

Susceptibility of *Cimex lectularius* (Hemiptera: Cimicidae) to Pyrethroid Insecticides and to Insecticidal Dusts With or Without Pyrethroid Insecticides

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ABSTRACT Relative increases of bed bug, *Cimex lectularius* L., populations are probably due in large measure to their resistance to pyrethroids, which have been used extensively against urban pests. A Connecticut population of bed bugs was assessed for sensitivity to pyrethroids and exposed to commonly-used commercial insecticides applied to various substrates on which the residues were allowed to age for 0–24 wk. Type I and type II pyrethroids differed in toxicity when applied at a high dosage (1 µg) per bed bug. Some type II pyrethroids (cyfluthrin, λ -cyhalothrin, *cis*-cypermethrin, and deltamethrin) caused >80% mortality, whereas exposure to type I pyrethroids caused <5% mortality over 72 h (with one exception, pyrethrins caused 23% mortality). Dust products were not affected by residue aging; mortality response over time of exposure closely fit ($R^2 > 0.95$) an exponential rise to a maximum model from which the survival half-life ($S_{1/2}$) was calculated directly. Tempo Dust (Bayer Environmental Science, Montvale, NJ) killed bed bugs relatively quickly, as did Syloid 244 (Grace Davison, Columbia, MD) and Drione (Bayer Environmental Science, Montvale, NJ) on hardboard and mattress fabric substrates ($S_{1/2} < 1$ d); DeltaDust (Bayer Environmental Science, Montvale, NJ) provided a relatively slow kill ($S_{1/2} \approx 3.5$ d). The sprayable pyrethroids, Cyonara 9.7 (Insecticide Control solutions, Pasadena, TX) and D-Force HPX Aerosol 0.06% (Waterbury Companies, Waterbury, CT), displayed reduced residual toxicity as they aged; the mortality was <50% on some substrates after 4 d. Desiccant dusts, with their physical mode of action and long residual activity, appear to be superior to sprayable pyrethroid products for killing bed bugs.

KEY WORDS bed bug, desiccant dust, pyrethroid, resistance, *Cimex lectularius*

Relative increases of bed bug, *Cimex lectularius* L., populations have been reported in the United States, Canada, and elsewhere in recent years (Hwang et al. 2005, Potter 2005, Doggett 2007, Pinto et al. 2007). Contact and residual insecticides are used extensively in apartments and other infested buildings in control efforts, including integrated pest management programs (Romero et al. 2007, Moore and Miller 2009, Wang et al. 2009). Effectiveness of these efforts, even with the reapplication of specific insecticides and other treatments, is often less than satisfactory (Moore and Miller 2009, Wang et al. 2009). Resistance of bed bugs to pyrethroids, which currently are used extensively for control of bed bugs and other urban pests, is a serious challenge to reducing populations (Romero et al. 2007, Yoon et al. 2008, Seong et al. 2010, Zhu et al. 2010, Adelman et al. 2011). To improve our

understanding of effective methods for controlling bed bugs, we measured susceptibility of a locally collected population of bed bugs to six type I and seven type II pyrethroids and to five insecticides currently registered for use in controlling bed bugs in the United States. Drione (pyrethrins and piperonyl butoxide formulated on a silica aerogel dust) was compared with its dust formulation ingredient. We also examined the residual activity of formulated products applied to three different substrates commonly found in the interior of houses.

Materials and Methods

Bed Bug Colony. Bed bugs collected in New Haven, CT, from an apartment building in 2007 were sustained using New Zealand white rabbits as hosts (Davis 1956). Rabbits were maintained in accordance with procedures approved by the Animal Care and Use Committee at The Connecticut Agricultural Experiment Station.

Pyrethroid Screening Trial. Pyrethroids tested consisted of *d-trans*-allethrin (90%, MGK Corp., Minneapolis, MN); cyfluthrin (Baythroid 50.2%, Bayer Corporation, Kansas City, MO); λ -cyhalothrin (98%,

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Zeneca Ag Products, Richmond, CA); *cis*-cypermethrin (99.3%, origin unknown); *trans*-cypermethrin (99.1%, origin unknown); deltamethrin (99.9%, Ultra Scientific, Kingston, RI); fenpropathrin (100%, USEPA); fenvalerate (99.4%, Shell Chemical, Houston, TX); permethrin (94.4%, FMC); d-phenoxyphenothrin (6% *cis*, 92% *trans*, Chem Service, West Chester, PA); pyrethrins (29.8% PY I, 22.2% PY II, Chem Service); resmethrin (Penick Corp., Lyndhurst, NJ); and tetramethrin (98.9%, FMC Corporation, Middleport, NY). Pyrethroids were analytical standards obtained from the Department of Analytical Chemistry at the Connecticut Agricultural Experiment Station, New Haven, CT. Each product was weighed (1–10 mg) with an analytical balance with 0.2-mg sensitivity and then diluted in 1 ml of histological grade acetone (Fisher, Pittsburgh, PA). This concentrate then was diluted further with acetone to obtain a 1,000-ppm standard solution.

Unfed adult bed bugs (five males and five females in each replicate, with three replicates) were held individually in separate wells of 13- by 8.5-cm untreated plastic 24-well flat bottom tissue culture plates (Corning, Inc., Lowell, MA); the bottom of each well was lined with a 15-mm-diameter filter paper. Sides of the wells were coated with Fluon (Asahi Glass Co., Charlotte, NC) to guarantee that bed bugs could not escape the enclosure. Bed bugs were maintained at room temperature (23°C) and a photoperiod of 16:8 [L:D] h. This test was conducted as a 14 by 2 factorial design, with the first factor being the pyrethroid treatment, and the second factor being exposure to piperonyl butoxide (Exponent Insecticide Synergist, 72% piperonyl butoxide, MCK Corp., Minneapolis, MN). Products were applied to the dorsum of the thorax as 1 µl of 1,000-ppm solution in acetone (1 µg of active ingredient per bed bug), by using a repeating dispenser (model PB-600, Hamilton Co., Reno, NV). Bed bugs receiving both insecticide and synergist were treated with synergist first (1 µl of 1,000-ppm solution in acetone), a few minutes before treatment with pyrethroids. Controls in each replicate received 1 µl of acetone or piperonyl butoxide in acetone applied to the dorsum of the thorax. Bed bugs were maintained at room temperature (23°C) and a daily photoperiod of 16:8 (L:D) h for 72 h before recording mortality.

Percent mortality from each group of 10 individuals was subjected to analysis of variance (ANOVA) as a randomized complete block experiment, blocked by treatment date (Analytical Software 2008). Two analyses were conducted. One analysis ignored pyrethroid type classification, which resulted in a 14 by 2 factorial design, and allowed comparisons among pyrethroids. The other analysis compared type I and type II pyrethroids in a 2 by 2 factorial design, essentially averaging over pyrethroid active ingredients within each pyrethroid type. The acetone and piperonyl butoxide controls were excluded from these analyses, because of their lack of either type I or II pyrethroid ingredient.

Exposure of Bed Bugs to Registered Insecticides. Ten adult unfed bed bugs (five males and five females) were used in each treatment. All treatments were

replicated three times. Bed bugs were maintained at room temperature (23°C) and a daily photoperiod of 16:8 (L:D) h.

Insecticides, formulated as liquids or dusts and labeled for control of bed bugs, were applied to three different substrates. The two liquid insecticides, trade names, formulations, and sources used in the residual tests were *λ*-cyhalothrin, (Cyonara 9.7 Insecticide, Control solutions, Pasadena, TX) and deltamethrin, (D-ForceHPX, aerosol 0.06%, Waterbury Companies, Waterbury, CT). The three dust insecticides, trade names, formulations, and sources used were cyfluthrin, (Tempo 1% Dust, Bayer Environmental Science, Montvale, NJ); deltamethrin (DeltaDust 0.05%, Bayer Environmental Science, Montvale, NJ); pyrethrins, (Drione Insecticide, 1% pyrethrins, 10% piperonyl butoxide, 40% amorphous silica gel, Bayer Environmental Science, Montvale, NJ.); and silica aerogel dust (Sylloid 244, Grace Davison, Columbia, MD), provided by Bayer Environmental Science as the dust formulation ingredient of Drione.

The three substrates were 4- by 4-cm hardboard panels, 4- by 4-cm mattress fabric, and 15-mm-diameter filter paper discs. Dusts were placed on a No. 20 soil sieve (Fisher, Pittsburgh, PA) and tapped lightly to evenly distribute the particles over the substrates. Similar volumes of dust were applied to surfaces, but product density varied. The weights (mean ± SE) of products applied were 8.9 ± 0.9, 9.8 ± 0.9, 3.3 ± 0.5, and 8.4 ± 0.9 mg/cm² for DeltaDust, Drione, Sylloid 244, and Tempo Dust, respectively. Liquids were applied by using label directions: for the aerosol D-Force, the can was held 30 cm from the surface and sprayed for 5 s over a 900-cm² area, to the point of run off. The Cyonara product was diluted in water to form a 0.03% (active ingredient) concentration and sprayed with an atomizer (model BRF4AB, Specialty Bottle, Seattle, WA) to a similar degree of saturation as for D-Force.

Bed bugs first were exposed 1 d after treatment of substrates (week 0), and then at 2, 4, 8, 16, and 24 wk postapplication. Treated substrates were kept in translucent covered deli cups at room temperature (23°C) and a photoperiod of 16:8 (L:D) h. A group of 10 bed bugs was placed in the center of the each treated mattress fabric or hardboard. The bottom of a Fluon-treated Falcon petri dish (35 mm by 10 mm) (Becton Dickinson Co., Franklin Lakes, NJ) was placed over the bed bugs and held in place with rubber bands to ensure their contact with the substrate. Bed bugs were confined to the treated filter paper disks by applying Fluon to the walls of the 24-well plastic tissue culture plates in which the filter paper disks were placed; one bug resided in each well. Mortality was recorded daily for up to 13 d after exposure. Ten bed bugs were placed on acetone-treated substrates as negative controls for each replicate.

Data were analyzed first by comparing treatments within an experimental series (those products that were tested concurrently) via randomized complete block design ($n = 3$ trials) factorial (insecticide × substrate) ANOVA (Analytical Software 2008), using

mortality at 13-d exposure as a single end-point measure of efficacy.

Each insecticide was further analyzed separately by logistic regression and analysis of deviance, using the proportion mortality as the dependent variable and residue age and duration of exposure of bed bugs to residues as independent variables. The deviance from the full regression model (including intercept, residue age, and days of exposure) was compared with regression models lacking residue age effect, duration of exposure effect, or both (the null model) to calculate one and two degree of freedom χ^2 statistics to test significance of these effects (Analytical Software 2008). Mortality data for materials unaffected by residue aging (dusts) were combined over residue age to model the influence of exposure duration on mortality via nonlinear regression. Cumulative percent mortality over time was fit with nonlinear regression to a model of exponential rise to a maximum, $Y_0 + 100*(1 - e^{-bx})$ (SPSS 1997) to obtain the exponential coefficient parameter "b," which then was converted to express the survival half-life for exposed bed bugs ($S_{1/2} = \ln(0.5)/-b$). The response for mortality of bed bugs exposed to sprayable products relative to residue age and days of exposure was more complex. To simplify analysis, the proportion of dead bed bugs after 13-d exposure for sprayable products was subjected to a homogeneity of regression test after linear regression of logit-transformed data (excluding zeros and ones) to determine whether residue aging effects differed among substrates (Analytical Software 2008).

Results

Pyrethroid Screening Trial. There were highly significant differences among pyrethroids in their toxicity to bed bugs ($F = 44.8$, df = 12, 48; $P < 0.0001$) (Table 1). Type II pyrethroids were more toxic than type I pyrethroids (63 ± 4.5 and $9.7 \pm 4.8\%$ mortality [mean \pm SE], respectively) at the relatively high dosage used in the screening trial (linear contrast Scheffé $F = 25.5$; df = 1, 48; $P < 0.0001$). However, not all type II pyrethroids were toxic; 20 and 3% of the tested population of bed bugs died after exposure to fenpropathrin and fenvalerate, respectively. Bed bug mortalities of 87, 90, 83, and 95% were recorded for cyfluthrin, λ -cyhalothrin, *cis*-cypermethrin, and deltamethrin, respectively, when not jointly exposed to piperonyl butoxide. The addition of piperonyl butoxide significantly increased mortality ($F = 9.32$; df = 1, 48; $P < 0.01$).

Efficacy of Registered Pesticides. Mortality in check groups in trials of D-Force and Drione was $6.9 \pm 1.6\%$ (mean \pm SE). Mortality in check groups in trials of the remaining products was $3.9 \pm 1.1\%$. Mortality of bed bugs was influenced by duration of exposure to insecticide residues ($\chi^2 = 3.85$, df = 1, $P = 0.05$ for Tempo Dust; $\chi^2 > 25$, df = 1, $P < 0.0001$ for all other insecticides). The liquid formulations of D-Force and Cyonara were less toxic than the four dust formulations (two separate trials: $F = 210$; df = 1, 10; $P < 0.0001$; and $F = 245$; df = 1, 22; $P < 0.0001$). Sprayable

Table 1. Percent mortality 72 h following topical exposures of bed bugs to 1 μ g of technical insecticides with or without piperonyl butoxide (PB)

Treatment	Mortality (%) \pm SE ^a	
	No PB	With PB
Type I Pyrethroids		
<i>d</i> -trans-allethrin	3 \pm 3c	3 \pm 3c
Permethrin	0 \pm 0c	20 \pm 15bc
<i>d</i> -phenothrin	3 \pm 3c	7 \pm 7c
Pyrethrins	23 \pm 19bc	37 \pm 20bc
Resmethrin	3 \pm 3c	7 \pm 7c
Tetramethrin	0 \pm 0c	7 \pm 7c
Type II Pyrethroids		
Cyfluthrin	87 \pm 9a	97 \pm 3a
λ -cyhalothrin	90 \pm 6a	100 \pm 0a
<i>cis</i> -cypermethrin	83 \pm 3a	83 \pm 3a
<i>trans</i> -cypermethrin	33 \pm 9bc	53 \pm 12ab
Deltamethrin	95 \pm 5a	100 \pm 0a
Fenpropathrin	20 \pm 12bc	37 \pm 4bc
Fenvalerate	3 \pm 3c	23 \pm 7bc

There was no mortality in acetone and piperonyl butoxide controls. There were 10 bed bugs per replicate ($n = 3$; except for deltamethrin, $n = 2$).

^a Means within the table followed by the same letter do not significantly differ, Tukey's HSD, $P < 0.05$. Acetone and PB check excluded from factorial analysis from which the mean separations were provided.

pyrethroids displayed reduced residual toxicity as they aged ($\chi^2 = 142$ and 32.4, df = 1, $P < 0.0001$ for Cyonara and D-Force, respectively), and even with freshly sprayed residues, the mortality was <50% on some substrates after at least 4 d of continual bed bug exposure (Cyonara on paper, D-Force on all substrates, Fig. 1).

Mortality from D-Force residues on filter paper was greater than that seen from residues on hardboard or mattress fabric (Fig. 1) as revealed by comparison of elevations in the homogeneity of regression test ($F = 4.30$; df = 2, 35; $P = 0.02$), but activity diminished in a similar manner on all three substrates during the 24-wk testing period (comparison of slopes, $F = 0.24$; df = 2, 33; $P = 0.79$). Cyonara activity diminished with time, as determined through linear regression on logit-transformed proportion mortality (age effect T value = 4.99, $P < 0.0001$). Cyonara was less active on filter paper than on the other two substrates (comparison of elevations, $F = 7.8$; df = 1, 16; $P = 0.012$ and $F = 16.6$; df = 1, 15; $P = 0.001$ for comparisons of filter paper with fabric and hardboard, respectively). Residues on hardboard had measurable toxicity 0–16 wk after treatment but were relatively ineffective when applied to paper, even at 0 wk after treatment.

Logistic regression analyses revealed that dust formulation efficacy was not significantly affected by residue aging ($\chi^2 < 1.0$, df = 1, $P > 0.3$ for all four dusts tested), and so mortality response was combined over residue age groups for further analyses. The cumulative percent mortality related to days of exposure for dust products was consistent with model [$Y = Y_0 + 100(1 - e^{-bx})$] for an exponential rise to a maximum (Fig. 2; Table 2). Nonlinear regression modeling was constrained to have the maximum value defined as 100%. This model describes first order kinetics, in

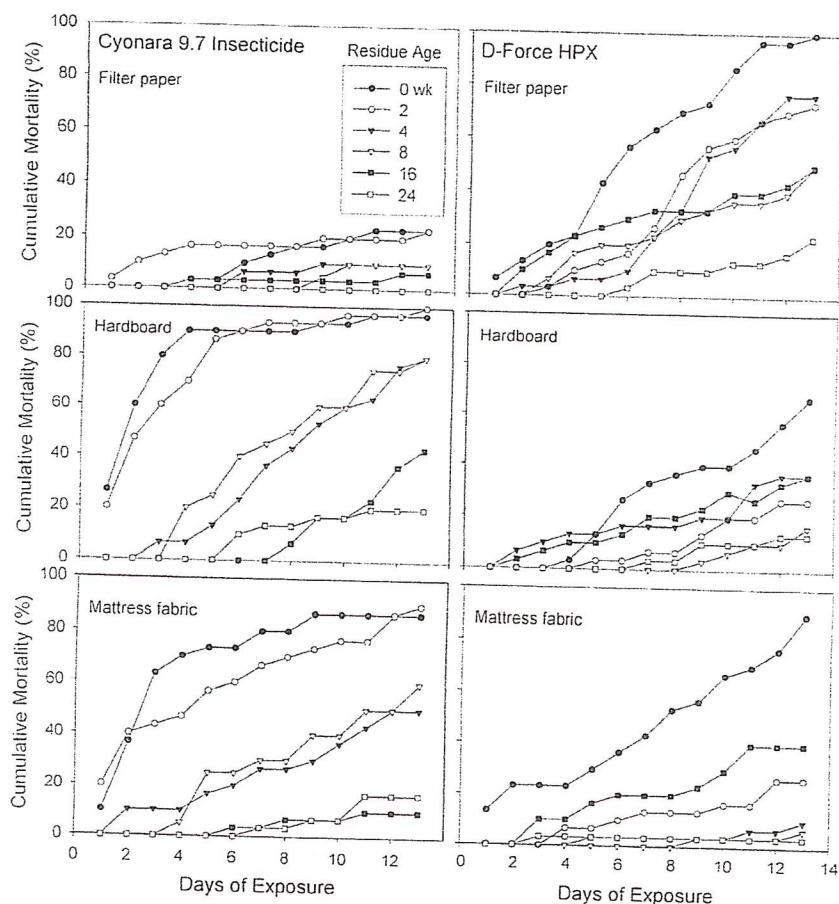


Fig. 1. Cumulative mortality responses on three substrates for bed bugs continuously exposed to various ages of residues of sprayable pyrethroid insecticides. Left column, Cyonara (λ -cyhalothrin); right column, D-Force (deltamethrin). Data are averaged over three replicates of 10 bed bugs each.

which a constant proportion of bed bugs died for each time interval. The dust products Drione, Tempo Dust, and Syloid 244 applied to hardboard and mattress fabric substrates killed most bed bugs (often 100%) within 48 h, even within 24 h (Fig. 2A). Drione exposure resulted in mortality similar but slightly slower than for Syloid 244 (Table 2). DeltaDust was also relatively effective, but bed bugs took longer to die when exposed to this product compared with the other dusts ($F = 12.4$; $df = 2, 16$; $P < 0.001$) (Table 2, Fig. 2B).

For Tempo Dust, there were no differences because of substrates ($F = 2.65$; $df = 2, 34$; $P = 0.08$), and so all data have been combined over age of residues and substrates (Table 2). There were significant differences because of substrates in determining bed bug survivorship half-life with the other dusts. For example, with DeltaDust, the hardboard had the fastest overall mortality, mattress fabric slightly slower, and filter paper the slowest ($F = 6.27$; $df = 2, 10$; $P = 0.017$). Exposure of bed bugs to residues on filter paper also

resulted in the slowest mortality for Drione and Syloid 244 (Table 2).

Discussion

Pyrethrins have been used to kill bed bugs since the mid-1800s (Potter 2011), but field-collected bed bugs in relatively recent years have been documented with pyrethroid resistance (Moore and Miller 2006, Romero et al. 2007, Yoon et al. 2008, Seong et al. 2010, Zhu et al. 2010, Adelman et al. 2011). Pyrethroid insecticides are often classified as type I and type II: type II pyrethrroids are primarily classified as such by the presence of an α -cyano group on the alcohol component of the pyrethroid ester, but they also can be classified based on mammalian and insect toxicology symptoms (Soderlund and Bloomquist 1989, Soderlund et al. 2002). Type II pyrethrroids tend to be more toxic and are more slowly metabolized in insects (Soderlund et al. 2002, Schleier and Peterson 2012). None of the six type I pyrethrroids measured in our

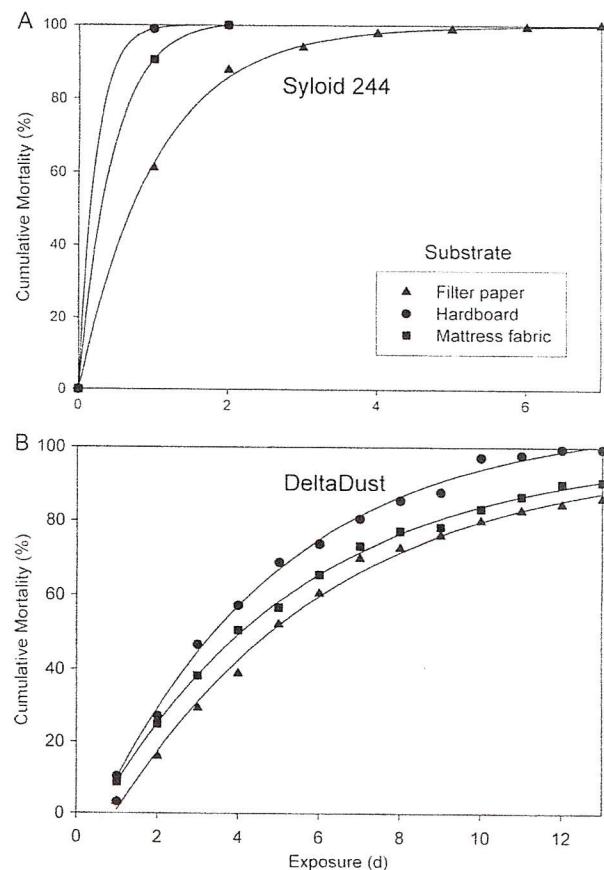


Fig. 2. Representative cumulative mortality responses of bed bugs continuously exposed to dust insecticides. A, Syloid 244 (silica aerogel dust); B, DeltaDust. Lines fitted through data are nonlinear regression results for the model: $Y = Y_0 + 100(1 - e^{-bx})$. Data are averaged over six residue age groups and three replicates of 10 bed bugs each.

study were highly toxic to the tested population of bed bugs. Four of the seven type II pyrethroids that were tested resulted in an 83% or greater mortality, although three of the type II pyrethroids were signifi-

cantly less toxic. Interestingly, two of these three less toxic type II pyrethroids, fenpropathrin and fenvalerate, have been classified according to their toxicity (versus structural) characteristics as intermediate pyrethroids, showing both type I and type II characteristics (Cammon et al. 1981, Scott and Matsumura 1983). Possibly the receptor binding similarities between these two pyrethroids and the type I pyrethroids may contribute to their poor activity.

Cyfluthrin, λ -cyhalothrin, *cis*-cypermethrin, and deltamethrin were all relatively toxic. Addition of piperonyl butoxide modestly increased toxicity, which suggests that the tested bed bug population has some underlying capability for pyrethroid detoxification based on cytochrome P450 or esterase enzymes, which are blocked by piperonyl butoxide (Young et al. 2005, Yoon et al. 2008). However, resistance levels of different strains of bed bugs vary as does the proportion of resistance attributable to cytochrome P450-based detoxification enzymes, which limits the usefulness of adding piperonyl butoxide as a synergist when targeting bed bugs (Romero et al. 2009a). Based

Table 2. Nonlinear regression parameters and regression R^2 , for the model $Y = Y_0 + 100(1 - e^{-bx})$, fit to the cumulative mortality of bed bugs exposed to dust insecticides

Product	Substrate	Y_0	b	R^2a	S_{10} (d)
Syloid 244	Paper	-0.12	0.98	0.999	0.71
	Fabric	-0.42	2.33	1.0	0.30
	Hardboard	0.01	4.51	1.0	0.15
Drione	Paper	-16.5	0.314	0.954	2.21
	Fabric	-92.6	0.85	0.956	0.82
	Hardboard	-32.6	0.95	0.985	0.73
Tempo Dust	All	-0.02	3.30	1.0	0.21
DeltaDust	Paper	-18.8	0.195	0.975	3.55
	Fabric	-10.8	0.200	0.990	3.46
	Hardboard	-13.7	0.210	0.966	3.30

The survival half-life (S_{10}) in days was calculated from the regression as $\ln(0.5)/-b$.

^a Correlation coefficients of 1.0 result from full parameterization of the regression fit through the data.

upon our results, the use of type I pyrethroids and three of the type II pyrethroids may be ineffective in controlling field populations similar to the one we tested.

D-Force (deltamethrin) and Cyonara (λ -cyhalothrin), the two liquid formulations tested, each contained a type II pyrethroid and were moderately effective in killing bed bugs. Four-week-old residues of Cyonara killed >50% of the bed bugs on hardboard and mattress fabric, and D-Force killed >50% of the bed bugs on filter paper. However, our laboratory bioassays restricted bed bugs so that they were in continual contact with insecticide residues. Some bed bug populations avoid continued contact with pyrethroid residues, which could limit their exposure and mortality (Romero et al. 2009b). Behavioral avoidance of residues by bed bugs would greatly limit toxicity to the sprayed pyrethroid products, because many days of continual contact may be required for mortality to occur. Delayed toxicity of the dust products was observed only with DeltaDust.

Efficacy of insecticides is affected by the substrates on which they are applied (Fletcher and Axtell 1993). Residues decay much more rapidly on certain substrates compared with others, and may bind more strongly to some substrates, which would prevent transfer to the insect. Cyonara was ineffective when applied to paper, but was more effective when applied to hardboard and mattress fabric. D-Force was more effective when applied to paper than to hardboard or mattress fabric. Desiccant components of dusts did not degrade; the dusts were equitoxic at 0–24 wk post-treatment on all three substrates. Our data confirm the assessment that dusts currently are effective against pyrethroid-resistant bed bug populations (Romero et al. 2009c).

Desiccant dusts for controlling insects are either nonsorptive (abrasive) or sorptive (Ebeling 1971). Many insects, including bed bugs, are more susceptible to sorptive dusts, particularly silica aerogels, which remove the lipid protective layer covering the epicuticle, causing relatively rapid water loss (Ebeling and Wagner 1959). An early sorptive formulation, Drione, which contains pyrethrins and piperonyl butoxide, was highly effective in rapidly killing cockroaches 45 yr ago (Ebeling 1971), and currently is highly effective in killing bed bugs. However, our studies show type I pyrethroids, with or without piperonyl butoxide, to be relatively ineffective in killing bed bugs. Comparison of Drione with Syloid 244, its sorptive dust ingredient, showed both to be similar in killing bed bugs up to 24 wk posttreatment. The calculated survival half-life for bed bugs exposed to Drione was \approx 2.5 times that of the Syloid 244, which is consistent with Drione containing only 40% as much silica aerogel. This finding is consistent with ineffectiveness of type I pyrethroids and suggests that the addition of pyrethrins to silica gel dust is unnecessary. Recent work (Benoit et al. 2007, Romero et al. 2009c) has demonstrated lethality of dusts to bed bugs, including time-dependent mortality curves for Drione and Tempo 1% Dust, is equivalent to our results. In that work (Romero et al.

2009c), pyrethroid-resistant populations exposed to DeltaDust took longer to die than a susceptible population, signifying that much of the insecticidal activity from DeltaDust may originate from the pyrethroid ingredient rather than a relatively efficient desiccant. An alternative explanation could be that pyrethroid-resistant bed bugs have adaptive changes to their integument that could provide some protection against desiccation.

An unfortunate consequence of formulating desiccant dusts with pyrethroids (especially type II pyrethroids) is that these dusts may only be applied in areas or in a manner, such as crack and crevice treatment, to minimize human exposure. Eliminating the pyrethroid from a silica gel dust formulation allows more permissive application of desiccant dust to fabrics, such as upholstery, mattresses, and box springs. Desiccant dusts, with their physical mode of action and long residual activity, are superior to pyrethroid insecticides that are sprayed on various substrates for the control of bed bugs.

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