insecticide dusts. Cardboard panels (22 by 22 cm^2) were treated with each insecticide dust by applying a 5.1-cm-wide dust band to the perimeter of each panel with a fine brush. The amount of dust required to treat a 5.1-cm-wide band (total area: 335.5 cm^2) was calculated based on the label rate. Twenty-five large nymphs were placed in each plastic dish. Fourteen dishes were used for DE dust, whereas 18 dishes were used for cyfluthrin dust (Table 1). A plastic ring (5 cm diameter and 2 cm height) was placed in the center of a cardboard panel. Twenty-five nymphs were taken out from the plastic dish and confined in the center with a plastic ring for 15 min to acclimate. The plastic ring was then removed, and the nymphs were allowed to cross the 5.1-cm dust band. This method simulates conditions in which bed bugs might be exposed to dust deposits. Brief exposure to a narrow dust band is one of the possible ways that bed bugs come in contact with the dust particles in a natural environment. The first 20 bed bugs that crossed the band were collected using forceps (Fig. 1B) and placed in clean Petri dishes for observing mortality. The control group was handled in the same manner as the treatment group using untreated cardboard panels. All other procedures were similar as direct spray bioassay including handling, transferring bed bugs to clean dishes, and recording mortality data.

Effect of Posttreatment Feeding on Mortality of Bed Bug Nymphs and Adult Males Directly Sprayed or Briefly Exposed to Insecticide Dust

At 1 d (chlorfenapyr, deltamethrin, Ecoraider, cyfluthrin, and DE) or 3 d (Bed Bug Fix) after treatment, half of the surviving bugs in the treatment and the control groups were grouped into 20 bugs per screened plastic container and blood fed separately. For large nymphs, four containers were used for deltamethrin, six containers each for chlorfenapyr, DE dust, and cyfluthrin dust, five containers for Bed Bug Fix, and four containers for Ecoraider. For adult males, six containers were used for chlorfenapyr, five containers for Bed Bug Fix, and four containers for Ecoraider (Table 1). After feeding, only fed bugs were picked and grouped into 20 bugs per dish. The bugs that did not feed were excluded from the study, as those bugs were behaviorally different or weaker than the bugs that fed successfully. The other half of the surviving bed bugs in the treatment and

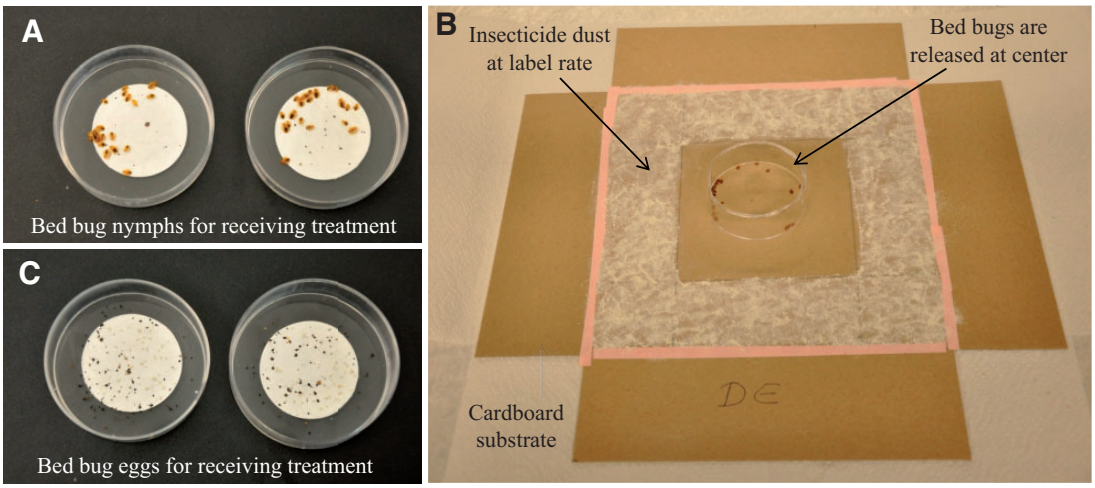

Fig. 1. Experimental setup: (A) Direct spray bioassay for bed bug nymphs, (B) Dust exposure bioassay for bed bug nymphs, and (C) Direct spray bioassay for bed bug eggs.

Table 1. An overview of the number of replications and bed bugs used for each treatment

Insecticide	Stage of bed bugs treated	No. of replications used in insecticide treatment[a]	No. of replications used in feeding/non feeding[b]	No. of replications used in postfeeding observation of mortality[c]
Deltamethrin spray	Fourth–fifth instars	14[d]	4[d]	3[d]
Chlorfenapyr spray	Fourth–fifth instars	14	6	5
Chlorfenapyr spray	Adult males	14	6	5
Chlorfenapyr spray	Eggs and first instars	6	4[e]	4
Bed Bug Fix spray	Fourth–fifth instars	14	5	4
Bed Bug Fix spray	Adult males	14	5	4
Ecoraider spray	Fourth–fifth instars	15	4	3
Ecoraider spray	Adult males	18	4	3
DE dust	Fourth–fifth instars	14	6	5
Cyfluthrin dust	Fourth–fifth instars	18	6	5

[a] Each experiment included treatment and control. Twenty bed bugs were used per replication.
[b] Each experiment included TF, CF, TNF, and CNF groups. Twenty bed bugs were used per replication unless stated otherwise.
[c] Each experiment included TF, CF, TNF, and CNF groups. Twenty bed bugs were used per replication.
[d] Refers to number of replications used for each category in that treatment.
[e] Ninety first-instars were used per replication in TF and CF bugs.

the control groups were not blood fed and also grouped into 20 bugs per dish. Each Petri dish represents a replicate. The grouped bed bugs were classified into four categories: 1) CF—control with feeding, 2) CNF—control without feeding, 3) TF—treatment with feeding, and 4) TNF—treatment without feeding. For large nymphs, five replicates were used in each category for the chlorfenapyr, cyfluthrin dust, and DE dust treatments. Four replicates were used per category for Bed Bug Fix. Three replicates per category were used for deltamethrin and Ecoraider. For adult males, five replicates were used in each category for the chlorfenapyr, four replicates for Bed Bug Fix, and three replicates were used for Ecoraider (Table 1). The body weight of 20 TF or CF bed bugs before and after feeding was measured using a high precision Metler Toledo PB153-S balance (Mettler-Toledo, LLC, Columbus, OH). The different number of replicates for feeding and then postfeeding was due to the varying numbers of survived or successfully fed bed bugs in different treatments. The different number of replicates for insecticide treatments and different feeding times (1 d after treatment in all insecticides and 3 d after treatment only in Bed Bug Fix) was due to different speed of kill by the insecticides based upon the findings by Singh et al. (2014) and our previous lab findings. All other procedures were similar as direct spray bioassay including handling, transferring bed bugs to clean dishes, and recording mortality data.

Effect of Posttreatment Feeding on Survival of First Instars Hatched From Chlorfenapyr-Treated Eggs

Chlorfenapyr was selected for this experiment because it resulted in 100% mortality in bed bug nymphs and adult males in the previous experiment. To collect eggs, thirty 3- to 4-wks-old recently blood fed mated Indy strain females were taken out from rearing containers and placed on filter paper in a small plastic dish. They were allowed to lay eggs for 1 d and then removed from the dish (Fig. 1C). Twelve dishes of females were used for collecting eggs. When eggs were 4 d old, six dishes (98.0 ± 4.2 eggs per dish) were randomly assigned to direct spray of chlorfenapyr, and the remaining six dishes (90.0 ± 3.7 eggs per dish) were sprayed with water

(control) following the procedures described in direct spray bioassay. The eggs were checked daily for nymph emergence.

The bed bug eggs hatched over a 24-h period (between seventh and eighth day). As soon as the nymphs emerged, they were immediately exposed to the chlorfenapyr residue because the filter papers along with the eggs were previously treated. Therefore, these nymphs had 0 - to 24-h exposure to spray residues on the filter paper before being transferred into cleans dishes. The bugs were divided into four categories: 1) CF—control with feeding, 2) CNF—control without feeding, 3) TF—treatment with feeding, and 4) TNF—treatment without feeding. Twenty first instars were randomly collected from the treatment or control dishes at 1 d after hatching and placed in screened dishes with a paper harborage for CNF and TNF groups, respectively. Both CNF and TNF included four dishes with 20 first instars each. Each dish represents a replicate. For the two feeding groups (CF and TF), 90 first instars were randomly collected from treatment and control dishes and placed in screened plastic containers for feeding. Each category included four containers of 90 first instars each. Nymphs were allowed to feed for 2 h. Twenty fed nymphs were randomly picked from each container and placed in a screened plastic dish with a paper harborage. Four replicates were used for CF and TF groups. The body weight of 20 CF or TF bed bugs before and after feeding was measured as described previously. All other procedures were similar as direct spray bioassay including handling, transferring bed bugs to clean dishes, and recording mortality data.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to compare: 1) amount of blood consumed or percent feeding between TF and CF bugs and 2) percent egg hatching between chlorfenapyr-treated and control eggs. For all feeding bioassays, the treatment mortality (TF and TNF) was corrected for control mortality (CF and CNF) using the Abbott's formula (Abbott 1925). For all insecticides, the repeated-measures analysis of the corrected mortality data was done using a mixed model to determine differences between treatments and their interaction with time (JMP 2014). The treatment, day, and treatment by day were included as main effect in the model. Replicate was included as random effect. When the interactions between the treatment and day were significant at $\alpha = 0.05$, Tukey's HSD test was used to separate the means among different time periods. In addition to repeated-measures analysis, the Kaplan–Meier method was also used to estimate survival time in chlorfenapyr- and Ecoraider-treated nymphs and adult males, where the corrected percent mortality values lie between 50–100% for both TF and TNF groups or the values for last observation periods between two groups were not significantly different. Survival differences were analyzed by the log-rank test. All analyses were conducted using JMP software, version 11 (SAS Institute 2012).

Results

Effect of Posttreatment Feeding on Mortality of Bed Bugs Exposed to Insecticides

Deltamethrin Direct Spray

Large Nymphs. Mortality (mean ± SEM) in insecticide-treated nymphs was 33.0 ± 4.8% at 24 h after treatment, and prior to feeding. Control mortality was 0%. The percentage of TF (96.3 ± 1.2%) and CF (95.0 ± 2.0%) nymphs that fed successfully after treatment was not significantly different ($F = 0.27$; df = 1, 6; $P = 0.62$). Similarly, the mean amount of blood consumed by each TF (142.0 ± 5.3 mg) replicate was not significantly different ($F = 0.87$; df = 1, 6; $P = 0.40$) from that of the CF replicates (148.0 ± 3.6 mg; Table 2). At 13, 16, and 20 d postfeeding, the corrected mortality in the TF nymphs was significantly lower ($F = 35.3$; df = 7, 28; $P < 0.0001$) than that in the TNF nymphs (Fig. 2). At 20 d postfeeding, the corrected mortality in the TF and TNF nymphs was 4.8 ± 1.7 and 69.3 ± 5.1%, respectively. Mortality in CF and CNF nymphs was 3.8 ± 1.2 and 2.5 ± 1.4%, respectively.

Chlorfenapyr Direct Spray

Large Nymphs. Mortality in insecticide-treated nymphs was 1.6 ± 0.5% at 24 h after treatment, and prior to feeding. Control mortality was 0%. The percentage of TF (90.0 ± 1.8%) and CF (95.0 ± 1.8%) nymphs that fed successfully after treatment was not significantly different ($F = 3.8$; df = 1, 10; $P = 0.08$). Similarly, the mean amount of blood consumed by each TF (144.2 ± 2.0 mg) replicate was not significantly different ($F = 0.65$; df = 1, 10; $P = 0.43$) from that of the CF replicates (146.8 ± 2.6 mg; Table 2). At 1, 2, and 4 d postfeeding, the corrected mortality in the TF nymphs was significantly higher than that in the TNF nymphs, whereas the corrected mortality in the TF nymphs was significantly lower than that in the TNF nymphs at 6, 9, and 13 d postfeeding ($F = 88.2$; df = 7, 56; $P < 0.0001$; Fig. 3A). At 20 d postfeeding, the corrected mortality in both TF and TNF nymphs was 100%. Mortality in CF and CNF nymphs was 3.8 ± 1.2 and 2.5 ± 1.4%, respectively. Based on the Kaplan–Meier analysis, the mean survival time in the TF nymphs (6.5 ± 0.6 d) was significantly ($\chi^2 = 6.1$; df = 1; $P = 0.01$) longer than that in the TNF nymphs (5.1 ± 0.2 d).

Adult Males. Mortality in insecticide-treated adult males was 3.5 ± 1.7% at 24 h after treatment, and prior to feeding. Control mortality was 0%. The percentage of TF (92.5 ± 1.1%) and CF (96.0 ± 2.0%) males that fed successfully after treatment was not significantly different ($F = 2.1$; df = 1, 10; $P = 0.17$). Similarly, the mean amount of blood consumed by each TF (224.5 ± 3.0 mg) replicate was not significantly different ($F = 3.0$; df = 1, 10; $P = 0.11$) from that of the CF replicates (230.0 ± 1.6 mg; Table 2). At 1 and 2 d postfeeding, the corrected mortality in the TF males was significantly higher than that in the TNF males, whereas conversely the corrected mortality in the TF males was significantly lower than that in the TNF males at 4 and 6 d postfeeding ($F = 110.5$; df = 5, 40; $P < 0.0001$; Fig. 3B). At 13 d postfeeding, the corrected mortality in both TF and TNF males was 100%. Mortality in CF and CNF males was 5.0 ± 2.9 and 8.3 ± 3.3%, respectively. Based on the Kaplan–Meier analysis, the mean survival time in the TF males (6.1 ± 0.4 d) was significantly ($\chi^2 = 56.5$; df = 1; $P < 0.0001$) longer than that in the TNF males (2.9 ± 0.1 d).

First Instars Hatched From Insecticide-Treated Eggs. The mean percentage of eggs hatched in the treatment group (98.1 ± 0.7%) was not significantly different ($F = 1.8$; df = 1, 10; $P = 0.21$) than that in the control group (96.6 ± 0.9%). Therefore, the chlorfenapyr direct spray did not affect egg hatching. Mortality in both insecticide-treated and control nymphs was 0% at 24 h after hatching. The percentage of TF (19.3 ± 1.2 %) and CF nymphs (96.1 ± 0.7%) that fed successfully after treatment was significantly different ($F = 3174.0$; df = 1, 6; $P < 0.0001$). Similarly, the mean amount of blood consumed by each TF (12.7 ± 0.5 mg) replicate was significantly different ($F = 10.0$; df = 1, 6; $P = 0.02$) from that of the CF replicates (14.5 ± 0.3 mg; Table 2). The treatment negatively affected first instars feeding capability. Similar to fourth–fifth

Table 2. Percent mortality (mean ± SEM) in bed bugs after treatment and prior to feeding, and food consumption of surviving bed bugs

Insecticide	Bed bug stage	Percent mortality before feeding		Percentage of bed bugs fed		Amount of blood consumed (mg)	
		Treatment	Control	Treatment (TF)	Control (CF)	Treatment (TF)	Control (CF)
Deltamethrin spray	Fourth–fifth instars	33.0 ± 4.8	0	96.3 ± 1.2	95.0 ± 2.0	142.0 ± 5.3	148.0 ± 3.6
Chlorfenapyr spray	Fourth–fifth instars	1.6 ± 0.5	0	90.0 ± 1.8	95.0 ± 1.8	144.2 ± 2.0	146.8 ± 2.6
Chlorfenapyr spray	Adult males	3.5 ± 1.7	0	92.5 ± 1.1	96.0 ± 2.0	224.5 ± 3.0	230.0 ± 1.6
Chlorfenapyr spray	Eggs and first instars	0	0	19.3 ± 1.2*	96.1 ± 0.7	12.7 ± 0.5*	14.5 ± 0.3
Bed Bug Fix spray	Fourth–fifth instars	11.1 ± 2.7	0.7 ± 0.7	99.0 ± 1.0	100.0 ± 0	150.6 ± 3.5	146.4 ± 2.1
Bed Bug Fix spray	Adult males	24.0 ± 3.9	0	99.0 ± 1.0	96.0 ± 1.9	237.0 ± 3.5	230.6 ± 1.4
Ecoraider spray	Fourth–fifth instars	42.0 ± 3.6	0	88.8 ± 2.4	95.0 ± 2.0	140.2 ± 3.3	147.0 ± 3.0
Ecoraider spray	Adult males	52.0 ± 3.1	0	93.8 ± 1.2	96.2 ± 2.4	227.0 ± 2.3	230.0 ± 1.6
DE dust	Fourth–fifth instars	2.0 ± 1.5	0	96.0 ± 2.5	94.1 ± 0.8	153.8 ± 3.2	149.5 ± 2.4
Cyfluthrin dust	Fourth–fifth instars	41.0 ± 3.0	0	95.3 ± 1.6	94.1 ± 0.8	146.8 ± 2.9	149.5 ± 2.4

Please refer to Table 1 for the number of replications and bed bugs used for each category.

* Indicates significant difference between TF and CF groups.

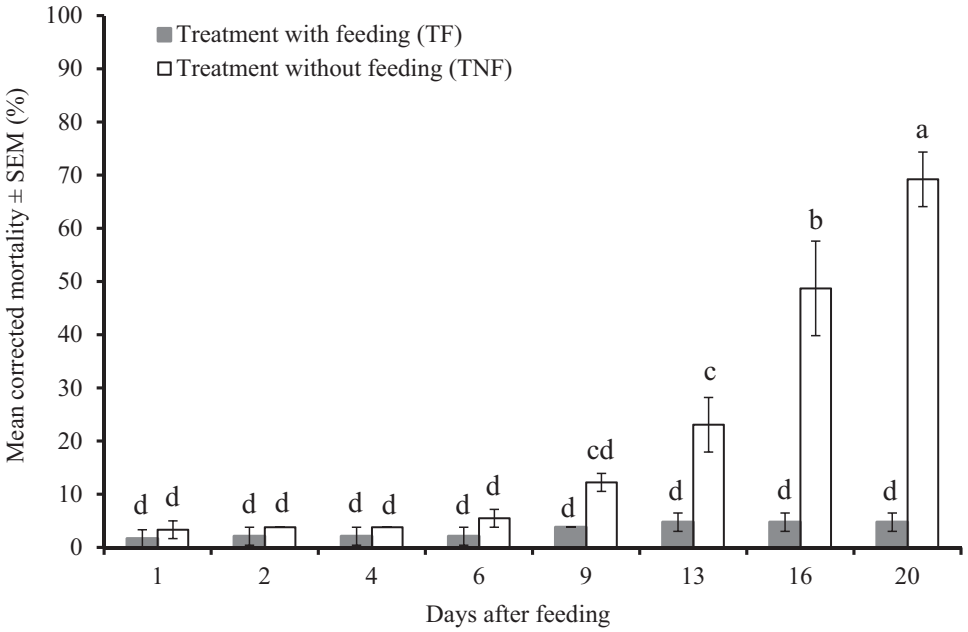

Fig. 2. Posttreatment mortality of deltamethrin spray-treated fourth–fifth instars with and without blood feeding. Means with the different letters are significantly different ($P < 0.05$, Tukey's HSD test).

instars, the corrected mortality in the TF nymphs was significantly higher than that in the TNF nymphs at 1 d postfeeding, whereas the corrected mortality in the TF nymphs was significantly lower than that in the TNF nymphs at 2, 4, 6, 9, and 13 d postfeeding ($F = 197.2$; df = 5, 36; $P < 0.0001$; Fig. 4). At 13 d postfeeding, the corrected mortality in TF and TNF nymphs was 87 and 100%, respectively. Mortality in both CF and CNF nymphs was 6.2 ± 1.2%. Based on the Kaplan–Meier analysis, the mean survival time in the TF nymphs (3.1 ± 0.5 d) was significantly ($\chi^2 = 7.5$; df = 1; $P = 0.006$) longer than that in the TNF nymphs (1.8 ± 0.04 d).

Bed Bug Fix Direct Spray

Large Nymphs. Mortality in insecticide-treated nymphs was 11.1 ± 2.7% at 3 d after treatment, and prior to feeding. Control mortality was 0.7 ± 0.7%. The percentage of TF (99.0 ± 1.0%) and CF (100.0 ± 0%) nymphs that fed successfully after treatment was not significantly different ($F = 1.0$; df = 1, 8; $P = 0.35$). Similarly, the mean amount of blood consumed by each TF (150.6 ± 3.5 mg) replicate was not significantly different ($F = 1.1$; df = 1, 8; $P = 0.33$) from that of the CF replicates (146.4 ± 2.1 mg; Table 2). At 4, 7, and 11 d postfeeding, the corrected mortality in the TF nymphs was significantly lower than that in the TNF nymphs ($F = 111.2$; df = 4, 24; $P < 0.0001$; Fig. 5A). At 11 d postfeeding, the corrected mortality in TF and TNF nymphs was 3.9 ± 1.3 and 92.3 ± 2.5%, respectively. Mortality in CF and CNF nymphs was 0 and 1.6 ± 1.6%, respectively.

Adult Males. Mortality in insecticide-treated adult males was 24.0 ± 3.9% at 3 d after treatment, and prior to feeding. Control mortality was 0%. The percentage of TF (99.0 ± 1.0%) and CF (96.0 ± 1.9%) males that fed successfully after treatment was not significantly different ($F = 2.0$; df = 1, 8; $P = 0.20$). Similarly, the mean amount of blood consumed by each the TF (237.0 ± 3.5 mg) replicate was not significantly different ($F = 2.8$; df = 1, 8; $P = 0.13$) from that of the CF replicates (230.6 ± 1.4 mg; Table 2). At 1, 2,

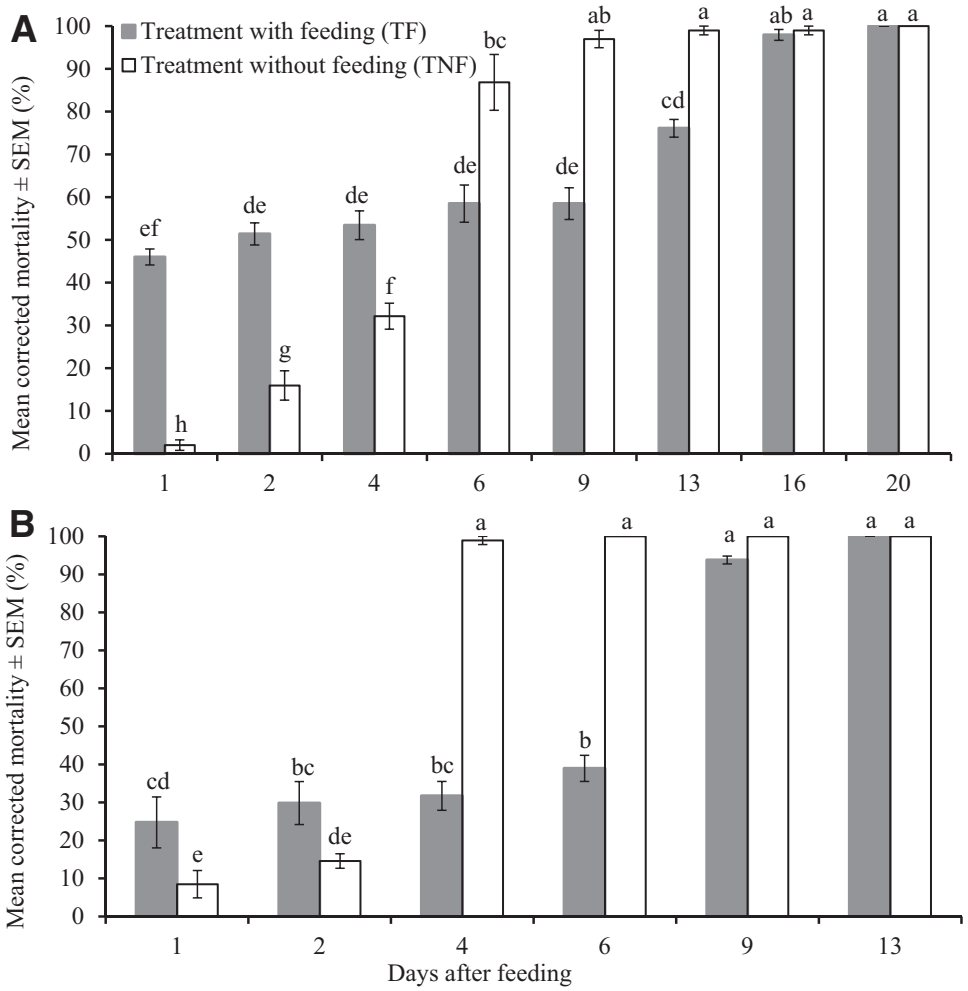

Fig. 3. Posttreatment mortality of chlorfenapyr spray-treated: (A) fourth–fifth instars and (B) adult males with and without blood feeding. Means with the different letters are significantly different ($P < 0.05$, Tukey's HSD test).

4, 7, and 11 d postfeeding, the corrected mortality in the TF males was significantly lower than that in the TNF males ($F = 40.6$; df = 4, 24; $P < 0.0001$; Fig. 5B). At 11 d post feeding, the corrected mortality in TF and TNF males was 8.7 ± 2.1 and 98.7 ± 1.3%, respectively. Mortality in both CF and CNF males was 5.0 ± 0%.

Ecoraider Direct Spray

Large Nymphs. Mortality in insecticide-treated nymphs was 42.0 ± 3.6% at 24 h after treatment, and prior to feeding. Control mortality was 0%. The percentage of TF (88.8 ± 2.4%) and CF (95.0 ± 2.0%) nymphs that fed successfully after treatment was not significantly different ($F = 3.9$; df = 1, 6; $P = 0.09$). Similarly, the mean amount of blood consumed by each TF (140.2 ± 3.3 mg) replicate was not significantly different ($F = 2.3$; df = 1, 6; $P = 0.18$) from that of the CF replicates (147.0 ± 3.0 mg; Table 2). At 1, 2, 4, 6, and 9 d postfeeding, the corrected mortality in the TF nymphs was significantly lower than that in the TNF nymphs ($F = 13.2$; df = 5, 20; $P < 0.0001$; Fig. 6A). At 13 d postfeeding, the corrected mortality in both TF and TNF nymphs was 100%. Mortality in CF and CNF nymphs was 3.8 ± 1.2 and 2.5 ± 1.4%, respectively. Based on the Kaplan–Meier analysis, the mean survival time in TF nymphs (7.9 ± 0.5 d) was significantly ($\chi^2 = 54.3$; df = 1; $P < 0.0001$) longer than that in the TNF nymphs (2.5 ± 0.3 d).

Adult Males. Mortality in insecticide-treated males was 52.0 ± 3.1% at 24 h after treatment, and prior to feeding. Control mortality was 0%. The percentage of TF (93.8 ± 1.2%) and CF (96.2 ± 2.4%) males that fed successfully after treatment was not significantly different ($F = 0.86$; df = 1, 6; $P = 0.40$). Similarly, the mean amount of blood consumed by each TF (227.0 ± 2.3 mg) replicate was not significantly different ($F = 1.2$; df = 1, 6; $P = 0.32$) from that of the CF replicates (230.0 ± 1.6 mg; Table 2). At 1, 2, 4, 6, and 9 d postfeeding, the corrected mortality in the TF males was significantly lower than that in the TNF males ($F = 28.2$; df = 5, 20; $P < 0.0001$; Fig. 6B). At 13 d postfeeding, the corrected mortality in both TF and TNF males was 100%. Mortality in CF and CNF males was 5.0 ± 2.9 and 8.33 ± 3.3%, respectively. Based on the Kaplan–Meier analysis, the mean survival time in the TF males (5.7 ± 0.4 d) was significantly ($\chi^2 = 64.1$; df = 1; $P < 0.0001$) longer than that in the TNF males (1.8 ± 0.1 d).

DE Dust

Large Nymphs. Mortality in insecticide-treated nymphs was 2.0 ± 1.5% at 24 h after treatment, and prior to feeding. Control mortality was 0%. The percentage of TF (96.0 ± 2.5%) and CF (94.1 ± 0.8%) nymphs that fed successfully after treatment was not significantly different ($F = 1.9$; df = 1, 10; $P = 0.20$). Similarly, the mean amount of blood consumed by each of the TF

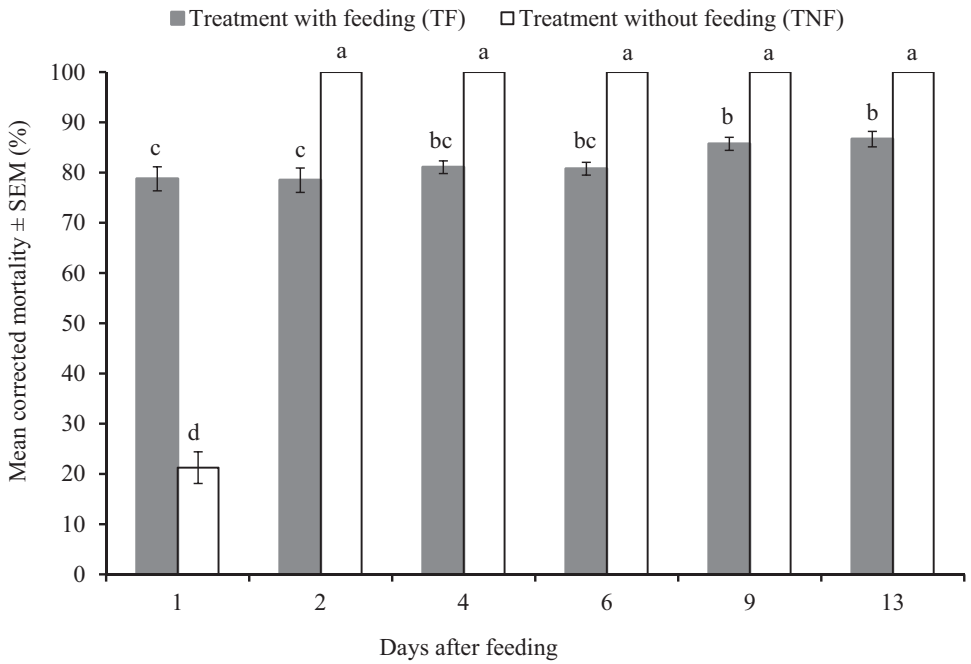

Fig. 4. Posttreatment mortality of the first instars hatched from chlorfenapyr-treated eggs with and without blood feeding. Means with the different letters are significantly different ($P < 0.05$, Tukey's HSD test).

(153.8 ± 3.2 mg) replicate was not significantly different ($F = 1.2$; df = 1, 10; $P = 0.30$) from that of the CF replicates (149.5 ± 2.4 mg; Table 2). At 13, 16, and 20 d postfeeding, the corrected mortality in the TF nymphs was significantly lower than that in the TNF nymphs ($F = 11.7$; df = 7, 56; $P < 0.0001$; Fig. 7). At 20 d postfeeding, the corrected mortality in TF and TNF nymphs was 2.2 ± 0.9 and 60.3 ± 15.3%, respectively. Mortality in CF and CNF nymphs was 3.3 ± 1.6 and 1.6 ± 1.6%, respectively.

Cyfluthrin Dust

Large Nymphs. Mortality in insecticide-treated nymphs was 41.0 ± 3.0% at 24 h after treatment, and prior to feeding. Control mortality was 0%. The percentage of TF (95.3 ± 1.6 %) and CF (94.1 ± 0.8%) nymphs that fed successfully after treatment was not significantly different ($F = 0.41$; df = 1, 10; $P = 0.53$). Similarly, the mean amount of blood consumed by each TF (146.8 ± 2.9 mg) replicate was not significantly different ($F = 0.51$; df = 1, 10; $P = 0.50$) from that of the CF replicates (149.5 ± 2.4 mg; Table 2). At 20 d postfeeding, the corrected mortality in TF and TNF males was 2.2 ± 1.7 and 3.8 ± 1.7%, respectively. Mortality in CF and CNF nymphs was 3.3 ± 1.6 and 1.6 ± 1.7%, respectively.

Cyfluthrin dust (41.0 ± 3.0%) caused significantly ($F = 136.0$; df = 1, 30; $P < 0.0001$) higher corrected mortality than DE dust (2.0 ± 1.5%) in TNF bugs at 24 h after treatment, and prior to feeding. In contrast, at 20 d postfeeding, DE dust (60.3 ± 15.3%) caused significantly ($F = 13.6$; df = 1, 8; $P = 0.006$) higher mortality than cyfluthrin dust (3.8 ± 1.7%) in TNF bugs.

Discussion

This study reveals that availability of a bloodmeal after insecticide exposure can have a major impact on bed bug survival. Our results indicated that posttreatment feeding significantly reduced or slowed down bed bug mortality. The effect of feeding on bed bug mortality varied among insecticides. Feeding reduced mortality in bed bugs treated with deltamethrin, Bed Bug Fix, and DE dust. However, in bed bugs treated with chlorfenapyr and Ecoraider, feeding only slowed down the speed of mortality, and there was 100% final mortality regardless of feeding status of the bed bugs. Nonetheless, the increased survival time resulting from feeding may provide more time for mated female bed bugs to lay eggs and also prolong the suffering of occupants from continued bed bug bites. The results from this study suggest that efficacy data collected from laboratory bioassays, where bed bugs are not fed posttreatment, may not be indicative of how the product will perform in the field when a host is present.

In our study, posttreatment feeding reduced mortality of deltamethrin-treated bed bugs. In a recent study, a bloodmeal one day prior to insecticide exposure did not increase the survival time when deltamethrin was topically applied to bed bugs compared with those bugs fed 9 d prior to the treatment (Choe and Campbell 2014). The discrepancy between these results could be the result of different study designs (i.e., feeding at 1 d before exposure vs. 1 d after exposure). The reduced mortality in deltamethrin-treated bugs after feeding could be explained by increased metabolic enzymes in response to blood feeding. Pyrethroid resistance has been linked with increased activity of *GSTs* in *Cu. quinquefasciatus* say (Xu et al. 2005, Che-Mendoza et al. 2009) and *P450s* in *Cu. quinquefasciatus* (Xu et al. 2005), *Cu. pipiens* (McAbee et al. 2003), *Anopheles gambiae* (Nikou et al. 2003), and *Anopheles funestus* (Brooke et al. 2001, Amenya et al. 2008). Blood feeding upregulated *GSTs* in *An. gambiae* (Marinotti et al. 2005) and *P450s* in *Cu. pipiens* (Baldridge and Feyereisen 1989) and *Ae. aegypti* (Sanders et al. 2003). Increased pyrethroid resistance in mosquitoes seems to be positively correlated with *P450s* and *GSTs* which in turn are elevated by blood feeding. In our study, the reduced mortality in deltamethrin-treated bugs following a bloodmeal also suggests the role of elevated *P450s* and *GSTs* after blood feeding in increased survival of bed bugs. Males were not tested with deltamethrin in this study. A study by Feldlaufer et al. (2013) found no significant difference in mortality

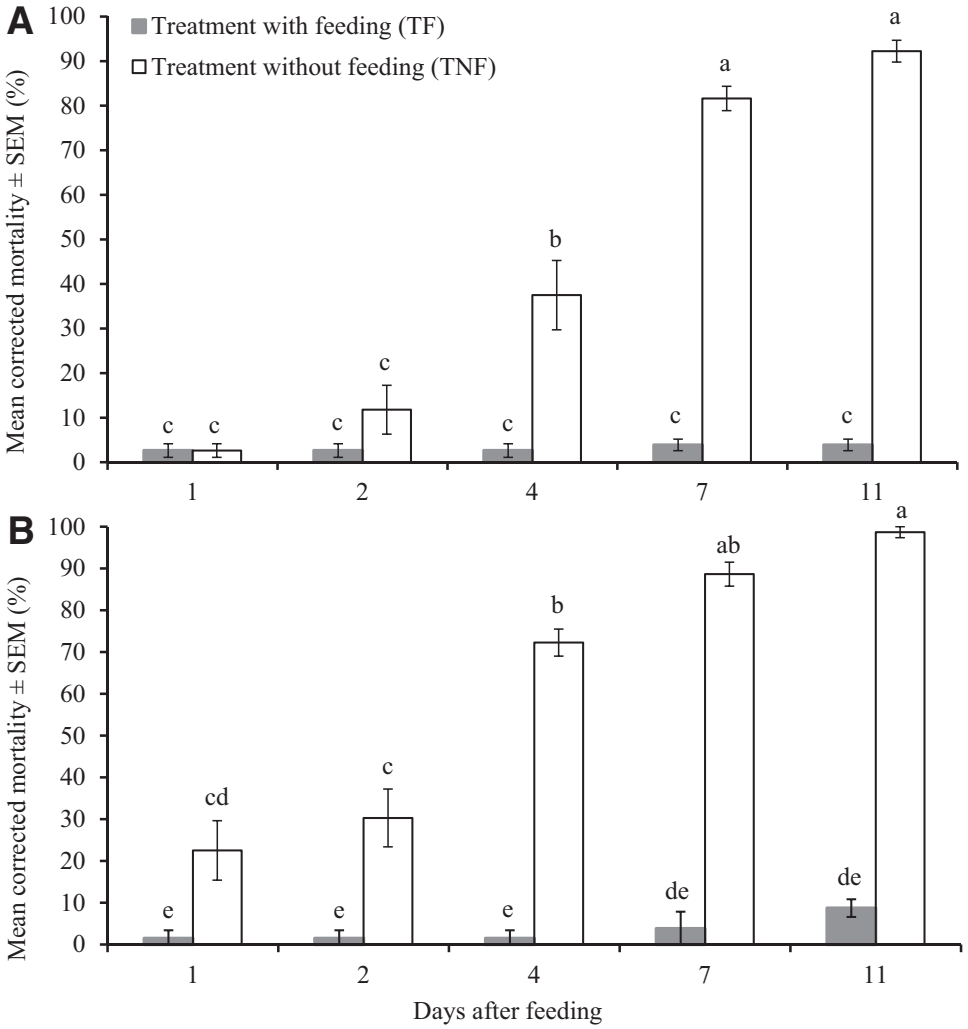

Fig. 5. Posttreatment mortality of Bed Bug Fix spray-treated: (A) fourth–fifth instars and (B) adult males with and without blood feeding. Means with the different letters are significantly different ($P < 0.05$, Tukey's HSD test).

between males, females, and nymphs exposed to various concentrations of deltamethrin at 24 or 168 h postexposure, and the survived bugs were capable of feeding when given the opportunity.

Mortality in chlorfenapyr-treated and fed first and fourth–fifth instars, and adult males increased during first few days after feeding and then decreased compared with mortality in treated nonfed bugs. This pattern of mortality can be explained by the mode of action of chlorfenapyr and the resistance status (pyrethroid resistant) of bed bugs used in this study. Chlorfenapyr is a proinsecticide that must be activated by *P450s* to a more active metabolite (Black et al. 1994). The overexpression of *P450s* and *GSTs* in pyrethroid-resistant bed bugs suggests a role of metabolic enzymes in resistance (Zhu et al. 2010, Adelman et al. 2011, Bai et al. 2011, Mamidala et al. 2011). Elevated levels of *P450s* in resistant bed bugs may result in increased sensitivity to chlorfenapyr (negative cross-resistance). In our study, a higher mortality during the first few days after feeding may also be caused by elevated *P450s* levels which in turn increased the activation of chlorfenapyr (Raghavendra et al. 2011). Negative cross-resistance between pyrethroids and chlorfenapyr has been shown in pyrethroid-resistant housefly and tobacco budworm strains (Pimprale et al. 1997, Scott et al. 2004).

Feeding after treatment increased the mean survival time in chlorfenapyr-treated bed bugs. However, 100% final mortality in treated bugs was reported regardless of the feeding status. Similarly, Choe and Campbell (2014) reported increased survival times in recently fed bed bugs over the 9 d starved bugs when they were exposed to topical application and fresh or aged residual deposits of chlorfenapyr. However, in their study the final mortality between chlorfenapyr-treated fed and nonfed bugs was significantly different.

Two essential oil-based products (Ecoraider and Bed Bug Fix) produced strikingly different responses among bed bugs subjected to posttreatment feeding. Ecoraider caused 100% mortality in treated bed bugs regardless of feeding or nonfeeding. In contrast, feeding significantly reduced the mortality of Bed Bug Fix-treated bed bugs. Ecoraider acted as a relatively fast-acting insecticide, as it caused 42 and 52% mortality at 24 h after treatment and prior to feeding in large nymphs and adult males, respectively. On the other hand, Bed Bug Fix was slow acting, as it only caused 11 and 24% mortality in large nymphs and adult males, respectively, at 3 d after treatment and prior to feeding. The effect of feeding seems to be more pronounced for slower-acting essential oil-based insecticides.

A recent field study indicates ineffectiveness of DE when used alone for controlling bed bugs (Potter et al. 2013). The results from our study reveal the probable reasons for the lack of field efficacy of DE dust. DE dust causes desiccation and death by removing the protective wax layer from the cuticle of an insect. In a naturally infested

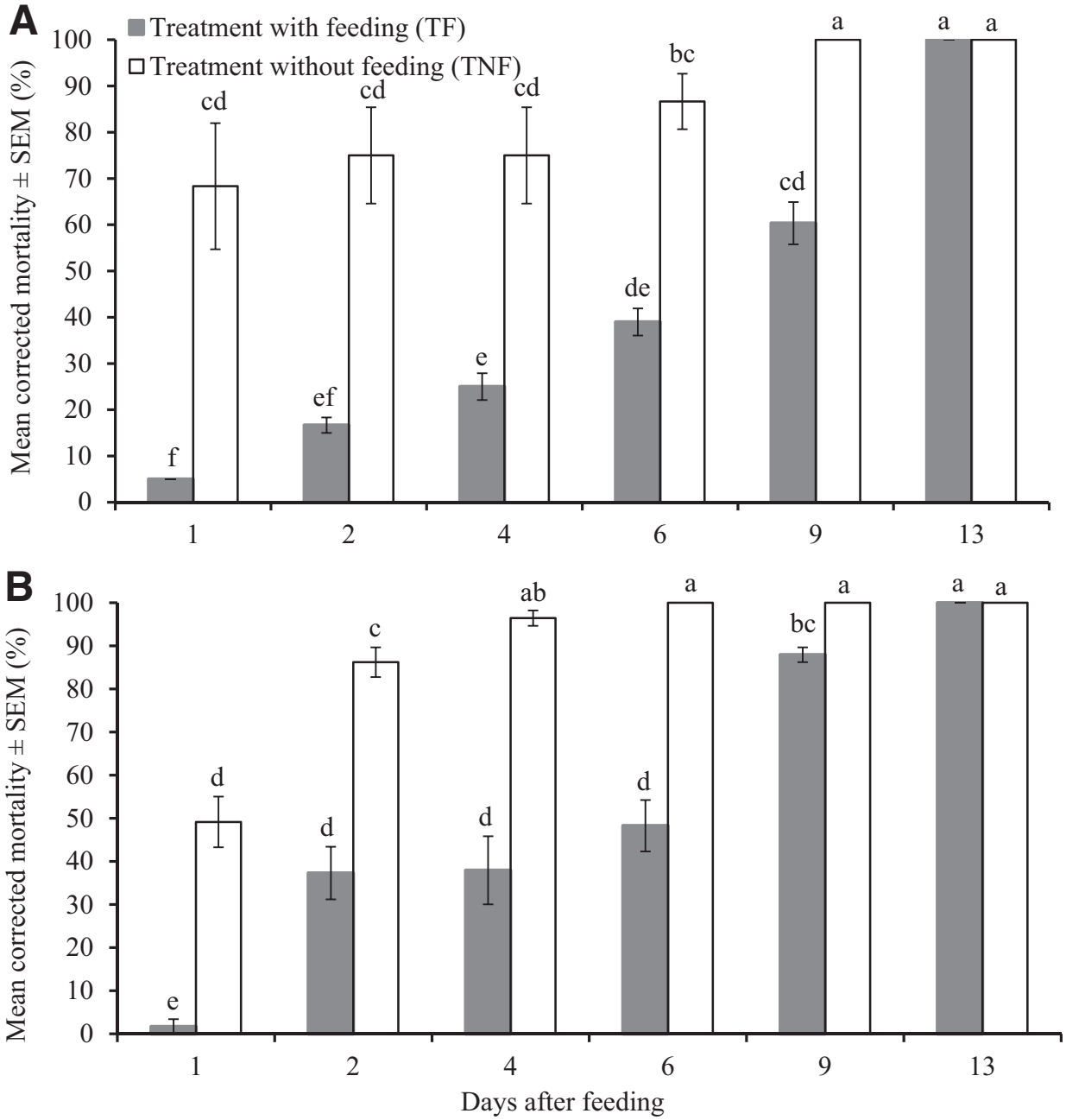

Fig. 6. Posttreatment mortality of Ecoraider spray-treated: (A) fourth–fifth instars and (B) adult males with and without blood feeding. Means with the different letters are significantly different ($P < 0.05$, Tukey's HSD test).

environment, the desiccant exposed bed bugs can replenish depleted water through periodic bloodmeals. Therefore, it is not surprising that the reported field efficacy of DE was lower than that suggested by Doggett and Russell (2008). Our results support the theory that feeding affects the efficacy of slow-acting desiccant dusts such as DE.

Posttreatment feeding had no effect on mortality of cyfluthrin dust exposed bed bugs. Bed bugs exposed to cyfluthrin dust had 41% mortality at 24 h after treatment and prior to feeding. The survived bugs in both fed and nonfed groups afterward had very little mortality even at 20 d (<5%) after feeding. Widespread resistance in bed bugs to pyrethroid insecticides has been reported (Romero et al. 2007; Yoon et al. 2008; Zhu et al. 2010, 2013; Adelman et al. 2011). Cyfluthrin dust treatment may have killed all the susceptible bed bugs in one day, and all the remaining survivors were highly resistant to cyfluthrin.

In our study, the increased tolerance in large bed bug nymphs and adult males to insecticides after feeding suggests the role of feeding in stimulating detoxification enzymes responsible for insecticide resistance. Choe and Campbell (2014) also suggested the role of detoxification mechanisms in increasing the tolerance of fed bed bugs to chlorfenapyr. The detoxification mechanisms required for neutralizing harmful components in the bloodmeal during blood digestion may also be involved in detoxifying insecticides as suggested by Spillings et al. (2008) in *An. funestus*.

Our findings have important implications for product evaluations, especially when evaluating slow-acting insecticides. Many

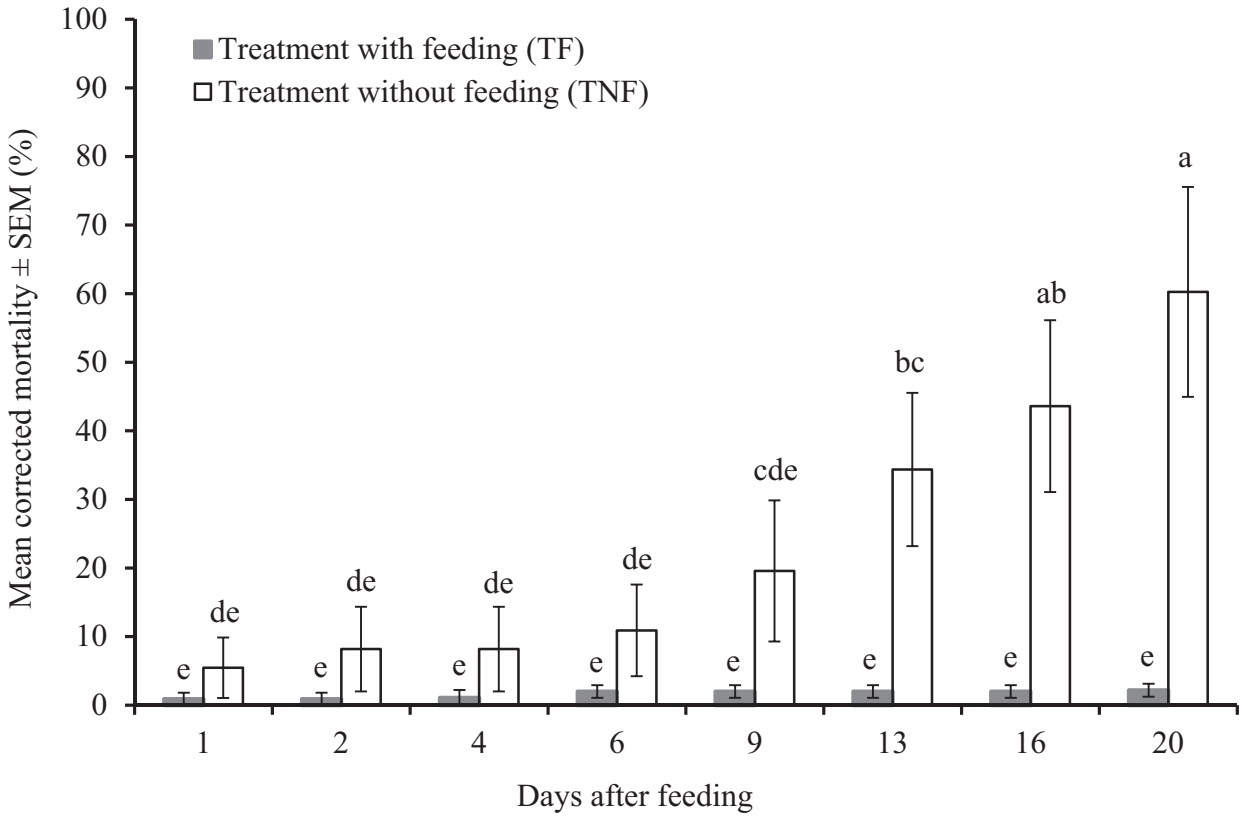

Fig. 7. Posttreatment mortality of diatomaceous earth dust exposed fourth–fifth instars with and without blood feeding. Means with the different letters are significantly different ($P < 0.05$, Tukey's HSD test).

laboratory studies evaluated the efficacy of insecticides by exposing bed bugs continuously to treated surfaces in small confinements without feeding (Todd 2006, Doggett and Russell 2008, Romero et al. 2009, Anderson and Cowles 2012). In light of the importance of posttreatment feeding, the usefulness of these study results become questionable in predicting field efficacy of the materials tested. Furthermore, with the exception of treatments directly onto bed bug harborages, it is unlikely that bed bugs will stay on a treated surface continuously in the natural environment. Based on the results from our study and the findings by Choe and Campbell (2014), insecticide efficacy testing protocols should include recently fed bed bugs (fed within 5 d) and offer posttreatment feeding within 1 to 3 d after treatment. In our study, most of the treated bed bugs were able to successfully feed on blood in the small setting provided. However, under field conditions treated bed bugs away from sleeping areas need to travel a significant distance to reach the host and may not be as successful in taking bloodmeals, as those with harborages located at host sleeping sites. Further investigations of the physiological and behavioral differences between the fed and nonfed insecticide-treated bed bugs will help elucidate the underlying mechanisms of the bed bugs' defense against insecticide treatments. Future research should investigate the effect of multiple posttreatment feedings on bed bug mortality.

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