

## Household and Structural Insects

# Comparative Efficacy of Selected Dust Insecticides for Controlling *Cimex lectularius* (Hemiptera: Cimicidae)

Narinderpal Singh,<sup>1</sup> Changlu Wang,<sup>1,2</sup> Desen Wang,<sup>1,3</sup> Richard Cooper,<sup>1</sup> and Chen Zha<sup>1</sup>

<sup>1</sup>Department of Entomology, Rutgers University, 96 Lipman Dr., New Brunswick, NJ 08901 (singh.narinderpal@gmail.com; cwang@aesop.rutgers.edu; wds830706@163.com; rcooper@aesop.rutgers.edu; chen.zha1@rutgers.edu), <sup>2</sup>Corresponding author, e-mail: cwang@aesop.rutgers.edu, and <sup>3</sup>Visiting student from South China Agricultural University

Received 24 January 2016; Accepted 19 May 2016

### Abstract

Bed bugs, *Cimex lectularius* L., are one of the most difficult urban pests to control. Pest management professionals rely heavily on insecticide sprays and dusts to control bed bugs. Dust formulations are considered to provide longer residual control than sprays. However, there are no scientific data available on the comparative efficacy of the commonly used insecticide dusts. We evaluated the efficacy of eight insecticide dust products using three exposure methods: 1) brief exposure—bed bugs crossed a 2.54-cm-wide dust-treated band, 2) forced exposure—bed bugs were continuously exposed to a dust-treated substrate, and 3) choice exposure—bed bugs were given a choice to stay on either dust-treated or untreated substrate. The brief exposure method was the most sensitive in detecting the differences among the insecticides. Only CimeXa (silica gel) dust caused 100% mortality from all three exposure methods. Other tested dusts (1% cyfluthrin, 0.05% deltamethrin, 0.075% zeta-cypermethrin + 0.15% piperonyl butoxide, 1% pyrethrins, 1% 2-phenethyl propionate + 0.4% pyrethrin, 0.25% dinotefuran + 95% diatomaceous earth, 100% diatomaceous earth) caused ≤65% mortality in a brief exposure assay. We also evaluated the horizontal transfer effect of the silica gel dust. Silica gel dust-exposed bed bugs transferred the dust horizontally to unexposed bed bugs resulting in 100% mortality at 4:6 donor: recipient ratio and 88.0 ± 5.0% mortality at 1:5 donor: recipient ratio. The results suggest silica gel is the most promising insecticide dust for controlling *C. lectularius*.

**Key words:** bed bug, insecticide dust, efficacy, horizontal transfer, choice exposure

Bed bug (*Cimex lectularius* L.) (Hemiptera: Cimicidae) infestations are very difficult and expensive to control (Potter et al. 2013a). Despite the many control materials and methods available for bed bug management, insecticide treatments remain the most popular among pest management professionals (Potter et al. 2013a,b). In a survey of pest control firms, 96% reported routinely using insecticide sprays and 90% mentioned using dusts for their bed bug control treatments (Potter 2013a). Applying insecticides to control bed bugs is often more economical and convenient compared to other bed bug control methods (Wang et al. 2015).

Dust formulations are believed to offer better residual protection than pesticide sprays because they are more readily picked up by bed bugs compared to dry spray residue (Potter et al. 2008, Romero et al. 2009a). There are only a few laboratory studies available comparing the effectiveness of dust insecticides. DeltaDust (0.05% deltamethrin), Tempo 1% Dust (1% cyfluthrin), Drione (1% pyrethrins, 10% piperonyl butoxide, and 40% amorphous silica gel), and MotherEarth D (100% diatomaceous earth) were evaluated by confining bed bugs continuously to dust-treated surfaces in small confinements (Romero et al. 2009a, Anderson and Cowles 2012).

While being useful for evaluating comparative efficacy of insecticides, this methodology can result in inflated mortality compared to what would be encountered in the field because it is highly unlikely that bed bugs will stay on dust-treated surface continuously in natural environments, where bed bugs always have a choice to avoid dust-treated surfaces.

A few field studies evaluated the efficacy of dust formulations in combination with other sprays (Wang et al. 2007) or nonchemical treatments (Wang et al. 2009, 2012, 2013; Singh et al. 2013; Stedfast and Miller 2014). All of these studies resulted in high percentage reduction in bed bug populations. To what degree, dust formulations contributed to the success is unclear and was not specifically measured in these studies. Most of the dust formulations contain pyrethroid or pyrethrins as active ingredients. However, widespread resistance in bed bugs to pyrethroid insecticides has been reported (Romero et al. 2007; Zhu et al. 2010, 2013; Adelman et al. 2011). Moreover, a recent study suggested insecticide mixtures containing a pyrethroid and a neonicotinoid can quickly lose efficacy after repeated applications (Gordon 2014). Additionally, pyrethroids can exhibit a repellent effect upon bed bugs, particularly in the absence of

harborage cues (Romero et al. 2009b). The use of nonpyrethroid or inorganic dust formulations (silica gel or diatomaceous earth dust [DE]) are suggested to slow down insecticide resistance development in bed bugs (Romero et al. 2011, Potter et al. 2014). There are only two preliminary field studies where DE (Potter et al. 2013b) or silica gel (Potter et al. 2014) was used as a standalone treatment to control bed bugs. Results showed DE was ineffective against bed bugs but silica gel dust was highly effective. Laboratory experiments also showed silica gel is superior to DE for controlling bed bugs (Potter et al. 2014).

To further increase our knowledge on the efficacy of various insecticide dusts, we evaluated the efficacy of eight commonly used insecticide dusts against field strains of bed bugs using several different bioassays: 1) brief exposure, 2) forced exposure, 3) choice exposure, and 4) horizontal transfer. The types of insecticide dusts examined included inorganic, pyrethrin, pyrethroid, pyrethroid + synergist, botanical oil + pyrethrin, and neonicotinoid + inorganic.

## Materials and Methods

### Bed Bugs and Experimental Conditions

Two field-collected bed bug strains, Indy and Irvington, were used. The Indy strain was collected in 2008 (six years prior to this study) from infested apartments in Indiana. The Irvington strain was collected three months prior to this study from infested apartments in New Jersey. Both strains were moderately resistant to deltamethrin. In a direct spray bioassay, deltamethrin at the highest label rate (0.06% deltamethrin; Suspend SC; Bayer Environmental Science, Durham, NC) caused <40% mortality in both strains at 7 d after treatment compared to 100% mortality in a laboratory susceptible strain (Ft Dix) at 3 h after treatment (Singh, unpublished data). Bed bugs were maintained in plastic containers (4.7 cm height and 5 cm diameter; Consolidated plastics, Stow, OH) with folded paper as harborages (3 cm long by 4 cm wide; Universal Stationers Supply Co., Deerfield, IL) at  $26 \pm 1^\circ\text{C}$ ,  $40 \pm 10\%$  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). All experiments were conducted in a nonventilated room measuring 4 m long and 2.3 m wide at  $26 \pm 1^\circ\text{C}$ ,  $40 \pm 10\%$  RH, and a photoperiod of 12:12 (L:D) h. Bed bugs were fed  $\sim 1$  wk prior to bioassays. All the bed bug nymphs had already molted at the time of the bioassay. Only males and second–fifth instars were used. Females were not used because there were not enough females for the experiments.

### Dust Insecticides

The trade names, active ingredient(s), label rate of application, and manufacturer information for eight dust insecticides, representing different classes of insecticides, are as follows, Pyrethroid: Tempo 1% Dust (1% Cyfluthrin, 4.9 g/m<sup>2</sup>; Bayer Environmental Science, RTP, NC) and DeltaDust (0.05% Deltamethrin, 4.9 g/m<sup>2</sup>; Bayer Environmental Science, RTP, NC); Pyrethroid + Synergist: Cynoff (0.075% Zeta-Cypermethrin + 0.15% Piperonyl Butoxide); Pyrethrin: Pyganic (1% Pyrethrin, 12.2 g/m<sup>2</sup>; McLaughlin Gormley King Company, Minneapolis, MN); Botanical oil + Pyrethrin: EcoPCO D.X (1% 2-Phenethyl Propionate + 0.4% Pyrethrin, 6.1 g/m<sup>2</sup>; EcoSMART Technologies Inc., Alpharetta, GA); Neonicotinoid + Inorganic: Alpine (0.25% Dinotefuran + 95% Diatomaceous Earth, 10 g/m<sup>2</sup>; BASF Corporation, St. Louis, MO); Inorganic: MotherEarth D (100% Diatomaceous Earth, 18.3 g/m<sup>2</sup>; BASF Corporation, St. Louis, MO),

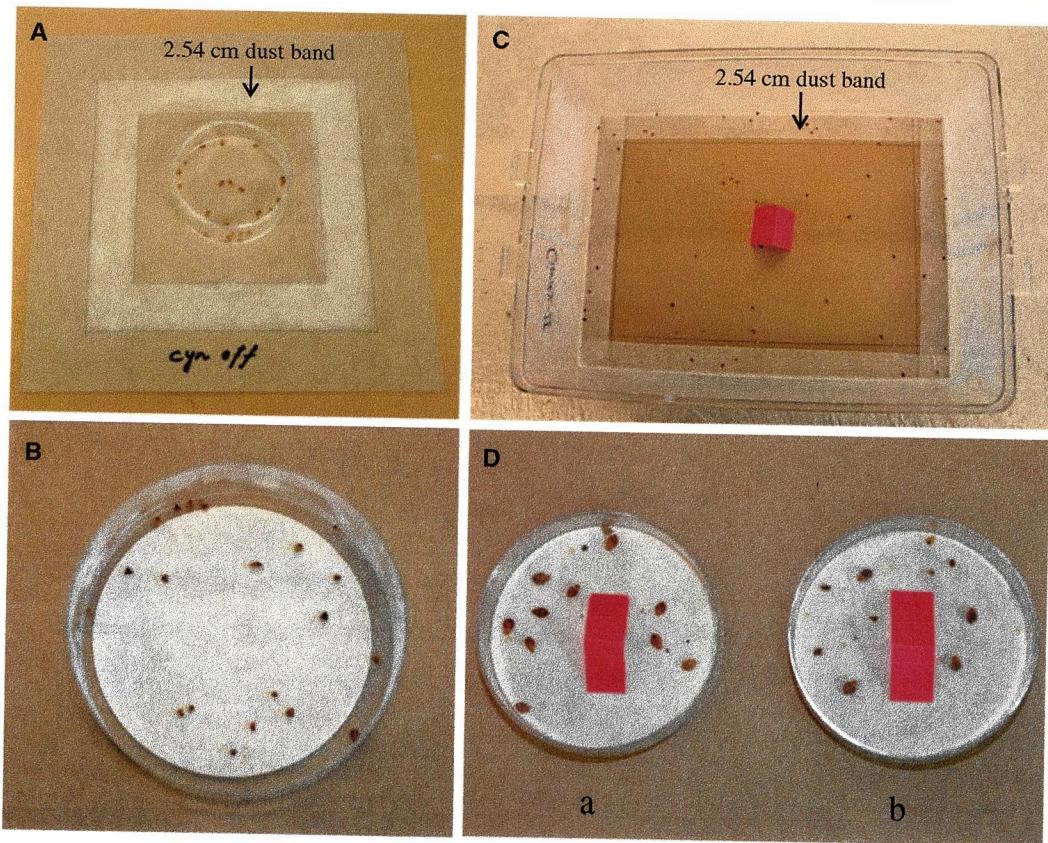
and CimeXa (100% Amorphous Silica Gel, 6.1 g/m<sup>2</sup>; Rockwell Labs Ltd, North Kansas City, MO). These dusts are among the most common dusts used by professionals for bed bug treatments in the U.S. (Porter et al. 2014). The products were obtained either directly from manufacturers or from commercial distributors. Dusts were used following the product label rates.

### Experiment 1. Efficacy of Dusts in Brief Exposure Bioassay

This experiment simulates field conditions where bed bugs only come in contact with dust applications for a brief amount of time. Indy and Irvington bed bug nymphs were tested at two different times within a 6-mo period. Cardboard panels (21 by 21 cm) were used as the substrate material. A 2.54-cm-wide dust band was applied with a fine brush on the panels leaving 3-cm untreated area between the outer edge of band and the panel perimeter. The amount of dust required to treat a 2.54-cm-wide dust band (total area: 125 cm<sup>2</sup>) was calculated based on the label rate. A plastic ring (5 cm diameter and 2 cm height) was placed in the center of a cardboard panel. Twenty-five nymphs (fourth–fifth instars) were 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 dust band. The first 20 nymphs that crossed the band were collected using soft forceps (BioQuip products, Rancho Dominguez, CA; Fig. 1A). The control group was handled in the same manner as the treatment group using untreated cardboard panels. Each petri dish with 20 nymphs represents a replicate. Each treatment was replicated three and four times for Indy and Irvington bed bugs, respectively. All of the dusts were tested against both strains except CimeXa and Cynoff dusts, which were only tested against Irvington strain bed bugs. Treated nymphs were immediately transferred to clean plastic petri dishes (3.8 cm diameter and 1 cm height; Fisher Scientific, Pittston, PA) 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. Time taken to cross the dust band (from touching the inner border of the band until leaving the outer border of the band) was recorded for each replicate of 20 bed bugs. Mortality data were recorded daily until 21 d after treatment. A bed bug was considered dead if there was no movement when it was prodded with forceps.

### Experiment 2. Efficacy of Dusts in Forced Exposure Bioassay

The methodology used in this experiment is similar to what had been used in previous studies, where bed bugs were continuously exposed to dusts in small confinements. This bioassay mimics field conditions where bed bugs do not avoid dust and reside more permanently on the dust treated area. Irvington bed bug nymphs were used. Two inorganic and two pyrethroid dusts that caused high mortality in Experiment 1 were tested: CimeXa, Tempo 1% Dust, MotherEarth D, and Cynoff. Plastic dishes (9 cm diameter and 2.5 cm height; Fisher Scientific, Pittston, PA) with a 2-cm-diameter screened area on the lid were used. Bottoms of the dishes were lined with a filter paper (7.6 cm diameter; VWR International, Arlington Heights, IL). Dust was applied thoroughly on the filter paper at the label rate (total area: 45 cm<sup>2</sup>). Twenty 4th–5th-instar bed bug nymphs were placed in the dishes (Fig. 1B). Control dishes did not receive insecticide dust. Each insecticide dust was replicated four times. Carbon dioxide (CO<sub>2</sub>; Airgas East Inc., Piscataway, NJ) was released at 200 ml/min for 4 h daily in the room to stimulate bed bug



**Fig. 1.** Experimental setup. (A) Brief exposure bioassay. (B) Forced exposure bioassay. (C) Choice exposure bioassay. (D) Horizontal transfer bioassay: a) dust-exposed males placed with unexposed males, and b) dust-exposed males placed with unexposed second–third-instar nymphs.

activity. Bed bug mortality was observed daily until 14 d after treatment.

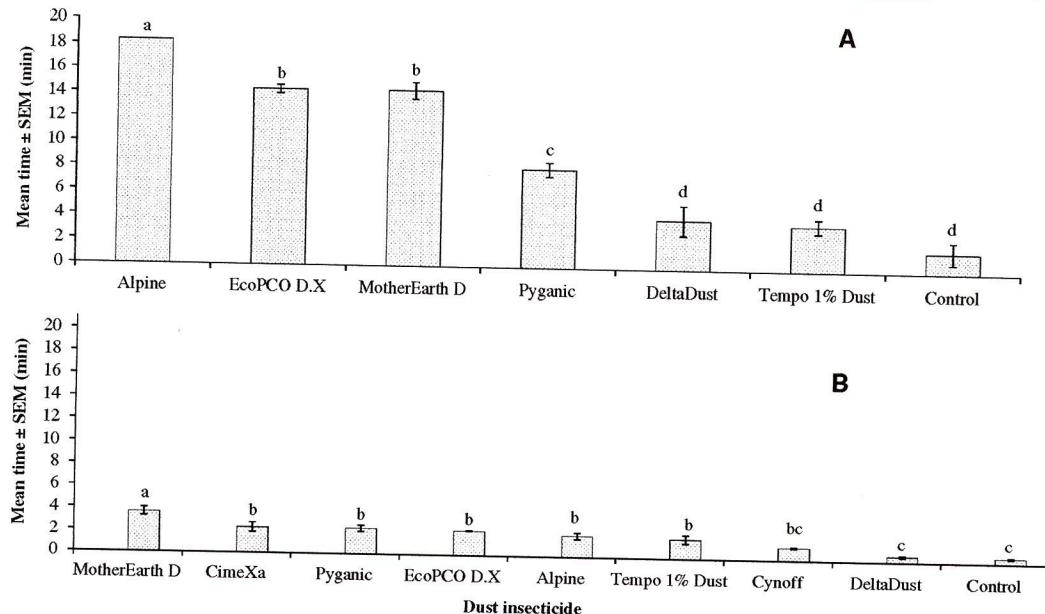
### Experiment 3. Efficacy of Dusts in Choice Exposure Bioassay

This experiment simulated field application of dusts where bed bugs may come in contact with a narrow band of dust multiple times, or avoid the dust deposit, when they have a choice to stay between treated and untreated substrates. Irvington strain bed bug nymphs were used. CimeXa, Tempo 1% Dust, MotherEarth D, and Cynoff dusts were tested. Clear plastic arenas (31 by 21.6 by 10 cm; Pioneer Plastics, Dixon, KY) with four 2-cm-diameter screened ventilation holes on the four corners of lid were used. The bottoms were lined with cardboard (Fig. 1C). A layer of fluoropolymer resin (BioQuip products, Rancho Dominguez, CA) was applied to inner walls of the arenas to prevent bed bugs from escaping. Paper surgical tape (Caring International, Mundelein, IL), 2.54 cm wide, was placed on floor along the perimeter of an arena. The dust was applied on this tape at the label rate (total area: 101 cm<sup>2</sup>) with a fine brush. A folded paper harborage (6 cm long by 5 cm wide) containing bed bug feces (conditioned harborage) was placed in the center of each arena. These conditioned harborages had been used in rearing containers for immature bed bugs, thus mimicked natural harborages with bed bug assembling scents. At 1 h after dark cycle, 50 fourth–fifth-instar nymphs were placed onto the conditioned harborage in the center of a plastic ring (5 cm diameter and 2 cm height) to confine the bed bugs for 30 min. The plastic ring confining the bed bugs

was then removed to initiate the experiment. CO<sub>2</sub> was released as described in Experiment 2. Control arenas received all the materials as test arenas except insecticide dust. Each type of insecticide dust was replicated four times. Bed bug mortality was observed daily until 14 d after treatment. Bed bugs were not fed during the 14-d trial.

### Experiment 4. Efficacy of CimeXa Through Horizontal Transfer

This experiment was conducted to determine whether bed bug adult males exposed to CimeXa dust could transfer dust to unexposed males or nymphs. CimeXa alone was included because only CimeXa dust caused 100% mortality in brief exposure, forced exposure, and choice exposure bioassays. Plastic dishes (5.5 cm diameter and 1.5 cm height) lined with filter paper (5.5 cm diameter) were used. Dust was applied at the label rate on the filter paper (total area: 24 cm<sup>2</sup>). Adult males destined to be used as donors were introduced in petri dishes containing the dust for 5 min. They were then introduced into clean petri dishes along with the recipient (unexposed) bed bugs. Each petri dish contained a folded paper harborage (3 cm long by 1 cm wide) to provide resting and hiding places for bed bugs (Fig. 1D). Three different treatments were used: A) 4 male donors and 6 male recipients, B) 4 male donors and 6 2nd–3rd instar recipients, and C) 1 male donor and 5 male recipients. In the positive control, the same numbers of exposed males used in the three treatments were introduced in petri dishes containing the dust for 5 min and then transferred to clean dishes with paper harborages. No unexposed bed bugs were mixed with the exposed bed bugs.



**Fig. 2.** Mean time to cross dust band. (A) Indy strain bed bugs, and (B) Irvington strain bed bugs. Means with different letters are statistically different ( $P < 0.05$ , Tukey's HSD test).

In the negative control, the same numbers of unexposed bed bugs of the same stage as those in the three treatments were placed in plastic dishes. Five replicates were used for each treatment. Bed bug mortality was observed daily until 14 d after treatment. Dust exposed bed bug males were excluded from the analysis because there was 100% mortality in positive control males.

#### Statistical Analysis

The Abbott (1925) formula was used to calculate corrected mortality. Percentage corrected mortality values were arcsine square root transformed to meet the assumptions of normality and homogeneity of variances. The repeated-measures analysis of the mortality data was done using Mixed model (JMP 2014) to determine differences between dust treatments and their interaction with time. Treatment, interaction day and treatment were included as the main effects in the model. Replicate was included as the random effect. One-way analysis of variance was used to compare the mean time taken by bed bugs for crossing the bands of various dusts and also to compare the time taken to cross dust band by two bed bug strains, to compare mortality in bed bugs among three bioassay methods, and to compare mortality caused by different dusts at only one observation period. When the interactions were significant, Tukey's HSD test ( $\alpha = 0.05$ ) was used to separate the means. All analyses were performed using JMP version 11 (SAS Institute 2012).

## Results

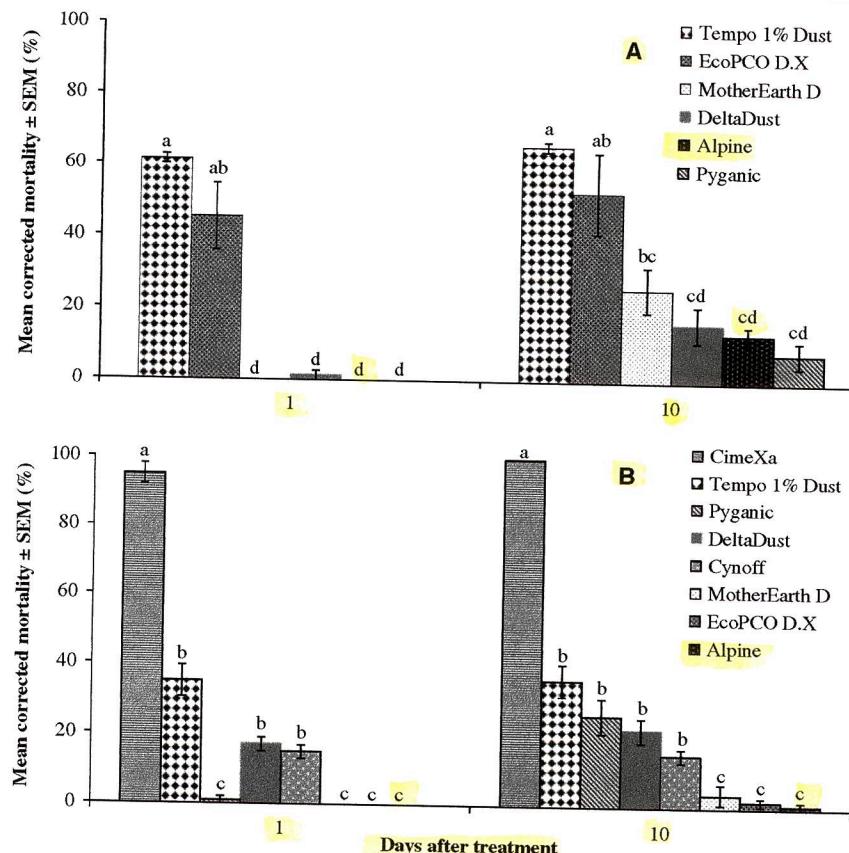
### Efficacy of Dusts in Brief Exposure Bioassay

After removing the confinement rings, bed bug nymphs soon started to move. When bed bugs reached the dust band, they would cross the band in a straight line perpendicular to the band, or followed an irregular path across the band and pass, or turned away from the band. For Indy bed bug nymphs, it took between 1.6–18.3 min for the first 20 bed bugs to cross the dust band (from the first bed bug touching the inner border of the band until the 20th bed bug leaving

the outer border of the band) in all treatments. The mean time for the first 20 bed bugs to cross Alpine, EcoPCO D.X, MotherEarth D, and Pyganic dust bands was significantly longer than the control ( $F = 11.5$ ;  $df = 6, 14$ ;  $P = 0.0001$ ), indicating bed bugs show some avoidance behavior to these dust treatments (Fig. 2A). For Irvington bed bug nymphs, the time taken for the first 20 bed bugs to cross a band was 0.52–3.6 min in all treatments. Among the eight products tested, the mean time for crossing a dust band was significantly longer ( $F = 13.5$ ;  $df = 8, 27$ ;  $P = 0.0001$ ) than the control except for Cynoff and DeltaDust (Fig. 2B).

Indy bed bugs were less active than Irvington bed bugs. Indy bed bug nymphs took more time to cross the bands compared to Irvington bed bug nymphs for all the dusts and control: Tempo 1% Dust ( $F = 12.5$ ;  $df = 1, 5$ ;  $P = 0.01$ ); Pyganic ( $F = 91.5$ ;  $df = 1, 5$ ;  $P = 0.0002$ ); MotherEarth D ( $F = 225.7$ ;  $df = 1, 5$ ;  $P = 0.0001$ ); EcoPCO D.X ( $F = 144.4$ ;  $df = 1, 5$ ;  $P = 0.0001$ ); Alpine ( $F = 414.1$ ;  $df = 1, 5$ ;  $P = 0.0001$ ); DeltaDust ( $F = 48.4$ ;  $df = 1, 5$ ;  $P = 0.0009$ ); and Control ( $F = 15.5$ ;  $df = 1, 5$ ;  $P = 0.01$ ).

Significant differences in bed bug mortality were caused by the six insecticide dusts for the Indy strain ( $F = 14.3$ ;  $df = 5, 12$ ;  $P = 0.0001$ ). At 1 d and 10 d after treatment, efficacy of Tempo 1% Dust and EcoPCO D.X was significantly higher than the other dusts (Tukey's HSD test,  $P < 0.05$ ). The highest mortality caused by the six treatments was  $65.0 \pm 1.6\%$  at 10 d (Fig. 3A). Against Irvington strain, CimeXa dust caused  $95.0 \pm 3.0\%$  and 100% mortality at 1 and 10 d, respectively. It was significantly more effective than all other seven dusts, which caused less than 40.0% mortality ( $F = 9.3$ ;  $df = 7, 24$ ;  $P = 0.0001$ ) (Fig. 3B). Comparing the mortality data between Indy and Irvington bed bug nymphs at 10 d after treatment showed that the following insecticide dusts were significantly more efficacious against Indy nymphs than to Irvington nymphs: Tempo 1% Dust ( $F = 29.2$ ;  $df = 1, 5$ ;  $P = 0.002$ ); Pyganic ( $F = 7.1$ ;  $df = 1, 5$ ;  $P = 0.04$ ); MotherEarth D ( $F = 12.5$ ;  $df = 1, 5$ ;  $P = 0.01$ ), EcoPCO D.X ( $F = 27.9$ ;  $df = 1, 5$ ;  $P = 0.003$ ), and Alpine ( $F = 30.3$ ;  $df = 1, 5$ ;  $P = 0.002$ ).



**Fig. 3.** Efficacy of dust insecticides in a brief exposure bioassay. (A) Indy strain nymphs, and (B) Irvington strain nymphs. Means with different letters are statistically different ( $P < 0.05$ , Tukey's HSD test).

#### Efficacy of Dusts in Forced Exposure Bioassay

Bed bugs tended to stay on treated or untreated filter paper during the observation period. Bed bugs found outside the filter paper were all dead. At 1 d after treatment, significant differences in bed bug mortality were observed among the four insecticide dusts against Irvington bed bug nymphs ( $F = 33.6$ ;  $df = 3, 12$ ;  $P = 0.0001$ ). CimeXa ( $100 \pm 0\%$ ) and Tempo 1% Dust ( $94.0 \pm 2.4\%$ ) were significantly more effective than Cynoff ( $76.3 \pm 2.4\%$ ) and MotherEarth D ( $70.0 \pm 3.5\%$ ; Fig. 4A). At 5 d after treatment, all treatments caused 100% mortality.

#### Efficacy of Dusts in Choice Exposure Bioassay

There were significant differences among the four treatments ( $F = 205.1$ ;  $df = 3, 12$ ;  $P = 0.0001$ ; Fig. 4B). CimeXa ( $96.5 \pm 2.0\%$ ) was significantly more effective than Tempo 1% Dust ( $82.5 \pm 2.2\%$ ), MotherEarth D ( $3.3 \pm 1.1\%$ ), and Cynoff ( $39.5 \pm 4.1\%$ ) at 1 d after treatment. At 10 d after treatment, CimeXa ( $100 \pm 0\%$ ), Tempo 1% ( $97.0 \pm 1.0\%$ ), and MotherEarth D ( $94.0 \pm 2.0\%$ ) caused a similar level of mortality and were significantly more effective than Cynoff ( $75.2 \pm 2.0\%$ ). Mortality by CimeXa was similar at 1 d and at 10 d after treatment, whereas mortality by Tempo 1% Dust, MotherEarth D, and Cynoff was much lower at 1 d compared to that at 10 d after treatment (Fig. 4B).

#### Comparison Among Three Bioassays

For Irvington bed bug nymphs, comparing 10-d mortality data among the three bioassay methods revealed only CimeXa dust caused 100%

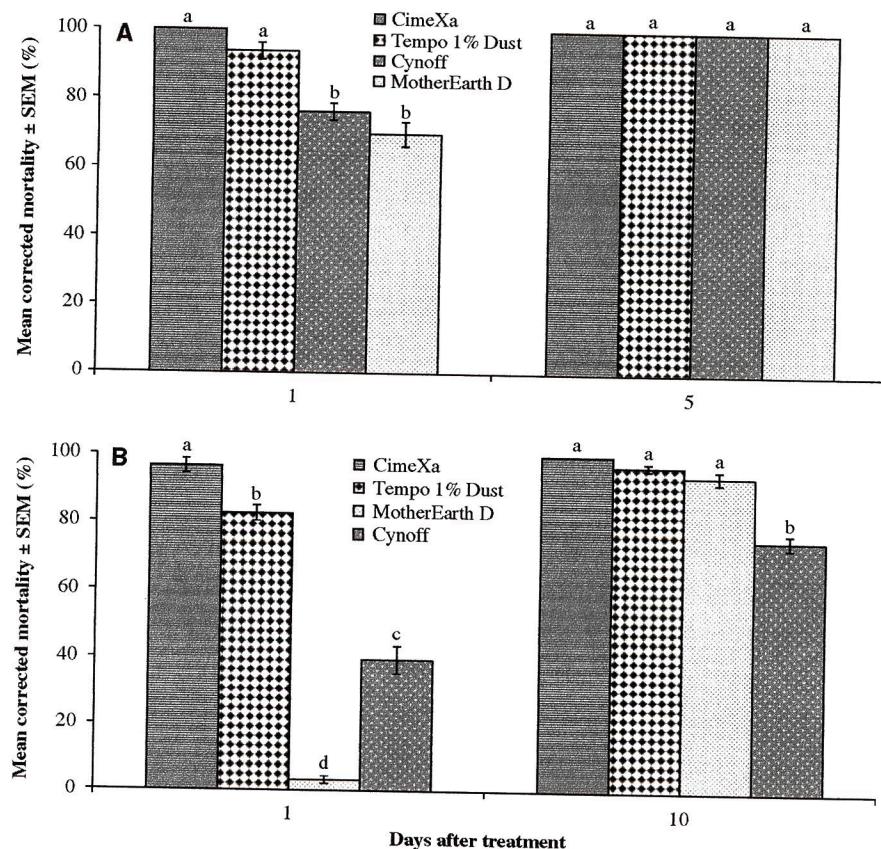
mortality. MotherEarth D ( $F = 725.4$ ;  $df = 2, 9$ ;  $P = 0.0001$ ) and Tempo 1% Dust ( $F = 180.3$ ;  $df = 2, 9$ ;  $P = 0.0001$ ) were much more effective in forced exposure and choice exposure bioassay than the brief exposure bioassay. For Cynoff dust, its efficacy among the three bioassay methods was: forced exposure > choice exposure > brief exposure ( $F = 716.4$ ;  $df = 2, 9$ ;  $P = 0.0001$ ).

#### Efficacy of CimeXa Through Horizontal Transfer Treatment A

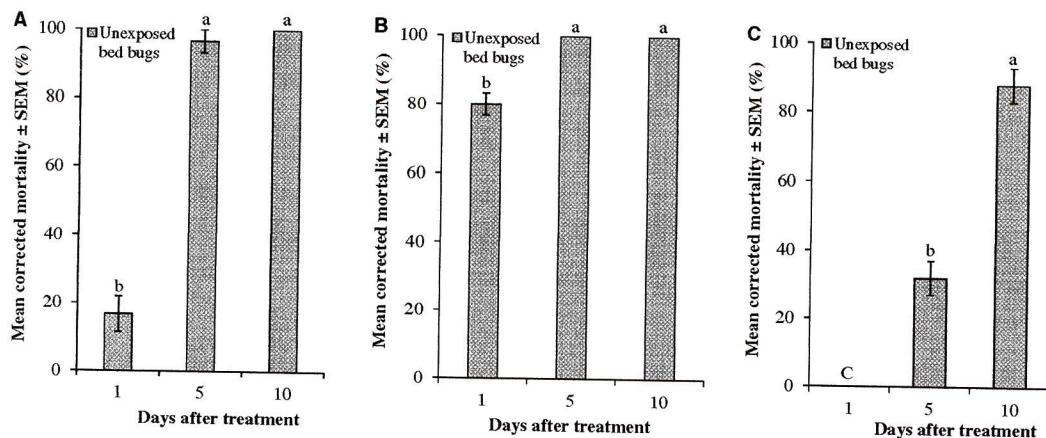
When compared with the negative control (unexposed bed bugs), significant mortality occurred at 1, 5, and 10 d after treatment when six unexposed males were placed with four dust-exposed males ( $F = 267.1$ ;  $df = 2, 16$ ;  $P = 0.0001$ ; Fig. 5A). The mortality at 5 d ( $96.7 \pm 3.3\%$ ) and 10 d ( $100 \pm 0\%$ ) after treatment was significantly higher than that at 1 d ( $16.7 \pm 5.3\%$ ). In the positive control, 100% mortality was recorded at 1 d after treatment. There was no mortality in the negative control at 10 d after treatment.

#### Treatment B

When compared with the negative control (unexposed bed bugs), significant mortality occurred at 1, 5, and 10 d after treatment when six unexposed second–third-instar nymphs were placed with four dust-exposed males ( $F = 10.2$ ;  $df = 2, 16$ ;  $P = 0.0001$ ; Fig. 5B). The mortality at 5 d ( $100 \pm 0\%$ ) and 10 d ( $100 \pm 0\%$ ) was significantly higher than that at 1 d ( $80.2 \pm 3.2\%$ ). In the positive control, 100%



**Fig. 4.** Efficacy of dust insecticides against Irvington strain nymphs. (A) Forced exposure bioassay. (B) Choice exposure bioassay. Means with different letters are statistically different ( $P < 0.05$ , Tukey's HSD test).



**Fig. 5.** Efficacy of CimeXa through horizontal transfer. (A) Four dust-exposed males placed with six unexposed males. (B) Four dust-exposed males placed with six unexposed second-third-instar nymphs. (C) One dust-exposed male placed with five unexposed males. Means with different letters are statistically different ( $P < 0.05$ , Tukey's HSD test). Mortality data were based on unexposed bed bugs.

mortality was recorded at 1 d after treatment. There was no mortality in the negative control at 10 d after treatment.

#### Treatment C

When compared with the negative control (unexposed bed bugs), significant mortality occurred at 5 and 10 d after treatment when

five unexposed males were placed with a single dust-exposed male ( $F = 114.4$ ;  $df = 2, 16$ ;  $P = 0.0001$ ; Fig. 5C). The mortality at 10 d ( $88.0 \pm 5.0\%$ ) was significantly higher than that at 5 d ( $32.0 \pm 5.0\%$ ). In the positive control, 100% mortality was recorded at 1 d after treatment. There was no mortality in the negative control at 10 d after treatment.

## Discussion

Our results provide scientific basis for using different types of laboratory bioassays for the evaluation of dust insecticides as a bed bug management tool. We used three different exposure methods for testing insecticide dusts to simulate field environment conditions where bed bugs may be exposed to dust applications, directly or indirectly, and where bed bugs can choose to avoid treated substrates or interact with them periodically over time. The short exposure bioassay resembles a perimeter application in which a narrow band of dust is applied to the floor perimeters in the rooms. Bed bugs hiding at the room perimeter may only be briefly exposed to the pesticide as they cross the dust band in order to access the host. In the short exposure bioassay, CimeXa dust caused 95.0% mortality at 1 d after treatment compared to <40.0% mortality for all other dusts. This demonstrates the rapid kill of CimeXa and suggests the bioassay is very sensitive for identifying effective insecticide products. CimeXa has the most potential of being effective under field conditions.

The mean time taken to cross dust bands in the brief exposure bioassay was significantly longer in Indy bed bug nymphs compared to Irvington bed bug nymphs. This could be due to the fact that Indy bed bugs had been kept in the laboratory for six years, while the Irvington bed bugs were collected three months prior to bioassays. Recently field-collected bed bugs were found to be more active and responsive to CO<sub>2</sub> stimulus and chemical lures than laboratory kept colonies (Wang et al., unpublished data).

The DE performed poorly in the brief exposure bioassay. Potter et al. (2013a) also found that the effects of DE are greatly reduced by abbreviated exposure to treated surfaces. Similar to our results, DE was less effective than silica gel when bed bugs were exposed briefly to dust deposits (Potter et al. 2014). Poor efficacy following short term exposure to DE also is reported by Benoit et al. (2009). However, in forced and choice exposure experiments, DE caused 100 and 94.0% mortality, respectively. This suggests that DE still can be effective under field conditions if applied thoroughly throughout in locations where bed bugs may experience continuous or repeated exposures over time.

In the forced exposure bioassay, all dusts killed 100% of the bed bugs at 5 d after treatment, indicating the high insecticidal efficacy of insecticide dusts against bed bugs. In forced exposure and choice bioassays, CimeXa and Tempo 1% Dust were significantly more effective than MotherEarth D and Cynoff at 1 d after treatment, suggesting that besides CimeXa, Tempo 1% Dust is potentially effective under field conditions.

Horizontal transfer of DE dust has already been reported in bed bugs (Akhtar and Isman 2013). For the first time, we showed silica gel is effective when it is horizontally transferred from dust exposed bed bugs to unexposed bed bugs. Because horizontal transfer can facilitate the spread of insecticide into hard to reach spaces, it could contribute greatly to the success of the treatment programs using silica gel.

Understanding the mode of action of each insecticide dust is very crucial to understand why some insecticide dusts were more effective than others against bed bugs. Pyrethroids (Tempo 1% Dust, DeltaDust, Cynoff) and pyrethrins (Pyganic, EcoPCO D.X) are sodium channel modulators that causes paralysis of the insect (IRAC 2016). Neonicotinoids, such as Alpine dust, mimic the agonist action of acetylcholine at nicotinic acetylcholine receptors (Nauen and Bretschneider 2002, Liu et al. 2005) that leads to overstimulation of the nervous system causing involuntary muscle contraction, cessation of feeding, paralysis, and death (Bloomquist 2009, Thompson et al. 2009). Inorganic insecticides such as MotherEarth D and

CimeXa cause desiccation and death by removing the ultra-thin, protective layer of wax from the cuticle of an insect (Potter et al. 2014). Synergists, such as piperonyl butoxide, are P450-dependent monooxygenase inhibitors that suppress the detoxification mechanism of insects and thus increase the toxicity of an insecticide (IRAC 2016). Romero et al. (2007) reported dust formulations containing silica gel or DE are effective against pyrethroid-resistant bed bugs. This may be the reason that CimeXa was the most effective dust against moderately resistant Irvington bed bugs. Some pyrethroids dusts such as Tempo 1% dust and Cynoff were also effective when bed bugs were exposed to dust continuously or multiple times. This shows that pyrethroid dusts can still be used effectively against resistant bed bugs.

Most insecticide sprays lack residual activity against bed bugs, and therefore require reapplication to achieve direct contact with bed bugs that may have been missed in previous treatments. Repeated applications are also needed to kill nymphs emerging from eggs (Potter et al. 2013b, Akhtar and Isman 2013). Dust insecticides can provide higher residual control than insecticide sprays. Potter et al. (2014) suggest that dust formulations containing silica gel retain their potency for months to years when applied into wall, cabinet voids, under baseboard, and the inner framework of box springs and sofas. This property, along with the results from our study, supports the use of silica gel in bed bug management programs.

Recent studies have shown that bed bugs of all developmental stages travel extensively within and between apartments within a building (Wang et al. 2010, 2012; Potter et al. 2013c; Cooper et al. 2015). Within apartments, bed bugs travel to host sleeping areas and in areas away from host sleeping and resting sites. Genetic studies also have revealed that spread of infestations in multi-unit buildings is due to active dispersal more often than repeated introduction by building residents (Booth et al. 2012). In such a scenario, applying silica gel dust around the floor perimeter of apartments as a preventative measure can help reduce introductions from neighbors within multiunit buildings especially for underserved communities that have limited resources to combat bed bug infestations. Our results support the findings by Potter et al. (2014) and suggest that CimeXa has high potential to be a cost-effective material in the management of bed bugs. Further field testing is warranted to confirm its effectiveness.

## Acknowledgements

This project was supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Agriculture Hatch project 1001098 through the New Jersey Agricultural Experiment Station, Hatch project NJ08127. The product manufacturers had no role in designing or sponsoring this study. The authors declare no conflict of interest. This is New Jersey Experiment Station publication # D-08-08127-10-15.

## References Cited

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.
- Akhtar, Y., and M. B. Isman. 2013. Horizontal transfer of diatomaceous earth and botanical insecticides in the common bed bug, *Cimex lectularius* L.; Hemiptera: Cimicidae. PLoS ONE 8: e75626.
- Anderson, J. F., and R. S. Cowles. 2012. Susceptibility of *Cimex lectularius* (Hemiptera: Cimicidae) to pyrethroid insecticides and to insecticidal dusts with or without pyrethroid insecticides. J. Econ. Entomol. 105: 1789–1795.
- Benoit, J. B., S. A. Phillips, T. J. Croxall, B. S. Christensen, J. A. Yoder, and D. L. Denlinger. 2009. Addition of alarm pheromone components improves

- the effectiveness of desiccant dusts against *Cimex lectularius*. J. Med. Entomol. 46: 572–579.
- Bloomquist, J. R. 2009. Insecticides: chemistries and characteristics. In E. B. Radcliffe, W. D. Hutchinson and R. E. Cancelado (eds.), Radcliffe's IPM world textbook. University of Minnesota, St. Paul, Minnesota.
- Booth, W., V. L. Saenz, R. G. Santangelo, C. Wang, C. Schal and E. L. Vargo. 2012. Molecular markers reveal infestation dynamics of the bed bug (Hemiptera: Cimicidae) within apartment buildings. J. Med. Entomol. 49: 535–546.
- Cooper, R., C. Wang, and N. Singh. 2015. Mark-release-recapture reveals extensive movement of bed bugs (*Cimex lectularius* L.) within and between apartments. PLoS ONE 10: e0136462.
- Gordon, J. R., M. H. Goodman, M. F. Potter, and K. F. Haynes. 2014. Population variation in and selection for resistance to pyrethroid-neonicotinoid insecticides in the bed bug. Sci. Rep. 4. doi:10.1038/srep03836.
- IRAC. 2016. Insecticide resistance action committee mode of action working group IRAC MoA classification scheme, version 8.1. (<http://www.irac-online.org/documents/moa-classification/?ext=pdf>) (accessed 15 April 2016).
- JMP. 2014. Analyzing repeated measures data in JMP® software (<http://www.jmp.com/support/notes/30/384.html>) (accessed 12 June 2015).
- Liu, Z., M. S. Williamson, S. J. Lansdell, I. Denholm, Z. Han, and N. S. Millar. 2005. A nicotinic acetylcholine receptor mutation conferring target-site resistance to imidacloprid in *Nilaparvata lugens* (brown planthopper). Proc. Natl. Acad. Sci. USA 102: 8420–8425.
- Nauen, R., and T. Bretschneider. 2002. New modes of action of insecticides. Pestic. Outlook 13: 241–245.
- Potter, M. F. 2008. The business of bed bugs. 2008. Pest Manage. Prof. 76: 24–25, 28–32, 34, 36–40.
- Potter, M. F., K. F. Haynes, A. Romero, E. Hardebeck, and W. Wickenmeyer. 2008. Is there a new answer? Pest Control Technol. 36: 116, 118–124.
- Potter, M. F., K. F. Haynes, J. Fredericks, and M. Henriksen. 2013a. Bed bug nation: are we making any progress? Pestworld 5: 5–11.
- Potter, M. F., K. F. Haynes, C. Christensen, T. J. Neary, C. Turner, and L. Washburn. 2013b. Diatomaceous Earth: Where do bed bugs stand when the dust settles? Pest Control Technol. 41: 72–74, 76–78, 80.
- Potter, M. F., J. R. Gordon, M. H. Goodman, and T. Hardin. 2013c. Mapping bed bug mobility. Pest Control Technol. 41: 72–74, 76, 78, 80.
- Potter, M. F., K. F. Haynes, and J. R. Gordon. 2014. Silica gel: a better bed bug desiccant. Pest Control Technol. 42: 78–94.
- Romero, A., M. F. Potter, D. A. Potter, and K. F. Haynes. 2007. Insecticide resistance in the bed bug: a factor in the pest's sudden emergence? J. Med. Entomol. 44: 175–178.
- Romero, A., M. F. Potter, and K. F. Haynes. 2009a. Bed bugs: Are dusts the bed bug bullet? Pest Manag. Prof. 77: 22, 23, 26, 28, 30.
- Romero, A., M. F. Potter, and K. F. Haynes. 2009b. Behavioral responses of the bed bug to insecticide residues. J. Med. Entomol. 46: 51–57.
- Romero, A. 2011. Moving from the old to the new: insecticide research on bed bugs since the resurgence. Insects 2: 210–217.
- SAS Institute. 2012. JMP statistics and graphics guide, version 11 of JMP. SAS Institute, Cary, NC.
- Singh, N., C. Wang, and R. Cooper. 2013. Effectiveness of a reduced-risk insecticide based bed bug management program in low-income housing. Insects 4: 731–742.
- Stedfast, M. L., and D. M. Miller. 2014. Development and evaluation of a proactive bed bug (Hemiptera: Cimicidae) suppression program for low-income multi-unit housing facilities. J. Int. Pest Manag. 5(3): E1–E7. doi: <http://dx.doi.org/10.1603/IPM14003>.
- Wang, C., M. Abou El-Nour, and G. W. Bennett. 2007. Controlling bed bugs in apartments-A case study. Pest Control Technol. 35: 64, 66, 68, 70.
- Wang, C., T. Gibb, and G. W. Bennett. 2009. Evaluation of two least toxic integrated pest management programs for managing bed bugs (Heteroptera: Cimicidae) with discussion of a bed bug intercepting device. J. Med. Entomol. 46: 566–571.
- Wang, C., K. Saltzmann, E. Chin, G. W. Bennett, and T. Gibb. 2010. Characteristics of *Cimex lectularius* (Hemiptera: Cimicidae), infestation and dispersal in a high-rise apartment building. J. Econ. Entomol. 103: 172–177.
- Wang, C., K. Saltzmann, T. Gibb, and G. Bennett. 2012. Comparison of three bed bug management strategies in a low-income apartment building. Insects 3: 402–409.
- Wang, C., N. Singh, R. Cooper, C. Liu, and G. Buczkowski. 2013. Evaluation of an insecticide dust band treatment method for controlling bed bugs. J. Econ. Entomol. 106: 347–352.
- Wang, C., N. Singh, and R. Cooper. 2015. Field study of the comparative efficacy of three pyrethroid/neonicotinoid mixture products for the control of the common bed bug, *Cimex lectularius*. Insects 6: 197–205.
- 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 U.S. Arch. Insect Biochem. Physiol. 73: 245–257.
- Zhu, F., H. Gujar, J. R. Gordon, K. F. Haynes, M. F. Potter, and S. R. Palli. 2013. Bed bugs evolved unique adaptive strategy to resist pyrethroid insecticides. Sci. Rep. 3. doi:10.1038/srep01456.